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Host components of Tn7 transposition

Author
Hughes, Owen,

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Host Components of Tn7 Transposition

by

Owen Hughes

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Host Components of Tn7 Transposition

Owen Hughes

Abstract

The goal of this work was to identify E. *coli* components involved in Tn7 transposition. A new variant of transposon Tn7, miniTn7-*lac*, was constructed to enable rapid visual assessment of Tn7 transposition . MiniTn7-*lac* is composed of a promoterless *lac*ZYA operon and an independently transcribed gene conferring kanamycin resistance between Tn7 ends.

Using miniTn7-*lac*, we examined orientation specificity of transposition to *att*Tn7. Tn7 insertion in *att*Tn7 has been characterized as orientation-specific, with the right end of the transposon inserting adjacent to the TnsD binding site in *att*Tn7. Insertion of miniTn7-*lac* into *att*Tn7 in the opposite orientation results in a Lac^+ phenotype, allowing us to identify rare opposite orientation insertions into *att*Tn7. Opposite orientation insertion of miniTn7-*lac* in *att*Tn7 was confirmed through Southern blot analysis. The frequency of opposite orientation insertion was found to be 1% the frequency of the standard insertion orientation.

Mutations in E. *coli* genes known to affect other bacterial transposons were examined for an effect on Tn7 transposition. We found that miniTn7-*lac* transposition is increased in *dam*-13 strains. DAM methylation may link miniTn7-*lac* transposition to DNA replication through a site of DAM methylation in the TnsB binding transposon end sequences. We also observed that Tn7 transposition is decreased by null alleles of the genes encoding FIS, H-NS, and Integration Host Factor (IHF), suggesting that these proteins may also participate in Tn7 transposition.

16 genetic loci associated with altered Tn7 transposition frequencies were identified in a screen of miniTn10-*tet* insertions in the E. *coli* chromosome. The

miniTn10-*tet* insertions were localized by sequencing the chromosomal DNA flanking each insertion. One of the miniTn10-*tet* insertions associated with altered Tn7 transposition was in *hns*, and another was in *min*D. MiniTn10-*tet* insertions were also found in or near six genes which affect the choice between aerobic and anaerobic growth (*arc*B, *sox*RS, *sdh*, *suc*, *men*, and *unc*). MiniTn10-*tet* insertions were also found in genes involved in nucleotide metabolism (*pur*MN , *gua*B, and *dcd*), and transcription (*lys*S, and the *rps*L-*tuf*A operon). These mutants suggest a possible link between the metabolic state of the cell and Tn7 transposition.

Table of Contents

Chapter 1: Introduction

The goal of the work described in this thesis was to better understand the interaction between transposon Tn7 and its host *Escherichia coli.* Interactions between a transposon and its host can be either indirect, through regulation of the expression of the transposon encoded transposition machinery or its activity, or involve direct participation of host-encoded proteins in the recombination complex (reviewed by Kleckner, 1990). The purpose of this introduction is to present our current understanding of transposition and host-transposon interactions. Transposition mechanisms of the different families of transposons will be reviewed in this chapter, with an emphasis on where host involvement may contribute to regulation and facilitation of transposition.

To develop an understanding of inheritance, we have to reconcile conflicting demands of genome stability and evolution. One of the major mechanisms for the evolution of genomes is genetic recombination (Aquadro, 1992; Berg et al., 1984; Ladunga, 1992). Genetic recombination comes in three varieties: generalized homologous recombination, conservative site-specific recombination, and transposition (Craig, 1988). Generalized recombination occurs between large stretches of homologous DNA sequences while conservative site-specific recombination occurs between specific pairs of short homologous sequences such as phage λ's *att*P and bacterial *att*B. In transposition, a segment of DNA moves from one genetic location to another, requiring no homology between the two locations.

Transposition was first described cytogenetically by Barbara McClintock (McClintock, 1956). She identified genetic sites, named *Dissociation* (*Ds*) and *Activator* (*Ac*), which could break *Zae mays* chromosomes. McClintock found that *Ds* and *Ac* could move to new chromosomal locations, causing breakage at their new locations upon

subsequent transposition. The ability of a genetic element to transpose was unexpected and generally unaccepted until examination of spontaneous *Escherichia coli gal* and *lac* mutants generated by insertions disrupting these genes provided an physical understanding of the genetic consequences of transposition (Malamy, 1966; Adhya and Shapiro, 1969; Shapiro, 1969; Saedler et al.; 1972; Hirsch et al. 1972; Hedges and Jacob, 1974; Jordan et al., 1967; Kopecko et al., 1976). Since McClintock's discovery, transposons have proven to be a ubiquitous biological phenomenon .

Transposons have been described in all prokaryotic and eukaryotic organisms where they have been looked for and often constitute a substantial portion of the genome (reviewed in Berg and Howe, 1989). In E. *coli*, IS elements comprise over 0.5% of the genome (Sawyer, 1987), while in *Drosophila melanogaster* transposons make up 10% of the genome (Manning, 1975). Two families of transposons, LINEs and SINEs, comprise several percent of the mammalian genome (Bennett, 1984; Fanning, 1982; Houck, 1979; Rinehart, 1981), and a variety of proviral DNAs accounts for as much as another 1% (Brown et al., 1989). Through their ubiquity, transposons present a significant dynamic in the evolution of genomes (Chao et al., 1983; Syvanen, 1984).

Mechanism of Transposition

Though transposons come in a wide variety of forms, the examples that have been examined in detail are unified by their basic mechanism of DNA recombination. From the study of a limited set of examples, a view of transposition as defined by three mechanistic steps can be developed and is illustrated in Figure 1-1, though variations on these mechanistic processes may yet be found.

1) The recombination mechanism of all transposons so far examined involves recognition of the transposon ends by transposase, followed by DNA strand cleavage exposing transposon terminal 3' hydroxyls (Mizuuchi, 1984; Fujiwara, 1988; Brown,

1989; Eichinger, 1990; Bainton et al., 1991; Engelman et al., 1991; Surette et al., 1991). Strand cleavage is the result of nucleophilic attack (most likely by water) on the phosphate group linked to the transposon terminal 3' hydroxyl, promoted by the transposase. For Mu and HIV, it is known that there is no covalent protein/DNA linkage to the transposase (Adzuma and Mizuuchi, 1991; Engelman et al., 1991).

2) The transposon terminal 3' OH groups exposed by the previous cleavage step directly attack phosphate links of the target site, simultaneously breaking the target strand and forming a target-transposon phosphate linkage in a concerted reaction. Since the number of bonds broken and formed is conserved, no exogenous energy source is required for the concerted reaction. For Mu and HIV, evidence for this direct nucleophilic attack by the transposon 3' OH groups on the target site phosphate has been seen in the form of the resulting single inversion of the phosphate center (Mizuuchi, 1984; Adzuma and Mizuuchi, 1991; Engelman et al., 1991). The two transposon terminal 3' OH groups attack target phosphates staggered 5' to one another (Craigie, 1987; Mizuuchi, 1984; Adzuma and Mizuuchi, 1991; Engelman et al., 1991).

3) Target site duplication occurs by filling in the single stranded DNA that results from the staggered break using the host DNA maintenance and repair machinery (Benjamin and Kleckner, 1989; Shapiro, 1979). The length of the duplication is determined by the degree of staggering and is characteristic for each transposon (Berg and Howe, 1989).

Transposition can be defined by these three mechanistic steps common to the transposons examined to date. However, these three steps alone are not sufficient to complete all the events of a transposition reaction. Distinct families of transposons can be grouped by the similarity of mechanistic steps required to complete transposition. Two of the major mechanistic families are non-replicative and replicative transposition.

Figure 1-1

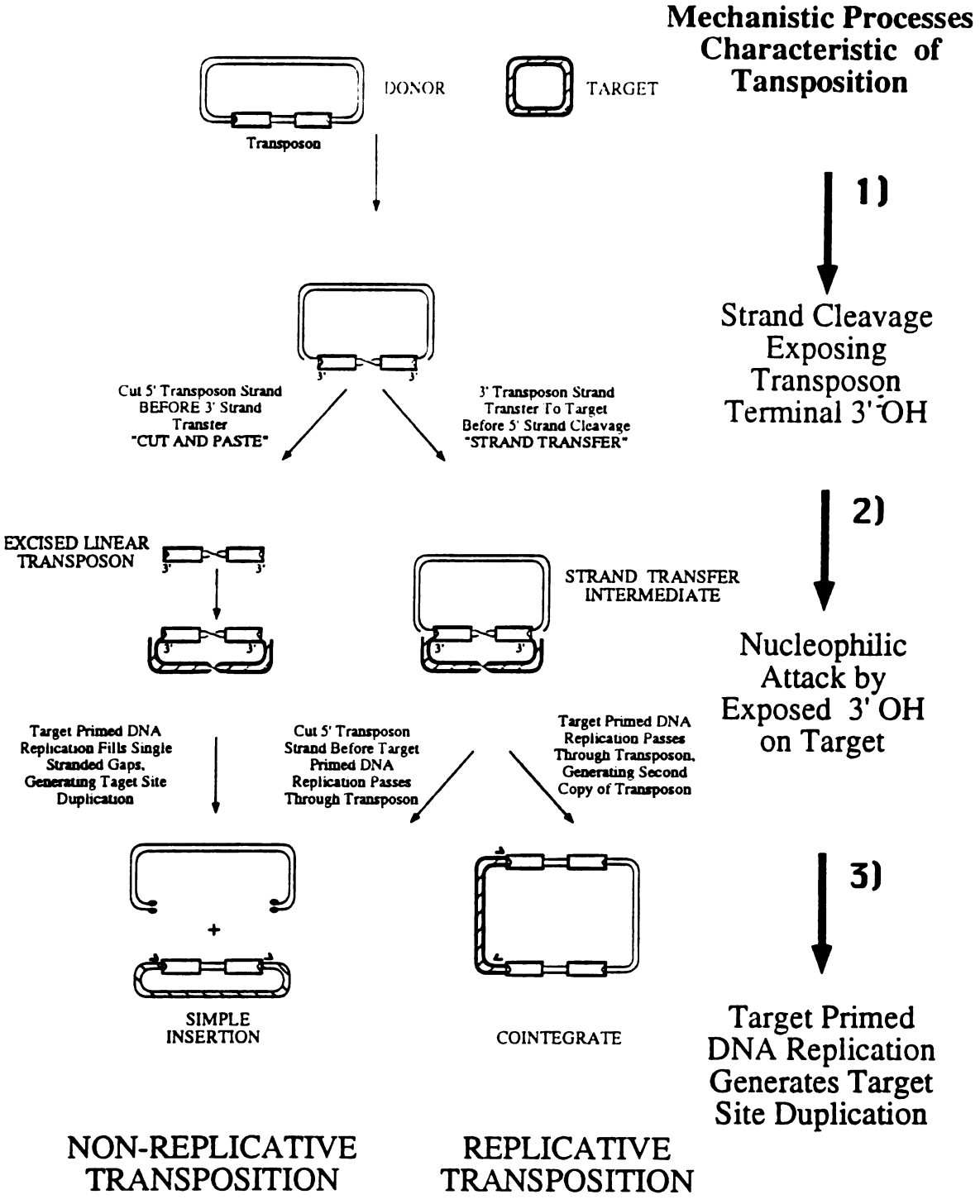

Mechanistic Processes Characteristic of Tansposition
DONOR
TARGET
Transposon
1)
Strand Cleavage Exposing Transposon Terminal 3'OH
Cut 5' Transposon Strand BEFORE 3' Strand Transfer "CUT AND PASTE"
3' Transposon Strand Transfer To Target Before 5' Strand Cleavage "STRAND TRANSFER"
2)
EXCISED LINEAR TRANSPOSON
STRAND TRANSFER INTERMEDIATE
Nucleophilic Attack by Exposed 3'OH on Target
Target Primed DNA Replication Fills Single Stranded Gaps, Generating Taget Site Duplication
Cut 5' Transposon Strand Before Target Primed DNA Replication Passes Through Transposon
Target Primed DNA Replication Passes Through Transposon, Generating Second Copy of Transposon
3)
SIMPLE INSERTION
COINTEGRATE
Target Primed DNA Replication Generates Target Site Duplication
NON-REPLICATIVE TRANSPOSITION
REPLICATIVE TRANSPOSITION

Figure 1-1 Legend

Mechanistic Processes Characteristic of Transposition

The three mechanistic steps common to the transposons examined to date are illustrated here in the context of the major pathway choices which define the replicative and non-replicative families of transposons. The three mechanistic steps characteristic of transposition are noted on the right of the figure. Text beside the thin arrows note the major pathway choices. Simple insertions and cointegrates structures are the products of transposition. As illustrated, both simple insertions and cointegrates can be formed via replicative transposition, while non-replicative transposition produces only simple insertions. These product distinctions between the results of replicative and non-replicative transposition can be blurred by further site-specific and general homologous recombination reactions (not illustrated - discussed in text). Portions of this figure adapted from Bainton, 1992.

Non-replicative Transposition

In non-replicative transposition, the transposon is cut from the donor site and inserted in the target site without replicating the transposon. Both strands of the transposon DNA are inserted into the target site, requiring DNA replication across only the single-stranded gaps left by the 5' staggered attack on the target (Bender and Kleckner, 1986). Non-replicative transposition can be achieved in two ways: by a cut and paste mechanism or by a strand transfer mechanism.

Cut and Paste Transposition

In the first step of transposition described above, one strand of DNA is cleaved at each end of the transposon, leaving exposed transposon terminal 3' OH groups. At this point, the DNA is nicked, i.e. containing a single strand break at each end of the transposon, but the transposon is still attached to the donor DNA by the unbroken strand. If both strands of DNA at each end of the transposon are cut from the donor site, the transposon is freed from the donor, and both DNA strands can then be inserted into the target site. *In vitro*, Tn10 and Tn7 have both been isolated as excised transposons which can subsequently complete transposition into a target site (Bainton et al., 1991; Hanniford et al., 1991). Tn10 cuts both strands flush at each end of the transposon, leaving both the terminal 3' and 5' strands of the transposon detached from donor DNA (Benjamin and Kleckner, 1989). Tn7 makes 5' staggered cuts at each end of the transposon, leaving 3 nucleotides of donor DNA attached to the 5' ends of the transposon (Bainton et al., 1991). These 5' donor site nucleotides are removed presumably during host repair of the target gap. The 5' donor site nucleotides seem to be unimportant in that synthetically-generated Tn7 with flush ends (lacking the 5' donor site nucleotides) and Tn7 elements with the 5' donor nucleotides are equally competent of transposition *in vitro* (Bainton et al., 1991).

Non-replicative Strand Transfer

A second mechanism of inserting both donor transposon strands into the target site involves the formation of a strand transfer intermediate. A strand transfer intermediate is formed by attack of the transposon terminal 3' hydroxyls on the target phosphates before the 5' transposon terminal strands are cleaved. In the strand transfer intermediate, both the donor and target molecules are joined by the transposon. To complete non-replicative strand transfer transposition, the donor molecule is released from the strand transfer intermediate by cleavage of the transposon's 5' terminal strand. 5' terminal strand cleavage must happen before host repair mechanisms fill the single stranded gap and continue replication across the transposon (Shapiro, 1979).

Non-replicative Transposition and the Host

Non-replicative transposition can have dire consequences for the host. Both non-replicative transposition mechanisms leave a broken gap, or double strand break, in the donor molecule (Morisato and Kleckner, 1984). Double strand breaks may lead to the destruction of the donor replicon (Berg and Drummond, 1978; Morisato and Kleckner, 1984; Weinert et al., 1984). In E. *coli*, SOS induction as a response to transposition has been shown for Tn7 and Tn10 (Haniford et al., 1989; Stellwagen and Craig, personal communication). SOS is a complex set of emergency responses to DNA damage and challenges which interfere with DNA replication (Defais, 1971; reviewed by G. Walker, 1987). Along with SOS induction, double strand gap repair is stimulated by transposition of Tn10 and Tn7 (Bender et al., 1991; Hagemann and Craig, 1993). The transposition of the *Drosophila* P element and the Tc1 transposon of C. *elegans* has also been reported to be associated with repair of the donor site directed by homologous DNA (Engels et al., 1990; Plasterk and Groenen, 1992).

Non-replicative transposition mechanisms do not leave a copy of the transposon at the donor site. Because of this, transposons "move" rather than "spread" though non-

replicative transposition. However, duplication of the transposon can result from non-replicative transposon if either the donor molecule from which the element is excised undergoes host mediated double strand gap repair, or if transposition occurs from a site behind a replication fork to a site in front of a replication fork on another replicon (Kleckner, 1981; Kleckner, 1990). As has been demonstrated for Ac transposition in *Zea mays*, though one copy of the donor chromosome may be lost as a result of non-replicative transposition from a site behind a replication fork to a site in front of a replication fork, one copy will remain and it will contain elements at both the donor and target sites (Greenblatt, 1966; Greenblatt and Brink, 1963; Greenblatt and Brink, 1962).

Replicative Transposition

Strand Transfer

In replicative transposition, only one strand of the transposon DNA is transferred from the donor site to the target site. After replicative transposition, both the donor and target sites have a copy of the transposon. Replicative transposition proceeds through a strand transfer intermediate, in which the transposon is attached to both the donor site at its 5' termini, and attached to the target site at its 3' termini . The mechanistic step that defines replicative transposition and creates the strand transfer intermediate is the absence of 5' transposon terminal strand cleavage (Craigie and Mizuuchi, 1985; Craigie and Mizuuchi, 1986; Kleckner, 1990). After formation of the strand transfer intermediate, the host replication machinery uses the broken 3' OH of the target strands as primers to initiate replication of the transposon. Replication across the transposon is completed by strand ligation to the donor molecule.

If the donor molecule is circular, the result of replicative transposition is the formation of a cointegrate (Arthur and Sherratt, 1979; Shapiro, 1979). The cointegrate structure has two directly repeated transposons flanking the donor molecule inserted into

the target site. Replicative transposition results in two copies of transposon, each with one newly replicated strand of DNA and one strand from the original copy.

A second "resolving" recombination event can excise the donor molecule and one transposon, leaving the target molecule with one transposon inserted between the target site duplication. The donor molecule is regenerated with the transposon in its original site. This resolving recombination event can be accomplished either by general homologous or conservative site-specific recombination between the two transposons in a cointegrate molecule. In addition to transposase, the Tn3 family of transposons encode a second recombination protein, *resolvase*, to promote site-specific recombination between *res* sites in the transposons of a cointegrate (Arthur and Sherratt, 1979; Grindley, 1983).

Replicative Transposition and the Host

Unlike non-replicative transposition, replicative transposition does not endanger either the transposon donor or the target replicon integrity if the donor molecule is circular (Kleckner, 1981; Kleckner, 1990). If, however, the donor molecule is linear, the target replicon will be linearized if it is circular or undergo a translocation with the donor if it is linear. Replicative transposition from an entering conjugal DNA segment into the bacterial chromosome results in linearization of the chromosome which can be rescued by recombination between the two resulting transposon copies. Recombination between two transposon copies is greatly enhanced by *res*/resolvase systems (Arthur and Sherratt, 1979; Grindley, 1983).

Inherent in replicative transposition, both donor and target sites end up with copies of the transposon generated by semiconservative replication. Replicative transposition is thus characterized by "spread" rather than "movement" of a transposon (Kleckner, 1990).

Retro-Transposition

Another family of transposons combines elements of both replicative and non-replicative transposition. Retro-transposons and retroviruses transcribe the donor site DNA element into RNA before reverse-transcribing the RNA into a DNA copy of the donor element. The reverse transcribed DNA copy of the element is then non-replicatively inserted at another genetic location by an element-encoded protein designated integrase (IN) (Varmus and Brown, 1989). Retroviral integrases are analogous to, and in some cases show sequence similarity to, bacterial transposases. Retro-transposons and retroviruses "spread" by producing a copies of the transposon while leaving the original intact. The DNA copy of retro-transposons and retroviruses is double-stranded, and both strands are integrated into the target site in a manner exactly analogous to non-replicative cut and paste transposition (Fujiwara, 1988; Adzuma and Mizuuchi, 1991; Engelman et al., 1991). Retroviruses and retro-transposons are mainly found in eukaryotes (Varmus and Brown, 1989). This thesis will focus on prokaryotic transposons.

Non-replicative vs. Replicative Transposition

In summary, non-replicative, replicative and retro-transposons differ in the fate of the donor transposon DNA strands (Campbell, 1980). Non-replicative transposition moves both strands to the insertion site. Replicative transposition moves one strand to the insertion site and leaves one at the donor site. Retro-transposition leaves both DNA strands at the donor site.

Some transposons can move by both non-replicative and replicative transposition. The choice between non-replicative or replicative transposition can be either developmentally determined as is the case with bacteriophage Mu, or may be "leakage" from one to the other mechanism of transposition due to their inherent similarity (differing only in the timing of the 5' strand cleavage), as is thought to happen with

insertion sequences (Craigie and Mizuuchi, 1985; Kleckner, 1981; Kleckner, 1990; Ohtsubo et al., 1981; Shapiro, 1979). A brief description below of phage Mu and Insertion Sequence transposition highlights the choice of non-replicative vs. replicative transposition and the genomic rearrangements that follow.

Phage Mu

Bacteriophage Mu is a temperate phage which employs both replicative and non-replicative transposition. After infection, Mu forms a lysogen by integrating into the bacterial chromosome through non-replicative transposition (Harshey, 1984). In lytic growth of Mu, Mu generates over 100 copies of itself through replicative transposition (Pato and Waggoner, 1981).

Mu encodes many proteins, but only two have been directly implicated in its transposition (Pato, 1989). MuA is the transposase, binding the ends of Mu and performing the strand cleavage/transfer reactions. MuB binds nonspecific DNA and is required for efficient use of intermolecular targets. Mu can transpose in the absence of MuB, though transposition is inefficient and mostly intramolecular (Maxwell et al., 1987; Mizuuchi, 1983). How Mu developmentally switches from non-replicative to replicative transposition is not understood, but is easy to rationalize in terms of "move" vs. "spread" phases of development (Chaconas et al., 1985).

IS, composite elements, and Tn3

Insertion sequences , or IS elements, are among the shortest fully competent transposons. The most basic, independent transposon is defined by a DNA sequence encoding a "transposase" to promote transposition flanked by DNA "ends" which are recognized by the transposase and utilized as the sites of DNA strand cleavage and transfer into a target DNA site. IS elements may encode only a single protein, the transposase, though many also encode a transposase regulator (Galas and Chandler, 1989).

Two IS elements can act together to mobilize a segment of DNA between them, forming a composite transposon (Galas and Chandler, 1989). With composite transposons, transposase encoded by an IS acts on the outside ends of the flanking IS elements. The information relevant to composite element transposition is encoded within the IS element ends, and information that is of selective advantage to the host cell is often encoded within the intervening DNA.

Some IS elements can move by both replicative and non-replicative transposition. IS1 and IS903 generally move *via* non-replicative transposition, though at low frequency can also form cointegrates through replicative transposition (Weinert, 1983; Weinert, 1984; Grindley and Reed, 1985). The use of both replicative and non-replicative transposition mechanisms for IS elements may not be developmentally regulated as with Mu but rather result from variability in the timing of 5' strand cleavage. After formation of a strand transfer intermediate, the decision between replicative and non-replicative transposition is determined by whether 5' transposon terminal strand cleavage happens before the target primed replication can pass through the transposon.

The Tn3 family of transposons generally translocates *via* replicative transposition forming cointegrates (Sherratt, 1989). However, in the absence of both the RecA mediated host recombination system and the Tn3 encoded *res*/resolvase site-specific recombination system, a low (<2%) percentage of simple insertion products is formed (Bennett et al., 1983). Whether these simple insertions are the result of non-replicative transposition is debated. These simple insertions may be formed as the result of non-replicative transposition or by replicative transposition forming a cointegrate that is subsequently resolved by a pathway independent of RecA and *res*/resolvase.

Genome Rearrangement

Transposition is a process of genome rearrangement and is likely to be an important factor in genomic evolution. Entire genomic segments can be mobilized by transposition using the outside ends of two separate transposons as is seen with the conjunction of two IS elements to form composite transposons. Inserting a transposon in a new genetic locus interrupts and often destroys the function of that locus. In addition to the destruction of the target site gene, most transposons exert strong polar effects on the expression of downstream genes (Jordan et al., 1967; Malamy, 1966). Transposon insertion in gene control regions can also deregulate genes or bring them under the control of promoters within the transposon.

Transposition can also lead indirectly to genome rearrangement in the form of inversions, deletions and translocations by general, homologous recombination between transposons scattered throughout the genome (Galas and Chandler, 1989). Transposition is thus a major threat to the stability of genomes.

Regulation of Transposition

Only transposons which have developed specialized mechanisms for horizontal transmission, such as phage Mu and retroviruses, can afford to adversely affect their hosts. Most transposons, however, have a strong vertical component of transmission. Without specialized mechanisms for horizontal transmission, they must rely on transposition to viruses and conjugal plasmids for horizontal transmission. Because of vertical transmission, viability of the transposon is linked to viability of the host.

Levels of transposition low enough not to grossly affect host viability may be too low for evolutionary success (Campbell, 1981a; Campbell, 1981b). Horizontal entry of a transposon into a new bacterial lineage, on a virus or conjugal plasmid, is a situation when efficient transposition would benefit the transposon, overriding the need to protect

the host from high levels of transposition. If the horizontal vector DNA is restricted or cannot replicate in the new host, efficient transposition to the host chromosome would increase the likelihood of the entering transposon's establishment. So, regulation of transposition must balance genomic stability and the evolutionary need to over replicate the host to succeed (Charlesworth, 1983).

Regulation of Transposase Expression

In many systems, transposition is thought to be limited by the availability of active transposase. To modulate the level of active transposase, either its expression or activity may be regulated. To restrict transposition, transposase expression is generally very low. Inherently weak promoters have been implicated in the low basal levels of IS1, IS10 and IS50 transposase transcription (Zerbib et al., 1987; Raleigh and Kleckner, 1986; McCommas, 1988). The IS10 transposase gene is estimated to be transcribed only 0.25 times per cell per generation (Raleigh and Kleckner, 1986), yet it is required to form a multimeric (possibly tetrameric) structure for transposition. IS10, IS50, Tn3, and Mu achieve low levels of transposase expression partially through the use of poor ribosome binding sites (Casadaban et al., 1982; Kleckner, 1990). IS 903 uses the poorly recognized GUG initiation codon rather than the preferred AUG (Derbyshire, 1990).

Repression of transposase transcription is a another method of controlling expression. The Tn3 family of transposons uses the resolvase (TnpR) to repress transposase (TnpA) by situating the transposase promoter within *res*, the resolvase binding site (Chou et al., 1979; Gill et al., 1978; Heffron et al., 1979). Binding of TnpR to *res* occludes the TnpA promoter. Divergent transcription of resolvase from a promoter also within the *res* site allows for autogenous regulation of the transposase repressor, resolvase (Sherratt, 1989).

Regulation of transposase translation has been demonstrated for many transposons, including IS1, IS10, IS50, IS903, Tn3, Mu, and many retroviruses (Jacks, 1985; Berg and Howe, 1989; Kleckner, 1990). IS10 efficiently represses transposase translation. By encoding an antisense RNA (RNA-OUT), IS10 can sequester the ribosome binding and start sites of RNA-IN, the transposase transcript. Overlapping divergent transcripts produce RNA-OUT and RNA-IN. Transcription of RNA-OUT and RNA-IN is mutually exclusive, making antisense RNA control most effective in *trans* (Kleckner, 1990; Simons and Kleckner, 1983).

Translational frame-shifting is used by many retroviruses, retro-transposons, and some bacterial transposons to control the expression of transposase. Translation of retroviral reverse transcriptase and integrase (transposase) requires a frame shift in the -1 direction for RSV, MLV, MMTV, HTLV and HIV (Jacks, 1985; Varmus and Brown, 1989). Translation of TyA-TyB transposase fusion product of S. *cerevisiae* Ty retrotransposons seems to require a +1 frame shift (Wilson, 1986; Clare et al., 1988; Clare and Farabaugh, 1985; Happel, 1992). Bacterial insertion sequence IS1 reduces transposase expression by requiring translational frame-shifting to produce transposase (Zerbib et al., 1990; Sekine, 1989).

Retroviruses, and many transposons in eukaryotes, have the opportunity to control transposase production through mRNA splicing. P elements in *Drosophila melanogaster* transpose only in germ line cells. Tissue specificity comes about by differential splicing of the transposase message. Only in the germ line is the intron between the second and third transposase exons removed to form active transposase (Laski et al., 1986; Siebel, 1990).

Regulation of Transposase Activity

To respond quickly to environmental change in the host without requiring *de novo* gene transcription and message translation, and as a second safety catch for leaky

transposase expression control, transposons can tightly control transposase activity as well as expression (reviewed by Kleckner, 1990, and specific examples below). Transposase activities generally include binding the two transposon ends, binding the target site, bringing these three DNA segments together, and catalyzing strand cleavage/transfer reactions to integrate the transposon into the target site. Each of these steps in transposase action may be subject to regulation.

Transposase activity can be inhibited by factors that bind to one of the DNA segments which must be brought together to perform transposition. For example, Mu transposase (MuA) must bind an internal activating sequence (IAS) as well as Mu ends to efficiently promote transposition (Leung et al., 1989; Mizuuchi and Mizuuchi, 1989). Mu repressor (MuC) binds the IAS, efficiently inhibiting it from MuA binding. MuC has sequence similarity to MuA (Harshey et al., 1985) and can bind to MuA binding sites at the ends of Mu transposon (Craigie et al., 1984), suggesting that part of MuC repressor activity may also be at the level of competing with MuA for end binding.

Transposase activity can be repressed by classic dominant negative interaction with transposase truncations retaining partial activity, such as binding DNA, but no longer able to promote transposition. IS50 transcribes a transposase inhibitor, *inh*, from a start site 55 codons into the transposases, *tnp* (Isberg et al., 1982; Johnson et al., 1982). IS50 inhibitor seems to function by forming mixed Tnp-Inh oligomers which are incapable of promoting transposition (DeLong and Syvanen, 1990).

IS1 transposition is negatively regulated by a transposase truncation. Failure to perform translational frame-shifting on the InsAB transposase message produces a truncation, InsA, missing the C-terminus of the full InsAB transposase (Machida and Machida, 1989; Sekine and Ohtsubo, 1989; Zerbib et al., 1990). InsA binds specifically to the ends of IS1, inhibiting transposition by directly competing with transposase for the binding sites (Zerbib et al., 1990).

Multicopy Transposition Inhibition

To reduce the evolutionary load of transposable elements on host cells, most transposons have developed regulatory mechanisms to inhibit the accumulation of multiple copies of the transposon. One common multicopy inhibition mechanism characterized by many IS elements is the use of stable, *trans*-acting inhibitors to repress unstable, preferentially *cis*-acting transposase (Foster et al., 1981; Galas and Chandler, 1989; Isberg and Syvanen, 1981; Johnson et al., 1982; Morisato and Kleckner, 1984). When transposase acts preferentially in *cis*, each IS element sees only the transposase it makes. The combination of *trans*-acting transposase inhibitors and preferential *cis*-acting transposase reduces the rate of transposon accumulation because the effective concentration of the inhibitor goes up while the effective concentration of *cis*-acting transposase remains the same as copy number increases (Kleckner, 1990).

The use of *trans*-acting transposase inhibitors is an effective method of allowing a burst of transposition activity upon horizontal entry into a naive cell. *Trans*-acting repression of Tn3 transposase expression, as detailed above, is accomplished by occluding the transposase promoter with a repressor, TnpR. On Tn3 entry into a Tn3-free cell, the absence of repressor TnpR allows for a burst of transposase transcription and subsequent transposition activity occurs (Chou et al., 1979). IS10 also experiences a burst of transposition activity upon entry into a IS10-free cell. One of the possible mechanisms behind this burst of transposition is that IS10-free cells lack transposase translation repression *via* pOUT antisense RNA occlusion of the ribosome binding site for the transposase message from pIN (Kittle et al., 1989; Simons and Kleckner, 1983). Another mechanism of activating IS10 transposition upon transposon entry into a cell is the regulation of transposition by DNA adenine methylation (considered in detail below). The relative contributions of DAM and pOUT regulation to the burst of transposition observed upon entry into a cell are unknown.

Transposition Immunity

Bacteriophage Mu, the Tn3 family of transposons, and Tn7 have developed a special case of multicopy inhibition, transposition immunity (Adzuma and Mizuuchi, 1988; Hauer and Shapiro, 1984; Robinson et al., 1977; Sherratt, 1989). Transposition immunity is *cis*-specific in that it acts to inhibit transposition to a replicon already containing a copy of the transposon and generally does not affect transposition to other replicons. Immunity is conferred through the interaction of transposase with a transposon end (or a single end) resident in the target molecule (Arciszewska et al., 1989; Darzins et al., 1988; Kans and Casadaban, 1989; Wiater and Grindley, 1990b; Amemura, 1990; Nissley, 1991).

The mechanism of transposition immunity is best understood for Mu (Adzuma and Mizuuchi, 1988; Adzuma and Mizuuchi, 1989; Adzuma and Mizuuchi, 1991). Intermolecular transposition of Mu requires the transposase, MuA, and a targeting protein, MuB. MuB binding defines the target of Mu transposition by binding DNA nonspecifically in an ATP bound state, then recruiting the synaptic complex of two Mu transposon ends held by MuA. However, MuA can also associate with MuB in a manner which inhibits transposition. MuA associated with a single Mu end in the target molecule acts to stimulate MuB-ATP hydrolysis. Upon hydrolysis of ATP, MuB dissociates from the DNA, no longer making it a target. In the absence of MuB, Mu transposition is extremely inefficient. Mu mutants lacking MuB transpose inefficiently and mostly intramolecularly, having lost the ability to discriminate between immune and non-immune targets.

Host Components of Transposition

Recombination of many mobile elements requires or is regulated by host factors (Berg and Howe, 1989). Host component participation in transposition enables transposons to tie transposition to favorable host environment conditions. Like the regulation of transposition described above, host control of transposition activity can be through modulation of transposase expression or activity. Three types of host participation in transposition events have been described: regulation of the activity or expression of the transposon encoded transposition machinery, direct involvement in the recombination complex, and DNA maintenance and processing necessitated by the process of transposition (Kleckner, 1990). Examples of these types of host-transposon interactions are described below.

DNA Adenine Methylation, DAM

The bacterial DNA adenine methylation (DAM) system is one of the best understood examples of host regulation of transposition. Transposition of IS10, IS50, and IS903 is regulated by DAM (Roberts et al., 1985; Yin et al., 1988). The product of the *dam* gene, DNA adenine methyltransferase, methylates the N-6 position of adenine on both strands of the duplex sequence GATC (Kleckner, 1989; Roberts et al., 1985). The case of IS10 is an illustrative example of DAM regulation of transposition. In *dam*$^-$ bacterial strains, IS10 transposition activity increases about 100-fold. DAM has been shown to regulate IS10 transposition by controlling both expression and activity of transposase.

DNA adenine methyltransferase acts at two GATC sequences in IS10. One DAM site is near the outside end in the -10 region of the pIN transposase promoter. DAM methylation at this site decreases transposase expression by decreasing pIN transcription.

The other DAM site lies within the inside end transposase binding site. DAM methylation of the transposase binding site decreases transposase activity by inhibiting end binding (Roberts et al., 1985). IS50 transposition is regulated by DAM in an exactly analogous way as IS10, with DAM sites repressing transposase transcription (McCommas and Syvanen, 1988; Yin et al., 1988) and blocking transposase end binding activity (Dodson and Berg, 1989; Makris et al., 1988).

In wild type *dam*$^+$ strains, the passage of a replication fork leaves two copies of the transposon, each hemimethylated until DAM methylates the newly synthesized strands. The ratio of transposition activity of the fully methylated and the two hemimethylated (top strand methylated and bottom strand methylated) transposons is approximately 1:12:2,400-60,000 (Kleckner, 1989). Thus only one of the two hemimethylated elements transposes.

DAM regulation is an obvious method of linking non-replicative transposition to replication. Hemimethylated DNA generally persists after replication fork passage for less than 10% of the cell cycle. Non-replicative transposition leaves a double strand break at the donor site. Though non-replicative transposition moves both strands of the donor element into the target site, transposing after replication fork passage can effectively leave a copy at the donor site. Activation of only one of the hemimethylated transposons after replication fork passage acts to ensure that a copy of the transposon in the donor site remains from which double strand gap repair may be directed. P element, Tc1, and Tn7 transposition have been shown to stimulate double strand gap repair at the donor site (Engels et al., 1990; Plasterk, 1992; Hagemann and Craig, 1993). Failure to repair the donor molecule after replication fork passage, however, will cause loss of just one arm of the replication fork rather than loss of the entire replicon.

Activation of transposition by hemimethylation plays an important role in horizontal transfer. Horizontal entry by conjugal transfer of a single DNA strand followed by complementary strand synthesis would result in a burst of transposition

activity. A similar burst of transposition is seen from undermethylated phage infection (McCommas and Syvanen, 1988).

Regulation of transposition by DNA methylation has also been reported in plants. Ac and En/Spm transposase binding depend directly on the cytosine methylation state of their binding sites (Federoff, 1989).

Histone-Like Proteins

Histone-Like Factor U, HU

HU is the most abundant histone-like protein in E. *coli*. Histone-like proteins are small, basic, usually heat stable, DNA-binding proteins that are highly conserved between prokaryotes and eukaryotes at the primary sequence level (Drlica and Rouviere, 1987; Pettijohn, 1988). Histone-like proteins wrap DNA to form higher-order structures, and some can condense DNA to form nucleosome-like structures (Broyles and Pettijohn, 1986; Rouviere-Yaniv et al., 1979).

In E. *coli*, HU is formed as a heterodimer of HU-α and HU-β coded for by *hup*A and *hup*B (Kano et al., 1986). Single mutations in *hup*A or *hup*B are masked by formation of active homodimers and do not affect cell growth. E. *coli* null mutants in both *hup*A and *hup*B have recently been described, and though viable, grow very poorly (Huisman et al., 1989). This combination of no phenotype for the single mutant and near lethality of *hup*A *hup*B double mutants has made genetic investigation of HU difficult.

HU is required for Mu transposition *in vitro* (Craigie et al., 1985). HU requirement can be bypassed if the donor DNA is nicked at the 3' ends of Mu, suggesting that HU is required to organize Mu into a form capable of being nicked at the 3' termini by MuA *in vivo* (Craigie and Mizuuchi, 1987).

In vivo, *hup*A or *hup*B single mutants have no effect on Mu transposition, as might be expected of masking by HU homodimers. However *hup*A *hup*B double mutants do not support Mu transposition (Huisman et al., 1989).

HU has also been implicated in the transposition of Tn10. *In vitro*, HU or IHF is required in addition to Tn10 transposase for transposition activity of two outside ends (Kleckner, 1989). The mechanism of HU involvement in IS10 transposition is unknown, though it may function in place of or with IHF (see below).

Integration Host Factor, IHF

Integration host factor (IHF) belongs to the class of related "histone-like" proteins that can wrap DNA into higher-order structures (Drlica and Rouviere, 1987). IHF is a sequence-specific DNA binding protein involved in a variety of cellular processes including replication (Gamas et al., 1986), gene expression (Friedman, 1988), plasmid partitioning (Funnel, 1988) and genetic recombination (see below). IHF was identified by E. *coli* mutants that fail to support phage λ site-specific recombination (Miller et al., 1979). Many recombination systems have since been shown to utilize IHF. IHF is composed of two protein subunits, both small and heat stable, encoded by *him*A at minute 38 and *hip* at minute 25 on the E. *coli* chromosome (Mechulam et al., 1985; Miller et al., 1984).

IHF is involved in the transposition of IS1, γδ, Tn5, IS10 and Mu (Gamas et al., 1987; Gamas et al., 1985; Morisato and Kleckner, 1987; Krause and Higgins, 1986; Surette and Chaconas, 1989; Surette et al., 1989; Wiater and Grindley, 1988; Wiater and Grindley, 1990a; Makris et al., 1990). IHF may affect transposase activity either through direct contact or indirectly through manipulation of DNA structure. IHF binding to the transposon ends has been show to cooperatively facilitate transposase binding for γδ (Wiater and Grindley, 1988). IHF facilitation of γδ transposase binding also enhances γδ transposition immunity conferred by a transposase bound end (Wiater and Grindley, 1990a). IHF is required for IS10 outside end activity *in vivo* and *in vitro* (Morisato and Kleckner, 1987; Roberts et al., 1987; Huisman et al., 1989; Kleckner, 1989). As with γδ, IHF binds next to the Tn10 transposase binding site. Efficient Mu transposition *in vitro*

at *in vivo* levels of supercoiling requires IHF as well as HU (see above). IHF's participation is mediated through an IHF binding site in the Mu early promoter region. IHF binding generates a specific geometrical configuration, which includes a sharp bend in the DNA, and is required for optimal induction of synapsis of the Mu transposon ends (Surette and Chaconas, 1989; Surette et al., 1989).

IHF can also affect transposition through its effects on gene expression. IHF mutants have been shown to reduce transcription from the Mu early promoter (Krause and Higgins, 1986). IHF has also been implicated in multicopy inhibition control of IS10, *via* transposase promoter pIN (Kleckner, 1989).

Histone-Like Protein H1, H-NS

H-NS is a small, moderately abundant, DNA-binding, histone-like protein. Unlike HU, H-NS is neutral rather than basic, and is formed as a homodimer. H-NS is thought to act through the binding and stabilization of bent DNA for the in the facilitation of building protein-DNA complexes (Yamada et al., 1991). H-NS mutants have pleiotropic effects on gene expression and DNA supercoiling, reflected by the number of names it is known by: H1, *pro*U, *osm*Z, *pil*G, *bgl*Y, *vir*R and *drd*X (Hulton et al., 1990; Owen-Hughes et al., 1992).

H-NS has been implicated in the transposition of Mu. Mu transposition in H-NS mutants is elevated (Falconi et al., 1991). Purified H-NS stabilizes Mu repressor-DNA complexes *in vitro*, suggesting that *in vivo*, H-NS contributes to the Mu transposition repression *via* Mu repressor (Gama et al., 1992).

Factor for Inversion Stimulation, FIS

FIS (factor for inversion stimulation) is a small, heat-stable DNA-binding protein. Though FIS has many of the physical characteristics of histone-like proteins, it is often considered separately because it is unrelated at the primary sequence level (Drlica and Rouviere, 1987; Finkel and Johnson, 1992).

FIS was identified by its ability to stimulate Hin and Gin site-specific DNA inversion reactions (Johnson et al., 1986; Johnson and Simon, 1985; Kahmann et al., 1985). FIS was later shown also to be involved in Cin, Pin and phage λ site-specific recombination reactions (Ball and Johnson, 1991a; Thompson et al., 1987) as well as the translocation of a number of prokaryotic transposons. FIS is thought to facilitate the assembly of DNA binding complexes by bending the DNA. As FIS binds DNA it induces a 40-90° bend (Thompson and Landy, 1988; Gille et al., 1991). FIS is highly expressed only in early exponential growth, and so can be used to link recombination to growth (Ball and Johnson, 1991a; Ball and Johnson, 1991b; Thompson et al., 1987).

FIS stimulates phage λ excision over 200-fold. FIS bound adjacent to *att* directly enhances Xis binding and may alter *att* topology to favor excision (Ball and Johnson, 1991a; Thompson et al., 1987). FIS has been shown to bind the ends of phage Mu, and in *fis*⁻ mutants, Mu lysogens are less stable under some conditions (Alazard et al., 1992; Betermier et al., 1989). FIS is also involved in the regulation of Tn5. FIS stimulates Tn5 transposition approximately 10-fold during early exponential E. *coli* growth and can inhibit IS50 transposition when the inside end of the element is not methylated by DAM. The combination of Tn5 stimulation and IS50 inhibition biases transposition towards transposition of the composite element Tn5 (Weinreich and Reznikoff, 1992).

DNA Maintenance and Processing

The process of transposition involves manipulation of DNA molecules. Transposons employ many of the elaborate mechanisms cells have developed to replicate, repair, and maintain DNA integrity (Berg and Howe, 1989).

DNA Supercoiling and Transposition

DNA supercoiling is generated through the action of DNA gyrase (encoded by *gyr*A and *gyr*B in E. *coli*) (Cozzarelli, 1980). DNA supercoiling is known to affect gene expression and the development of higher-order DNA and DNA-protein structures (Craigie, 1986; Richet, 1986; Higgins et al., 1989). Transposons form elaborate DNA-protein complexes which may have specific supercoiling requirements *in vivo* (Isberg and Syvanen, 1982). *In vitro*, donor DNA molecule supercoiling is required or stimulatory, under some conditions, for efficient transposition of Mu, IS10, and Tn7 (Bainton, 1992; Craigie et al., 1985; Surette et al., 1987). *In vivo*, mutants of *gyr*A affect Tn5, IS10, and Mu transposition (Isberg and Syvanen, 1982; Lundblad and Kleckner, 1982; Ross et al., 1986). Tn10 transposition in *gyr*A temperature sensitive mutants is defective even at the permissive temperature (Lundblad and Kleckner, 1982). Gyrase is required for Mu development *in vivo* as demonstrated by studies with gyrase inhibitors or either *gyr*A or *gyr*B mutants (Ross et al., 1986).

DNA Replication and Transposition

As described above, the transposition mechanisms that have been examined involve staggered cuts in the target molecule, and thus generate target site duplications, requiring some DNA synthesis. The 5' staggering of the transposon's nucleophilic attack results in single stranded gaps left on each side of the transposon. Filling these gaps requires DNA synthesis primed by the target site's 3' OH group, which proceeds towards the transposon. In non-replicative transposition, this single stranded gap repair is

completed by ligation of the newly synthesized DNA to the 5' of the transposon, while with replicative transposition replication passes across the transposon and is ligated to 5' target DNA (Kleckner, 1981; Shapiro, 1979).

Replication and ligation mutants do not support transposition of a number of transposons. Mutations in *pol*A reduce Tn5 and Tn10 transposition 5- to 100-fold (Syvanen et al., 1982). *lig* mutants are also defective for Tn10 transposition (Lundblad, 1983).

Tn7

In understanding the mechanism and regulation of genetic recombination, bacterial transposon Tn7 transposition provides an attractively defined and tractable event to study. Tn7 was first isolated from drug resistant E. *coli* found in calves treated with large doses of trimethoprim and sulfonamides, and later from an outbreak of trimethoprim resistant *Klebsiella pneumoniae* in a London hospital (Barth et al., 1976; Datta et al., 1979). Attention was brought to Tn7 when it was observed to move with high efficiency to a single site (*att*Tn7) in the bacterial chromosome (Barth et al., 1976; Lichtenstein, 1982; Lichtenstein and Brenner, 1981). Insertions in this specific chromosomal site are flanked by a target site duplication, strongly suggestive of transposition rather than conservative site-specific recombination. This degree of target site specificity was unique among transposons. Efficient transposition combined with defined target, as well as donor sites, makes Tn7 attractive to study.

Tn7 is an unusually large (14 Kb) bacterial transposon. The large size suggests that it contains complex information for its translocation. Indeed, genetic studies using deletions and insertions in Tn7 revealed that the element encodes five genes involved in its transposition, *tns*ABCD and *tns*E (Hauer and Shapiro, 1984; Ouartsi et al., 1985; Rogers et al., 1986; Smith and Jones, 1984; Waddell and Craig, 1988). These studies demonstrated that the *tns* genes work efficiently in *trans* and that not all five *tns* genes

are required for a transposition event (Hauer and Shapiro, 1984). *tns*ABC are required for all Tn7 transposition events, while *tns*ABC +*tns*D mediate both transposition at high frequency to *att*Tn7 and at lower frequency to a few pseudo-*att*Tn7 sites, while *tns*ABC +*tns*E mediate transposition at low frequency to seemingly random sites (Rogers et al., 1986; Waddell and Craig, 1988; Kubo and Craig, 1990).

Roles of the Tn7 Encoded Genes

Substantial progress towards understanding the roles of the *tns* gene products has been made. Transposition of Tn7 requires that three DNA segments, the two transposon ends and a target site, be brought together. TnsB binds 30 bp repeats in the ends of Tn7 (Arciszewska and Craig, 1991; Arciszewska et al., 1991; Tang et al., 1991). TnsB may function to recognize Tn7 ends and promote transposon end synapses in a manner analogous to MuA in Mu transposition. MuA performs the DNA breakage and joining activities required for transposition (Pato, 1989). Whether TnsB also performs the DNA recombination is not yet clear.

While many transposons use the same protein to recognize the two transposon ends and a non-specific target site specifically, Tn7 recognizes its ends and target site with separate proteins. Because TnsD is required solely for transposition to chromosomal *att*Tn7 and pseudo-*att*Tn7 sites, TnsD's role was expected to be in the sequence recognition of the *att*Tn7 target (Waddell and Craig, 1988; Waddell and Craig, 1989). TnsD was found to specifically recognize and bind *att*Tn7 sequence between approximately +30 and +50 bases from the point of insertion (Bainton et al., 1993). In analogy with Mu, TnsD acts like MuB to recognize a target.

Via TnsABC+E, Tn7 can also transpose to apparently random DNA targets that have no sequence similarity to *att*Tn7 (Waddell and Craig, 1988; Kubo, 1990). By analogy to TnsD, TnsE bound to (sequence non-specific) DNA may be important in defining a target for TnsABC + TnsE promoted transposition.

Tn7's recognition of target sites is not limited to TnsD or TnsE. TnsC has been found to bind with TnsD to *att*Tn7 (Bainton et al., 1993). In the absence of TnsD, TnsC binds DNA nonspecifically. TnsC is known to bind ATP, and ATP hydrolysis is stimulated by DNA (Gamas and Craig, 1992). Recent work suggest that TnsC's ATP metabolism is important for target recognition. TnsC mutants have been obtained which support Tn7 transposition in the absence of TnsD or TnsE, and some no longer exhibit transposition immunity (Stellwagen, 1993). One of these TnsC mutants is in a sequence-defined ATP binding domain. The role of TnsA remains elusive.

Tn7 also carries three genes conferring antibiotic resistance (Fling and Richards, 1983; Simonsen, 1983; Sundstrom et al., 1991), dihydrofolate reductase - *dhfr*, streptothricin-acetyl-transferase - *sat*, and adenylyltransferase - *aadA*. These genes confer resistance to trimethoprim, streptothricin, and streptomycin or spectinomycin respectively. These drug resistance genes contribute to the success of Tn7 by conferring a selective advantage to bacteria carrying Tn7 and faced with a drug challenge.

Site-specific and Non-Specific Transposition

*att*Tn7, the site of high frequency Tn7 insertion, is located at minute 83.75 on the E. *coli* chromosome between *pho*S and *glm*S, very near *ori*C at minute 84 (Barth et al., 1976; Lichtenstein and Brenner, 1981; Lichtenstein and Brenner, 1982; Gay et al., 1986). The point of insertion lies in the region of DNA encoding the RNA stem of a stem-loop transcription terminator of *glm*S, and thus does not disrupt coding sequence (Gay et al., 1986; Gringauz et al., 1988). *glm*S encodes a necessary and conserved protein involved in cell wall biosynthesis (Walker et al., 1984). The DNA sequences which confer Tn7 transposition target activity to *att*Tn7 lie upstream of the point of insertion, in conserved *glm*S coding sequence (Bainton et al., 1993; Gay et al., 1986; Gringauz et al., 1988; McKown et al., 1988; Qadri et al., 1989). *pho*S encodes a phosphate binding protein which participates in phosphate-specific transport across the periplasmic membrane. Tn7

is capable of site-specific translocation in many different bacteria including *Vibro* species (Thomson et al., 1981), *Caulobacter crescentis* (Ely, 1982), *Pseudomonas aeruginosa* (Caruso and Shapiro, 1982), *Klebsiella pneumoniae* (Craig, 1989), *Salmonella typhimurium* (Craig, 1989), and *Agrobacterium tumefaciens* (Leemans et al., 1981). Where transposition has been examined in these different species of bacteria, Tn7 displays similar site specificity, presumably recognizing *glm*S homologs.

Most transposons move only to random sites, and so are prohibited from moving at a very high frequency by the negative pressure of disrupting essential host functions. By ensuring that transposition is to a non-detrimental site through the separation of recognition and insertion sites, Tn7 can transpose at a very high frequency. If, however, Tn7 was limited in movement only to *att*Tn7 sites, it would be unable to move onto horizontally transmitted shuttles which do not have *att*Tn7 sites. Tn7 transposition at a frequency a hundred-fold lower than site-specific transposition, and comparable to other transposons, enables Tn7 to access horizontal vectors and occupy bacteria missing *att*Tn7. In this manner, Tn7 has developed an effective strategy of dissemination through populations, using a random integration path to get between cells, and a efficient site-specific path once in a cell. Though it does not form an extracellular vehicle, as do phage, Tn7 is adapted for horizontal as well as vertical transmission.

Ends of Tn7

Tn7, unlike Tn5 or Tn10, is not a composite transposon made up of insertion sequence modules. The *cis*-acting ends of Tn7 (Tn7L and Tn7R) are structurally and functionally different from each other and cannot transpose independently (Gosti-Testu, 1982; Lichtenstein, 1982; Arciszewska et al., 1989; Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991). Tn7L contains three 30 bp TnsB binding repeats separated by 30 and 40 bp, while Tn7R contains four head-to-tail TnsB

binding repeats (Gosti-Testu, 1982; Lichtenstein, 1982; Arciszewska et al., 1989; Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991).

Tn7, like bacteriophage Mu and the Tn3 family of transposons, has developed the ability to inhibit its transposition to a replicon already containing a copy of itself. As described above, transposition immunity for Mu and γδ is conferred by interaction of transposase with a transposon end (or a single end) in the target molecule (Arciszewska et al., 1989; Darzins et al., 1988; Kans and Casadaban, 1989; Wiater and Grindley, 1990b). Tn7L-Tn7L constructs do not transpose or confer transposition immunity. Tn7R-Tn7R constructs do transpose, and the presence of a single copy of Tn7R is necessary and sufficient to confer transposition immunity (Arciszewska et al., 1989). Deletion of one of the four TnsB-binding repeats of the right end of Tn7 diminishes its ability to confer immunity.

Transposition Orientation

Transposition of Tn7 into both the bacterial chromosome at *att*Tn7, and some plasmids, is orientation specific. Orientation specificity is seen into both attTn7 (TnsD directed) and non-attTn7 (TnsE directed) sites. Tn7 inserts in *att*Tn7 with the left end of Tn7 adjacent to *pho*S and the right end adjacent to *glm*S and the recognition sequences of *att*Tn7 (Barth et al., 1978; Hauer and Shapiro, 1984; Krishnapillai et al., 1984; Lichtenstein and Brenner, 1981; McKown et al., 1988; Moore and Krishnapillai, 1982; Ogawa et al., 1984).

To achieve transposition orientation specificity, target and donor orientation must be distinguished. Transposition to plasmids carrying a cloned *att*Tn7 is also orientation specific with the right end of Tn7 proximal the *att*Tn7 recognition sequences, suggesting orientation specificity is partially defined by *att*Tn7 for TnsD directed transposition. Tn7 must also be able to distinguish between its right and left ends to achieve orientation specificity. As described above, the left and right ends of Tn7 are nonequivalent in their

ability to promote transposition and their ability to confer transposition immunity. The target cues orienting TnsE directed transposition are unknown (Hauer and Shapiro, 1984; Ouartsi et al., 1985).

Mu as a Model for Tn7

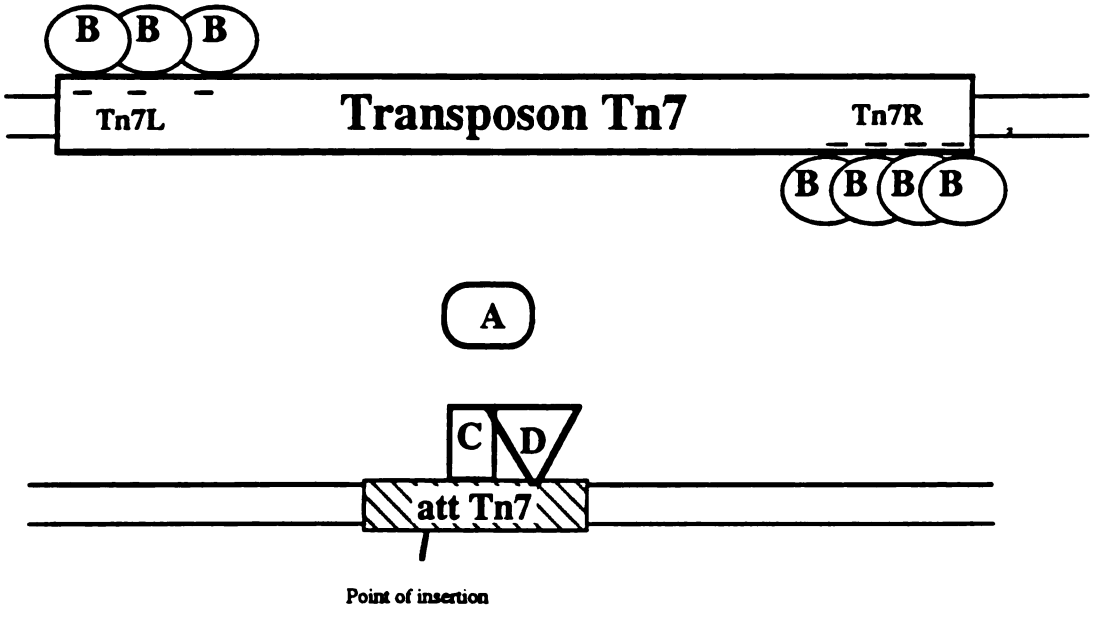
A speculative model of Tn7 transposition based on the information known about the Tns proteins and using Mu as a model can be developed. This model lacks the role of TnsA which may function with TnsB as the transposase. The speculative model is as follows:

Intermolecular transposition of Tn7 requires the transposase TnsB (and TnsA?), and the targeting complex TnsC + TnsD or E. Though the order of association is not known, TnsC+TnsD/E on DNA define the target of Tn7 transposition. TnsC binds DNA non-specifically in an ATP bound form. Binding of TnsC-ATP to DNA stimulates ATP hydrolysis and TnsC-ATP hydrolysis ejects TnsC off DNA. TnsD or E act to slow TnsC-ATP hydrolysis. TnsD binds specifically to *att*Tn7 and with reduced affinity to a few degenerate pseudo-attTn7 sites, and TnsC-ATP binds with TnsD. TnsC-ATP bound to DNA in a form stabilized by TnsD or E recruits the synaptic complex of Tn7 transposon ends held by TnsB+A. Order of assembly is unknown. The synaptic complex may form with all Tns proteins, or some sub-set before target site recognition.

In this speculative model of Tn7, transposition immunity is generated as TnsB bound to a Tn7 end in the target molecule acts to stimulate TnsC-ATP hydrolysis, overriding TnsC-ATP stabilization by TnsD or E. Upon hydrolysis of ATP, TnsC dissociates from the DNA, no longer making it a target.

Figure 1-2

Roles for the Tns Proteins



***att*Tn7 Target Site**

B —Binds site-specifically to the ends of Tn7.

D —Binds site-specifically to attTn7 site.

C —With TnsD, TnsC binds site-specifically to attTn7 site. May connect target sites to other Tns machinery.

A —Unknown.

E —Unknown. May bind DNA sequence non-specifically to define non-attTn7 targets.

Figure 1-2 Legend

Roles for the Tns Proteins

The known roles played by the Tns proteins in the transposition of Tn7 are illustrated here. Transposon Tn7, the *att*Tn7 transposition target site, and the Tns proteins are schematically illustrated. TnsB binds sequence-specifically to sequence repeats in the ends of Tn7 (Arciszewska and Craig, 1991; Arciszewska et al., 1991; Tang et al., 1991). TnsD binds sequence-specifically to *att*Tn7 (Waddell and Craig, 1989; Bainton et al., 1993). TnsC binds with TnsD to *att*Tn7 (Bainton et al., 1993). The roles of TnsA and TnsE are unknown. The role played by the Tns gene are discussed in greater detail in the text.

Overview

A review of the intricate control mechanisms used to set a low basal level of transposition activity responsive to specific host environmental signals shows us that transposons are highly evolved to minimize damage to the host and take advantage of opportunity.

The goal of this thesis was to understand better the interaction between Tn7 and its host *Escherichia coli.* Host components participate in the recombination of many mobile elements as has been reviewed in this chapter. When I began this work, the Tn7-encoded genes involved in transposition had been genetically identified, and their functions were being biochemically dissected. Completely undefined were any interactions of Tn7 with host components. To search for host components, we decided to look for E. *coli* mutants that affect the rate of Tn7 transposition. Our standard assay for transposition (Mating-Out) involves determining the fraction of conjugal plasmids in a population onto which Tn7 has hopped. To perform a genetic screen, we needed an assay for Tn7 transposition that does not require the independent manipulation of each population to be tested.

Chapter 2 describes the construction and characterization of a colony papillation assay for Tn7 transposition.

Chapter 3 describes the use of the papillation assay as an assay of Tn7 transposition orientation into chromosomal *att*Tn7 site.

Chapter 4 examines the effect of E. *coli* mutants known to affect other transposition/recombination systems.

Chapter 5 describes a search for new E. *coli* mutants affected for Tn7 transposition and the mutants that were found.

Chapter 6 concludes the thesis by summarizing our current understanding of host involvement in Tn7 transposition.

Chapter 2: A Visual Assay for Tn7 Transposition

Abstract:

Described here is the construction and characterization of a new variant of transposon Tn7 which enables rapid visual assessment of Tn7 transposition. MiniTn7-*lac* was constructed to carry between Tn7 ends a promoterless *lac*ZYA operon oriented with its 5'-end adjacent to the left end of the transposon and an independently transcribed gene conferring kanamycin resistance. MiniTn7-*lac* transposition from a non-transcribed to a transcribed genetic location in the appropriate orientation confers a Lac^+ phenotype to the cell in which the transposition occurred, and to its progeny. On MacConkey Lactose indicator plates, transposition is visualized as red Lac^+ papillae on a white Lac^- colony. We demonstrate here that miniTn7-*lac* transposes in a manner characteristic of Tn7, requiring Tn7 transposition genes *tns*ABC + *tns*D for high frequency transposition to *att*Tn7 and *tns*ABC + *tns*E for low frequency transposition to sequence non-specific sites. MiniTn7-*lac* educed papillation represents transposition events as revealed by its dependence on *cis*-acting Tn7 transposon ends and on *tns* genes, and its independence of the E. *coli* host homologous recombination system. The papillation assay provides a valuable tool for genetic studies of Tn7 transposition.

Introduction

Studies of transposition are influenced by the methods used to detect transposition events. Barbara McClintock discovered transposons with what turned out to be the first assay for transposition, a visual inspection of *Zea mays* kernels for the phenotypic effects of transposition. Examination of the chromosomes from kernels with altered phenotypes revealed physical changes of the chromosomes associated with the altered kernel phenotype. McClintock observed chromosome breakage and rearrangements that were the result of *Ds* and *Ac* transposition. By combining her physical observation of *Zea mays* chromosomes with genetic observations of altered gene activity, she postulated the transposition of controlling genetic elements (McClintock, 1956).

In vivo, phenotype based transposition assays can rely on either genetic loss or gain of function. The investigation of genetic loss of function in spontaneous *Escherichia coli lac* and *gal* mutants led to the general acceptance of transposons as mobile genetic elements (Adhya and Shapiro, 1969; Hedges and Jacob, 1974; Jordan et al., 1967; Kopecko et al., 1976; Malamy, 1966). These mutants resulted from physical insertions of insertion sequences (IS elements) which disrupted the *lac* or *gal* genes leading to Lac^- or Gal^- phenotypes. Though transposition events can be identified by screening for disruption of a specific target, when insertion site specificity is low, very few of the total transposition events are reported. Transposition assays based on genetic loss of function at the donor site have also been useful for measuring transposition frequency of elements that promote adjacent deletions (Roberts et al., 1985).

Assaying the transposition of a transposable element from one location to another can be complicated by the intracellular nature of transposition. Two popular *in vivo* assays for transposition have been developed based on different methods of separating donor from target replicons. Donor sites on conditional replicons, which require certain growth conditions or genetic backgrounds to replicate, can be separated from target

replicons under non-permissive conditions. The "λ-hop" assay for transposition employs conditional replicon strategy by assaying transposition from an infecting replication/recombination conditional phage λ onto the chromosome of a non-permissive host (Kleckner et al., 1978). Conjugal plasmids can be used in second method for separating target from donor replicons. In the "mating-out" assay, transposition frequency is measured by determining the fraction of conjugal plasmids onto which the transposon has hopped, after the conjugal plasmid has been transferred to another genetically separable strain (Foster et al., 1981).

λ-hop and mating-out assays have both been developed to measure Tn7 transposition (Barth et al., 1978; Smith and Jones, 1984; McKown et al., 1988; Rogers et al., 1986; Waddell and Craig, 1988). With these assays, a genetic deconstruction of Tn7 has been successful in characterizing the Tn7-encoded components required for transposition (Hauer and Shapiro, 1984; Kubo and Craig, 1990; Ouartsi et al., 1985; Rogers et al., 1986; Smith and Jones, 1984; Waddell and Craig, 1988). The goal of this thesis was to identify E. *coli* components affecting Tn7 transposition. Neither λ-hop nor mating-out assays are well-suited to genetic screens in the hope of identifying host mutants. To screen large numbers of mutants for those affecting Tn7 transposition requires an assay which does not necessitate independent testing of each strain.

Genetically tractable visual assays for Tn10 and Tn5 transposition have been described which involve the use of a promoterless *lac*Z gene carried by the mobile element (Huisman and Kleckner, 1987; Krebs and Reznikoff, 1988). Transposition of the *lac* containing element from a non-transcribed to a transcribed region results in a Lac^+ phenotype in the cell in which the transposition occurred, and in all its subsequent progeny. On MacConkey Lactose plates (a rich medium supplemented with lactose and a pH sensitive dye, Neutral Red), Lac^+ cells have a growth advantage enabling them to form papillae among Lac^- cells, and as a result of fermenting lactose, these papillae turn red. The red Lac^+ papillae are easily distinguished against the white Lac^- colony.

Though only a fraction of the transposition events will be into a transcribed region in the proper orientation, the rate of colony papillation is related to the rate of transposition. Scoring the rate of red papillae formation in a single colony can be used to visually assess transposition frequency in that colony.

Described here is the development and characterization of a visual assay for Tn7 transposition. Tn7 Lac^+ papillation is characterized for both *tns*ABC+D and *tns*ABC+E promoted transposition from two donor sites.

Results

Construction of MiniTn7-*lac*

MiniTn7-*lac* was constructed to carry promoterless *lac*ZY and partially truncated *lac*A genes, and a gene conferring kanamycin resistance between the ends of Tn7. The genes required for Tn7 transposition, *tns*ABC+D or *tns*ABC+E, are supplied to mini Tn7-*lac* in *trans*. MiniTn7-*lac* can be followed independent of *lac*ZYA expression by its ability to confer kanamycin resistance.

Cis-acting End Sequences of MiniTn7-*lac*:

The *cis*-acting transposon end sequences of miniTn7-*lac* are the minimal functional ends previously defined for Tn7. MiniTn7-*lac* is diagrammed in Figure 2-1; its construction is summarized by Figure 2-2. The left-end and right-end terminal nucleotides of Tn7 are referred to as **L**1 and **R**1 respectively. The left end of miniTn7-*lac* is composed of the terminal 166 base pairs of the left end of Tn7. These 166 base pairs contain three TnsB binding sites and are functionally indistinguishable from larger left Tn7 ends (Arciszewska et al., 1989) and is referred to as **L**166. The right end of miniTn7-*lac* consists of the terminal 70 base pairs of intact Tn7 (**R**70). These 70 base pairs of the right end contain only three of the four endogenous right end TnsB binding sites.

The fourth (internal most from R1) endogenous right end TnsB binding site is not required for Tn7 transposition, and contains the *tns*AB promoter (Arciszewska et al., 1989; Gay et al., 1986; Rogers et al., 1986). The deletion of the innermost TnsB binding site was expected to decrease the frequency of transposition five fold (Arciszewska et al., 1989), and remove the *tns*AB promoter, such that Lac$^-$ miniTn7-*lac* elements could be constructed with the *lac*ZYA/Km cassette in both the *lac* adjacent left end, and the *lac* adjacent right end orientations. MiniTn7-*lac* carries 5 Kb of the *lac*ZYA operon, beginning with the ribosome binding site and truncated 20 amino acids from the end of

*lac*A, as well as the aminoglycoside 3'-phosphotransferase gene (Km) from pUC-4k (see Figure 2-2).

Phenotypically Lac- MiniTn7-*lac* Isolates:

We wished to construct a miniTn7-*lac* element which confers a Lac$^+$ phenotype only when inserted into a transcribed region of DNA. Two miniTn7-*lac*-variants were constructed such that the *lac*ZYA/Km -encoding DNA was oriented with the ribosome binding site of *lac*Z adjacent to either the left end of the transposon (on pOH1 - Figure 2-2b) or the right end of the transposon (pOH2 - not shown). Both pOH1 and pOH2 confer a Lac$^+$ phenotype (data not shown). We then assayed whether phenotypically Lac$^-$ insertions could be isolated upon transposition of miniTn7-*lac* from either pOH1 or pOH2 to an F' plasmid, pOX38*gen*. Transposition was carried out in strain NLC51 with *tns*ABCDE supplied by Tn7 in *att*Tn7. The Lac phenotype of miniTn7-*lac* insertions in pOX38*gen* was assayed on MacConkey lactose media after conjugation to a new *lac*$^-$ strain, CW51.

Transposition of miniTn7-*lac* from pOH1 to pOX38*gen* resulted in a Lac$^-$ phenotype in 38 out of 50 insertions screened. In contrast, all transposon insertions (n≈200) from pOH2 into pOX38*gen* result in a Lac$^+$ phenotype. The reason the miniTn7-*lac* element constructed on pOH2 could not be isolated as a phenotypically Lac$^-$ insertion in pOX38*gen* is unknown. If there is residual transcription from within the 70 base pair right end, the miniTn7-*lac* element of pOH2 would be Lac$^+$, independent of transcription from outside the element. Since these studies require that miniTn7-*lac* reports a Lac$^-$ phenotype unless inserted into a transcribed region of DNA, throughout the rest of this thesis, miniTn7-*lac* refers to the element with its *lac*ZYA/Km genes oriented so that the ribosome binding site of the *lac*Z gene is adjacent to the left end of the transposon.

tns Genes for MiniTn7-*lac* Transposition:

MiniTn7-*lac* does not encode transposition proteins. Transposition of miniTn7-*lac* requires transposition proteins to be supplied in *trans* either from an intact Tn7 or from cloned *tns* genes. By requiring transposition functions to be supplied in *trans*, miniTn7-*lac* transposition promoted by either *tns*ABC+D or *tns*ABC+E can be independently examined.

Four pACYC based plasmids were used to supply *tns* genes in *trans* to miniTn7-*lac* in this work. pCW4 contains approximately 9 Kb of Tn7, encompassing all five *tns* genes (*tns*ABCDE) (Waddell and Craig, 1988). To examine transposition and papillation promoted by either *tns*ABC+D or *tns*ABC+E separately, pCW4 carrying miniMuΩ insertions disrupting *tns*E (pCW4::miniMuΩ^{76E}) or *tns*D (pCW4::miniMuΩ^{107D}) were used respectively (Waddell and Craig, 1988). The levels of *tns* gene expression from pCW4::miniMuΩ^{107D} (supplying *tns*ABC+E), and pCW4::miniMuΩ^{76E} (supplying *tns*ABC+D), are unknown and may be significantly different than from pCW4. pCW15 was used to examine transposition or papillation in the presence of only *tns*ABC (Waddell and Craig, 1988).

Figure 2-1

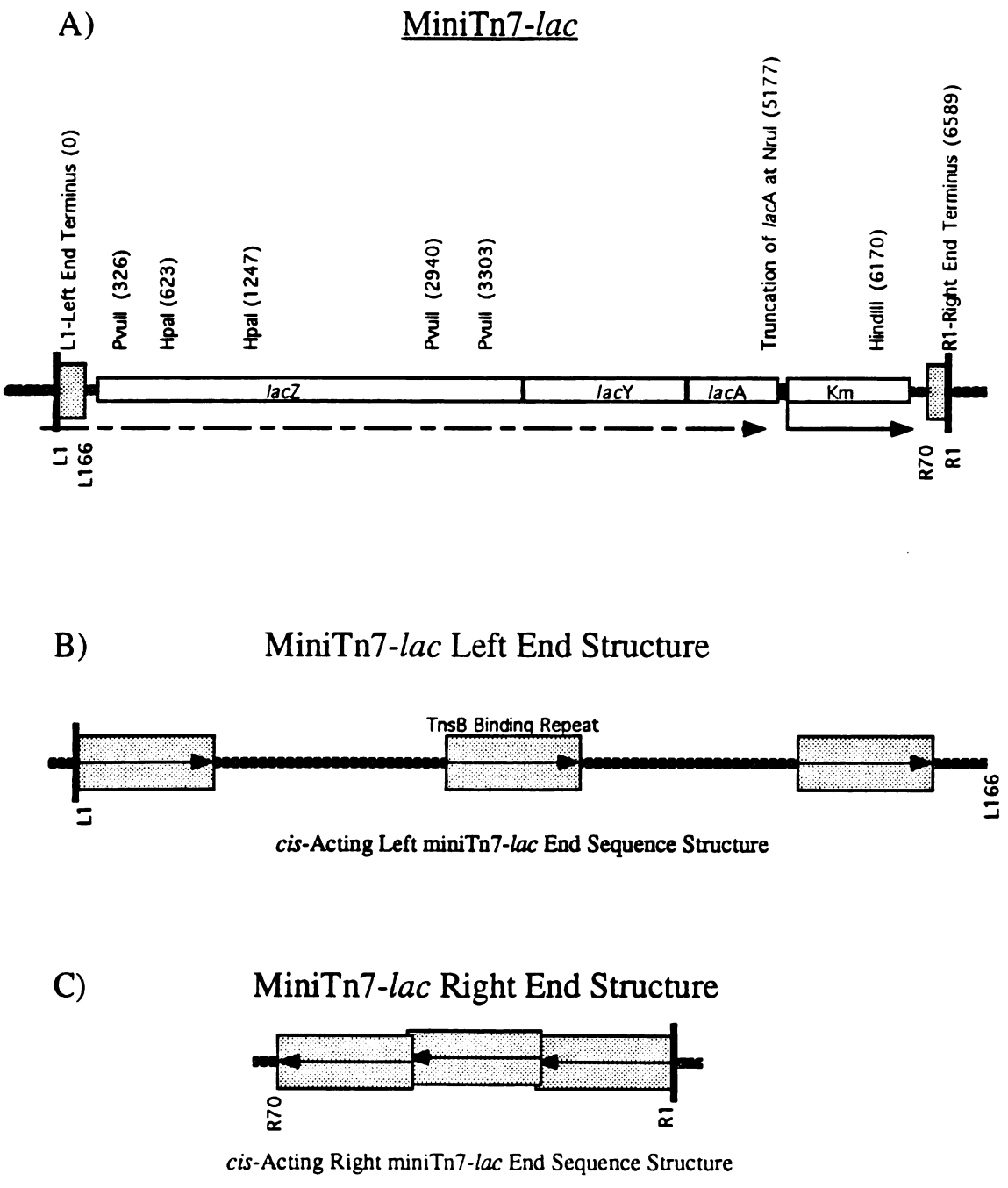

A)
MiniTn7-lac
L1-Left End Terminus (0)
PvuII (326)
HpaI (623)
HpaI (1247)
PvuII (2940)
PvuII (3303)
Truncation of lacA at NruI (5177)
HindIII (6170)
R1-Right End Terminus (6589)
lacZ
lacY
lacA
Km
L1
L166
R70
R1
B)
MiniTn7-lac Left End Structure
TnsB Binding Repeat
L1
L166
cis-Acting Left miniTn7-lac End Sequence Structure
C)
MiniTn7-lac Right End Structure
R70
R1
cis-Acting Right miniTn7-lac End Sequence Structure

Figure 2-1 Legend

Structure of MiniTn7-*lac*

MiniTn7-*lac* is diagrammed (not to scale) in Panel A. MiniTn7-*lac* is composed of a *lac*ZYA/Km cassette flanked by the *cis*-acting Tn7 end sequences. The promoterless *lac*ZYA gene cassette begins at the BamHI site of pRS415 (Simons et al., 1987), immediately 5' of the *lac*Z ribosome binding site, and is truncated at the NruI site 60 bases from the end of *lac*A (see Figure 2-2). Expression of *lac* requires transcription from outside miniTn7-*lac*. The Km gene is independently transcribed; thus miniTn7-*lac* can be followed by Km regardless of the Lac phenotype.

An enlarged view of the end structures of miniTn7-*lac* is presented in Panels B and C. The *lac*ZYA/Km cassette is flanked by the 70 outermost base pairs of the right end of Tn7 and 166 base pairs of the left end of Tn7. The 166 base pairs of the left end of Tn7 contain the three TnsB binding sites and is functionally indistinguishable from larger ends. 70 base pairs of the right end of Tn7 contains only three of the four endogenous TnsB binding sites, a deletion which has been seen to decrease five fold the transposition frequency of a shorter mini-Tn7 element (Arciszewska et al., 1989).

Figure 2-2a

Construction of miniTn*7*-*lac*'s *lac*ZY/Km cassette on pOH0

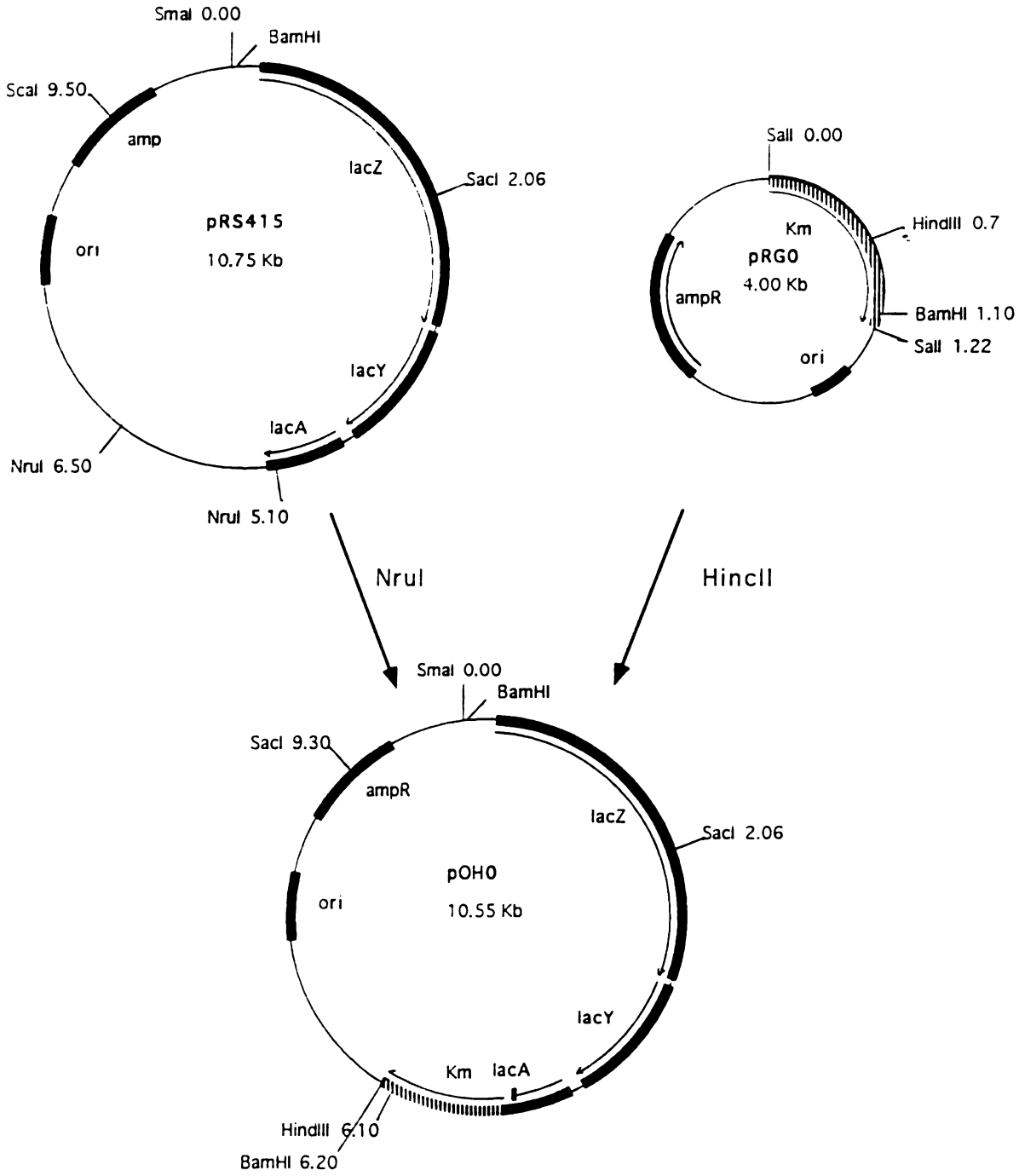


Figure 2-2b

Construction of miniTn7-*lac* on pOH1

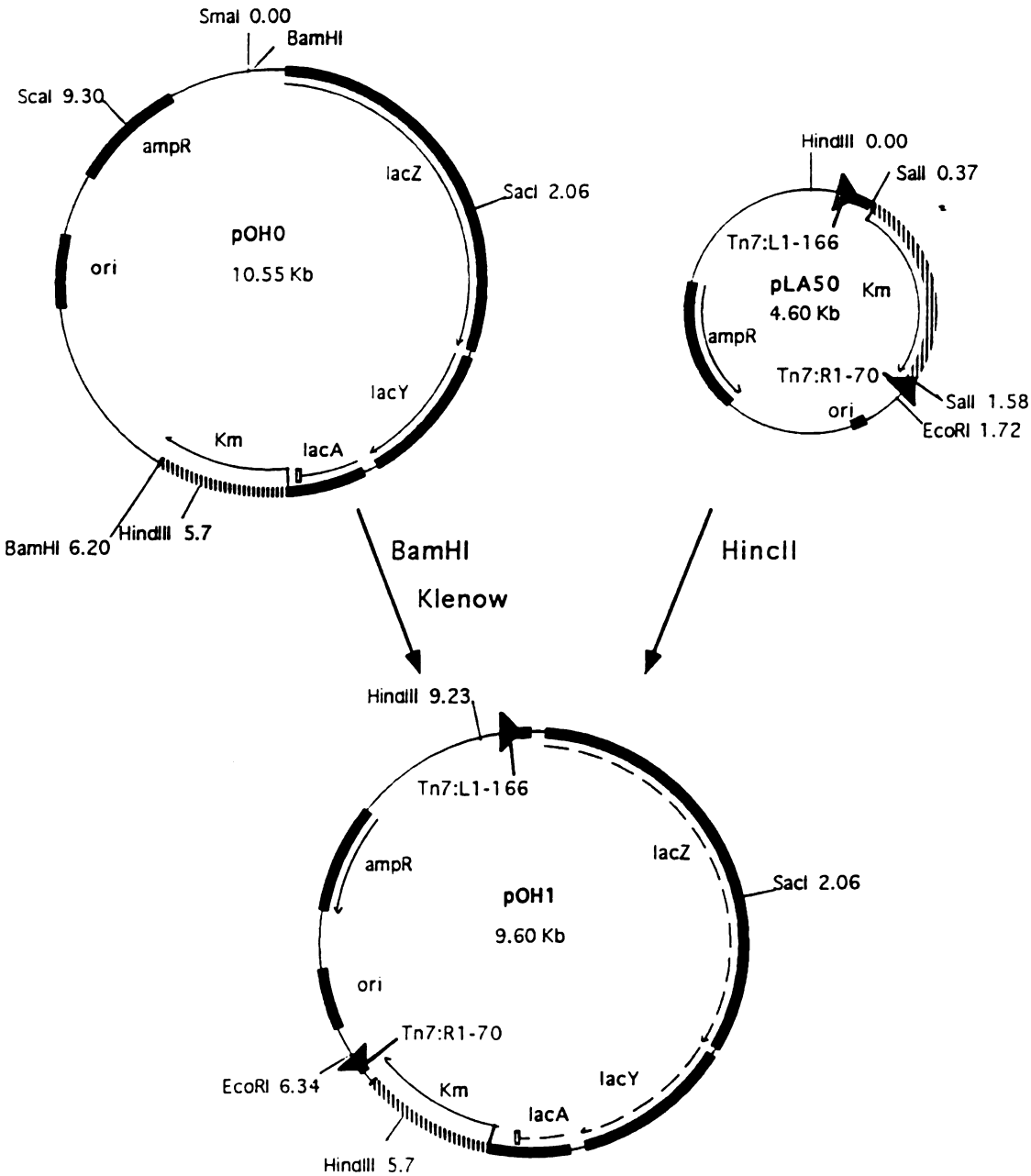


Figure 2-2 Legend

Construction of MiniTn7-*lac*

A 1.2 Kb SalI fragment carrying the aminoglycoside 3'-phosphotransferase (Km) from pUC-4k in pRG-0 was ligated to NruI digested pRS415 (see 2a). The resultant 10.4 Kb plasmid (pOH0) was digested with BamHI to produce a 6.3 Kb fragment carrying *lac*ZYA and Km. After treatment with the Klenow fragment of DNA pol I, the BamHI - Km/*lac*ZY fragment was ligated into HincII cut pLA50 to produce pOH1 and pOH2 (see 2b). pOH1 and pOH2 carry the *lac*/Km cassette between the *cis*-acting end sequences of miniTn7-**L**166-Km-**R**70 element from pLA50. In pOH1 the *lac*ZYA/Km cassette is oriented such that the *lac*Z ribosome binding site is adjacent to the left *cis*-acting end sequences. In pOH2 (not shown) the *lac*ZY/Km cassette is oriented with the *lac*Z adjacent to the right *cis*-acting end sequences.

MiniTn7-*lac* Transposition

MiniTn7-*lac* Does Transpose

MiniTn7-*lac* is capable of intermolecular transposition as demonstrated by its ability to move from pOH1 to another replicon. Table 1 reports the frequency of transposition of miniTn7-*lac* from pOH1 to pOX38*gen*. In the experiment described by Table 1, transposition functions are supplied from Tn7 in the chromosomal *att*Tn7 site; Tn7 also serves as an internal positive control for transposition.

In the presence of Tn7, miniTn7-*lac* can transpose from a donor plasmid to pOX38*gen* (Table 2-1, line 1); this transposition requires the *cis*-acting Tn7 end sequences (line 2).

Comparison here of the relative transposition frequency for miniTn7-*lac* from pOH1 and Tn7 from chromosomal *att*Tn7 is complicated by the differences in donor site context and copy number. A systematic comparison of miniTn7-*lac* to other mini-Tn7 elements is described in the next section of this thesis.

It is noted that the conjugation based ("mating-out") assay used here and throughout the rest of this thesis to examine transposition frequencies probably measures both translocation of the transposable element to the conjugal plasmid followed by conjugation of that plasmid to CW51, and a background frequency of F' donor strain mutation to nalidixic acid resistance (see Materials and Methods). Thus, numbers reported as transposition frequencies as measured by the mating-out assay in this thesis may not reflect only transposition events. This caveat is expected to be significant at measures transposition frequencies below 10^{-7} (Bob DeBoy and Nancy Craig, personal communication).

Table 2-1

MiniTn7-*lac* Transposition From pOH1

Plasmid	Km Element From Plasmid	Transposition Frequency	Tn7 Element From *att*Tn7	Transposition Frequency
1) pOH1	miniTn7-*lac*	3.5×10^{-6}	Tn7	4.4×10^{-6}
2) pOH0	"End-less" *lac*ZYA/Km Cassette	$<1 \times 10^{-8}$	Tn7	7.3×10^{-5}
3) (-)	(-)	(-)	Tn7	2.8×10^{-4}

Table 2-1 Legend

MiniTn7-*lac*'s ability to transpose was demonstrated by assaying translocation onto a F' plasmid (pOX38*gen*) using the mating-out assay. MiniTn7-*lac* transposes from pOH1, while Tn7 transposes from the chromosomal *att*Tn7 site (line 1). MiniTn7-*lac* transposition is promoted by *tns* functions from *att*Tn7::Tn7. Intact miniTn7-*lac* transposition is compared to a negative control in line 2 - the *lac*ZYA/Km cassette without Tn7 ends (from pOH0). Each value is the average of 3-5 independent trials. The F' donor strain for these mating-out assays was NLC51 *att*Tn7::Tn7 + Km Element plasmid + pOX38*gen*. The F' recipient strain was CW51. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

MiniTn7-*lac* Transposition in Comparison to Other mini-Tn7 Elements

MiniTn7-*lac* transposition was further examined to compare its transposition frequency relative to other previously characterized miniTn7 transposable elements (Arciszewska et al., 1989). We found that transposition frequency of miniTn7-*lac* is reduced in comparison to other miniTn7 transposable elements.

Table 2-2 shows a comparison of the transposition frequency to pOX38*gen* of miniTn7-Cm, miniTn7-Km**R**70, and miniTn7-*lac* . MiniTn7-Cm has all known *cis*-acting Tn7 end sequence information, containing of approximately 0.5 Kb of the **R**ight end of Tn7 (encompassing the four TnsB binding repeats at the right end of intact Tn7), and 1.9 Kb of the **L**eft end of Tn7 (encompassing the three left end TnsB binding repeats), flanking a chloramphenicol resistance determinant (Hauer and Shapiro, 1984; Arciszewska et al., 1989). MiniTn7-Km**R**70 is comprised the same Tn7 ends found in miniTn7-*lac* (**R**70 and **L**166), however the ends are separated by only a 1.5 Kb Km cassette (Arciszewska et al., 1989). As described above, miniTn7-*lac* is composed of 70 base pairs of the **R**ight end of Tn7 (consisting of only three of the four right end TnsB binding repeats from Tn7), and 166 base pairs of the **L**eft end of Tn7 (encompassing all three left end TnsB binding repeats from Tn7) separated by a 6.5 Kb *lac*ZY+Km cassette.

The miniTn7 elements compared in Table 2-2 were located in the chromosomal *att*Tn7 site, while transposition functions were supplied in *trans* from Tn7 in located in a second chromosomal location (at ϕ80dIII*lac*).

MiniTn7-*lac* Transposition is Impaired.

Several aspects of the results in Table 2-2 are notable. First, transposition of miniTn7-*lac* (Table 2-2, line 3) is reduced in comparison to the miniTn7-Cm element (line 1). This reduced transposition may due to a combination of the deletion of the fourth TnsB binding site in miniTn7-*lac*, reducing miniTn7-*lac* transposition to the level of miniTn7-Km**R**70 transposition (line 2), and further reduction of transposition by

greater separation of the ends of miniTn7-*lac* (6.5 Kb) vs. miniTn7-Km**R**70 (1.5 Kb). Also notable is the comparatively low frequency of transposition seen even for miniTn7-Cm, which here transposes at a frequency of about 10^{-5}, while in previous studies this element has been shown to transpose at a frequency of about 10^{-4} (Arciszewska et al., 1989). The reason for this 10-fold lower transposition frequency is unknown, but may lie in differences between the strains in which these measurements were done. Previous measurement of miniTn7-Cm transposition was done in a *rec*A$^-$ strain (NLC51), with *tns* proteins supplied from a plasmid, and with only one transposable Tn7 element present in the strain. Transposition frequencies reported in Table 2-2 are from experiments done in NLC28 which is a *rec*A$^+$ strain, with *tns* functions supplied by a second chromosomally located intact Tn7; moreover, there may be negative interaction between the two transposing Tn7 elements (see below).

Two Chromosomal Tn7 Elements Compete for Transposition.

Another notable aspect of the data in Table 2-2 is that as the transposition frequency of the miniTn7 element in *att*Tn7 decreases, the frequency of intact Tn7 transposition from ϕ80dIII*lac* increases. This result suggests competition exists between the mini-Tn7 elements in *att*Tn7 and Tn7 in ϕ80dIII*lac*.

Table 2-2

Comparison of MiniTn7-Cm, MiniTn7-KmR70, and MiniTn7-*lac* Transposition Frequency

Element from *att*Tn7	Transposition Frequency	Element from ϕ80dII*lac*	Transposition Frequency
1) miniTn7-Cm	9.4×10^{-6}	Tn7	4.5×10^{-6}
2) miniTn7-KmR70	1.7×10^{-6}	Tn7	9.7×10^{-6}
3) miniTn7-*lac*	2.7×10^{-7}	Tn7	1.3×10^{-5}

Table 2-2 Legend

The relative transposition frequencies of miniTn7-Cm, miniTn7-Km**R**70, and miniTn7-lac as measured by the mating-out assay are compared here. In the F' (pOX38gen) donor strains, miniTn7 elements transpose from *att*Tn7 (min. 84), while Tn7 transposes from a second chromosomal location, ϕ80dII*lac* , at minute 27.5 on the E. *coli* chromosome. Transposition of the miniTn7 elements was promoted by *tns* functions from ϕ80dII*lac*::Tn7 . Each value is the average of 5 independent trials. The F' donor strain for these mating-out assays was NLC28 *att*Tn7::miniTn7 Element ϕ80dII*lac*::Tn7 + pOX38gen. The F' recipient strain was CW51. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

MiniTn7-*lac* Transposes to *att*Tn7 Targets Via *tns*ABC+D, and non-*att*Tn7 Targets Via *tns*ABC+E

MiniTn7-*lac* transposition was found to closely mimic transposition of Tn7, requiring *tns*ABC + D for high frequency transposition to *att*Tn7, and *tns*ABC + E for transposition to sequence non-specific sites.

MiniTn7-*lac* Transposes to *att*Tn7 Targets Via *tns*ABC+D

As measured by the mating-out assay, miniTn7-*lac* transposition from chromosomal *att*Tn7 to an F' plasmid carrying a cloned *att*Tn7 site (pOX38*gen-att*Tn7) is promoted at high frequency (1.2×10^{-2}) by *tns*ABC+D (Table 2-3, line 1). However, no transposition to pOX38*gen-att*Tn7 is detected in the presence of only *tns*ABC, or in the absence of *tns* transposition functions (lines 3 and 4).

MiniTn7-*lac* Transposes to non-*att*Tn7 Targets Via *tns*ABC+E

By contrast to high frequency transposition to pOX38*gen-att*Tn7 via *tns*ABC+D, miniTn7-*lac* transposes at low frequency to both pOX38*gen* and pOX38*gen-att*Tn7 via *tns*ABC+E (lines 2 and 3). Again, no transposition is seen to pOX38*gen* in the absence of *tns* functions or in the presence of only *tns*ABC (lines 3 and 4). As expected for *tns*E-dependent transposition, there is no significant effect of the presence of *att*Tn7 on the transposition target plasmid (line 2).

These results show that miniTn7-*lac* transposes in the Tn7 characteristic manner: to *att*Tn7 at high frequency in the presence of *tns*ABC+D, and to non-*att*Tn7 sites at low frequency in the presence of *tns*ABC+E.

Impaired MiniTn7-*lac* Transposition Phenotype is Suppressed by Multicopy *tns* Source.

We note that when *tns* genes are supplied by a multicopy plasmid, miniTn7-*lac* transposition to pOX38*gen* is about 100-fold more frequent than when *tns* genes are supplied in *trans* from intact Tn7 integrated in the chromosome (compare Table 2-3, line 2 with Table 2-2, line 3). One explanation of this increased miniTn7-*lac* transposition frequency from 2.7×10^{-7} to 1.1×10^{-5} may be a suppression of an impaired miniTn7-*lac* transposition phenotype. When *tns* genes are supplied by a multicopy plasmid, miniTn7-*lac* transposition increased to levels comparable to intact Tn7 transposition (1.3×10^{-5}, Table 2-2, Line 3). Increase of miniTn7-*lac* transposition frequency to that of Tn7's may reflect suppression of decreased TnsB binding to the end sequences of miniTn7-*lac* or suppression of decreased miniTn7-*lac* synaptic transposition complex formation through overexpression of TnsB (see discussion).

Table 2-3

MiniTn7-lac Transposition Via *tns*ABC+D vs. *tns*ABC+E

	Transposition Genes	Transposition Target pOX38gen	pOX38gen-attTn7
1)	*tns*ABC+D	$<9x10^{-9}$	$1.2x10^{-2}$
2)	*tns*ABC+E	$1.1x10^{-5}$	$1.4x10^{-5}$
3)	*tns*ABC	$<9x10^{-9}$	$<9x10^{-9}$
4)	(-)	$<8x10^{-9}$	$<1x10^{-8}$

Table 2-3 Legend

MiniTn7-*lac* transposes to *att*Tn7 targets *via tns*ABC+D and to non-*att* targets *via tns*ABC+E. MiniTn7-*lac* transposition frequency from chromosomal *att*Tn7 to *att*Tn7 and non-*att*Tn7 targets was determined using the mating-out assay. pOX38*gen* was used to measure transposition to non-*att*Tn7 targets. pOX38*gen-att*Tn7 was used to measure transposition to an *att*Tn7 target. Transposition of miniTn7-*lac* is promoted by *tns* functions from plasmids as indicated above. Each value is the average of 5 independent trials. The F' donor strain for these mating-out assays was NLC28 attTn7::miniTn7-lac + *tns* Plasmid + pOX38*gen* or pOX38*gen-att*Tn7. The *tns* genes were present on the following plasmids : *tns*ABC = pCW15, *tns*ABC+D = pCW4::miniMuΩ ^{76E}, and *tns*ABC+E = pCW4::miniMuΩ ^{107D}. The F' recipient strain was CW51. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

MiniTn7-*lac* Papillation

Having shown in the previous section that miniTn7-*lac* transposes in a manner characteristic of Tn7 we show here that transposition of miniTn7-*lac* can result in Lac$^+$ colony papillation and, therefore, that papillation can report transposition.

Characteristics of the MiniTn7-*lac* Papillation Assay:

The papillation assay is based on the expectation that transporting *lac*ZYA genes from a non-transcribed to a transcribed location through Tn7 transposition will result in a Lac$^+$ phenotype. A Lac$^+$ phenotype requires the expression of *lac*Z (encoding β-galactosidase) and *lac*Y(encoding a permease for the uptake lactose and certain related sugars) but not *lac* A (function unknown). In the papillation experiments described here, miniTn7-*lac* carries the only copy of the *lac* genes in the cell, due to a deletion of the E. *coli* chromosome covering the *lac* genes (see Materials and Methods).

On MacConkey Lactose plates , Lac$^+$ cells generated as the result of miniTn7-*lac* transposition form red papillae on a white Lac$^-$ colony. Colony papillation was examined after plating cells at a dilution to achieve 100 colonies or less per plate on thickly poured MacConkey Lactose plates. MacConkey Lactose plates were protected from light and desiccation for the extended incubations at 37°C or 30°C. Papillation is reported here graphically as the number of papillae per colony over time or photographically after an indicated incubation period.

Lac- MiniTn7-*lac* Donor Sites:

Two different miniTn7-*lac* Lac$^-$ donors are used for all papillation experiments described here. One donor site was on a F' derivative: pOX38*gen*::miniTn7-*lac*. pOX38*gen*::miniTn7-*lac* was isolated as described above by screening for phenotypically Lac$^-$ miniTn7-*lac* insertions on pOX38*gen*.

A second miniTn7-*lac* donor site utilized here was an insertion in *att*Tn7, Tn7's specific insertion site located at minute 84 of the *E. coli* chromosome. The position of Tn7 insertion in *att*Tn7 lies about 20 bp from the carboxyl terminus of *glm*S and 300 bp from the amino terminus of *pho*S (Lichtenstein, 1982; Lichtenstein and Brenner, 1981; Walker et al., 1984). Insertion into *att*Tn7 is site- as well as orientation-specific: in *att*Tn7::Tn7, Tn7R is adjacent to *glm*S and Tn7L to *pho*S. Because no transcription impinges on *att*Tn7 from *pho*S, cells containing *att*Tn7::miniTn7-*lac* in its standard orientation are Lac$^-$ (see Chapter 3).

Target Sites:

All bacterial strains used to examine papillation in this chapter have Tn7 or a miniTn7 element in *att*Tn7. The presence of such elements in *att*Tn7 inactivates *att*Tn7 as a high frequency target (Hauer and Shapiro, 1984; Lichtenstein and Brenner, 1981). Thus, all miniTn7-*lac* papillation events examined here are to either pseudo-*att*Tn7 sites *via tns*ABC+D promoted transposition, or sequence non-specific sites *via tns*ABC+E promoted transposition.

Characterization of MiniTn7-*lac* Papillation: Papillation Reflects Transposition.

As described below, Lac$^+$ colony papillation by miniTn7-*lac* is greatly stimulated by *tns* gene combinations known to promote miniTn7-*lac* transposition and is independent of the host's homologous recombination system. In the absence of *tns* genes, no significant papillation is observed. Thus, miniTn7-*lac* Lac$^+$ papillation can reflect Tn7 transposition. Additionally, we find that the temperature of incubation has little effect on miniTn7-*lac* papillation and that papillation is similar for either F' or chromosomally located miniTn7-*lac* donor sites.

Papillation With MiniTn7-*lac* On a F' Donor Site (pOX38*gen*::miniTn7-*lac*):

Figure 2-3 graphically summarizes time courses of miniTn7-*lac* educed papillation, from a F' (pOX38*gen*) donor site in the presence of various combinations of the *tns* genes in *rec*A$^+$ and *rec*A$^-$ strains incubated and at 30°C and 37°C. Photographs of Lac$^+$ colony papillation from pOX38*gen*::miniTn7-*lac* after 72 hour incubations at 37°C are presented in Figures 2-4 and 2-5. Photographs from the 72 hour time point are presented rather than the 96 hour time point due to rising colony background redness. Background colony redness may be the result of low level *lac* expression from the donor site miniTn7-*lac* - a supposition supported by observation that these strains are slightly blue on X-gal indicator plates (data not shown). Background colony redness may also be the result of anaerobic metabolism within the colony which would drop the colony pH, and thus turn the colony red on MacConkey plates. In all strains, the rate of miniTn7-*lac* papillation is determined by which *tns* genes are present. MiniTn7-*lac* papillation is considerably stimulated by the presence of all five transposition genes *tns*ABCDE, while little to no papillation occurs in the absence of *tns* genes or the presence of only *tns*ABC. MiniTn7-*lac* papillation in the presence of *tns*ABC+E occurs slightly slower than in the presence of all five transposition genes. Papillation in the presence of *tns*ABC+D is

much slower than *tns*ABC+E, but significantly faster than *tns*ABC or no *tns* genes. Thus the requirement for *tns* functions for papillation is the same as those for transposition.

MiniTn7-*lac* Papillation is *rec*A Independent.

Comparison of papillation in *rec*A$^-$ strains (Figures. 3A, 3B, and 4) and *rec*A$^+$ strains (Figures. 3C and 5) reveals that papillation is generally equivalent in *rec*A$^-$ and *rec*A$^+$ strains. Thus papillation is independent of the E. *coli* host homologous recombination system.

The Effect of Temperature on MiniTn7-*lac* Papillation

Papillation occurs over the course of 4 days at 37°C (Figures 2-3B, 2-3C, 2-4, 2-5, 2-6b and 2-7) and 6 days at 30°C (Figures 2-3A and 2-6A). When normalized for colony size, the time course of papillation at 30°C is very similar to papillation at 37°C for all sets of transposition functions. Incubation at 30°C reduces colony background redness in comparison to incubation at 37°C (data not shown).

Characterization of Papillation With MiniTn7-*lac* in a Chromosomal *att*Tn7::miniTn7-*lac* Donor Site:

We determined that the characteristics of miniTn7-*lac* papillation described in the previous section are not particular to the F' donor site used by also examining papillation from a chromosomal miniTn7-*lac* donor site (see below). As observed with miniTn7-*lac* papillation from an F' donor site, the presence of *tns* genes known to promote transposition greatly stimulates Lac$^+$ papillation.

Figure 2-6 graphically describes the course of miniTn7-*lac* educed papillation from a chromosomal *att*Tn7 donor site, while Figure 2-7 photographically depicts this papillation. As with a pOX38*gen* donor site (above), the rate of miniTn7-*lac* papillation from *att*Tn7 is influenced by the set of *tns* genes supplied in *trans*. MiniTn7-*lac*

papillation is greatest in the presence of all five transposition genes *tns*ABCDE, while little to no papillation occurs in the absence of *tns* genes or the presence of only *tns*ABC. MiniTn7-*lac* papillation in the presence of *tns*ABC+E occurs slightly slower than in the presence of all five transposition genes. Papillation in the presence of *tns*ABC+D is much slower than *tns*ABC+E, but significantly faster than *tns*ABC or no *tns* genes.

We do notice that miniTn7-*lac* papillation from *att*Tn7 is slightly slower for all conditions tested (compare Figures 2-3 and 2-4). However the same hierarchy of the rate of miniTn7-*lac* papillation can be described as:

(*tns*ABCDE > *tns*ABC+E > *tns*ABC+D > *tns*ABC or no *tns*)

It is important to note that the relative levels of *tns* gene expression from each of the plasmids used (pCW4, pCW4::miniMuΩ^{76E}, pCW4::miniMuΩ^{107D}, and pCW15) is unknown. *tns* expression level is likely to affect the rate of miniTn7-*lac* papillation.

Variability of MiniTn7-*lac* Papillation

To serve as a useful assay, we must understand the degree of variability in the papillation assay. Examining the degree of variability within a given papillation experiment (as in Figures 2-3A and 2-6A) we see that as the number of papillae per colony increases during the course of incubation, the standard deviation of the average number of Lac$^+$ papillae increases - but the relative variability of the measurement (the relative variability of the number of papillae on the multiple colonies examined) dramatically decreases. Here relative variability is defined as the ratio of the standard deviation of the mean number of Lac$^+$ papillae per colony divided by the mean number of Lac$^+$ papillae per colony. These relationships are explicitly depicted in Figure 2-8.

Figure 2-8 depicts the degree of variability between papillation experiments. In Panel A we see that independent examinations of NLC28 *att*Tn7::miniTn7-*lac* p*tns*

papillation differ significantly enough to have non-overlapping standard deviations (compare day 6 *tns*ABC+E papillation). Panel B suggests the difference between the time courses observed in Panel A may be partially due to differences in incubation. Papillation time courses in Panel B were performed simultaneously on colonies from independent cultures of NLC28 *att*Tn7::miniTn7-*lac* p*tns* as described in the figure legend of Figure 2-9.

tns -Independent Papillation

Although papillation was greatly stimulated by *tns* gene combinations known to promote intermolecular transposition, a very low level of Lac^+ papillation was observed in the absence of *tns* genes (see Panels E in Figures 2-4, 2-5, and 2-7). Such *tns*-independent papillation could result from any event which could activate transcription of the *lac*ZYA genes in miniTn7-*lac* including non-transposition related genetic recombination and activation of a cryptic transcription promoter. The nature of the events that underlie *tns*-independent papillation events vs. *tns*-dependent papillation events is different (see below).

It should also be noted that there appears to be a small but reproducible increase of miniTn7-*lac* papillation in the presence of only *tns*ABC (no *tns*D or *tns*E). This papillation in the presence of only *tns*ABC is most evident in Figure 2-6A (and compare Panels D vs. E in Figures 2-4, 2-5, and 2-7). The *tns*ABC stimulated papillation may represent *tns*D and *tns*E independent transposition. However, no intermolecular transposition of miniTn7-*lac* was seen in the presence of only the *tns*ABC transposition genes (Table 2-3, line 3). As alluded to above, however, papillation may result from either inter- or intramolecular transposition (see below).

Figure 2-3

Papillation With MiniTn7-*lac* in a F' Donor Site
(pOX38*gen*::miniTn7-*lac*)

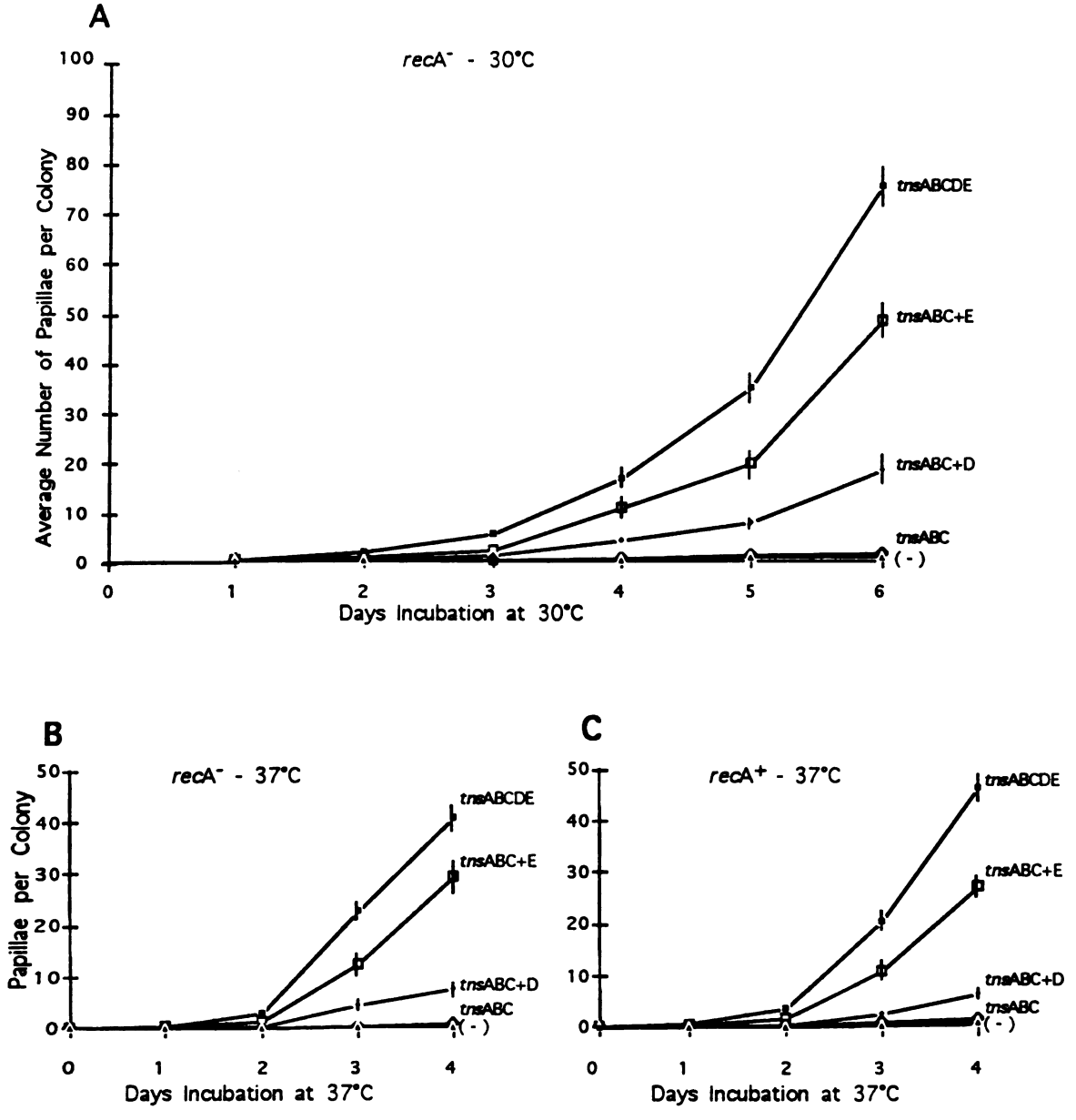


Figure 2-3 Legend

Papillation With MiniTn7-*lac* On a F' Donor Site

(pOX38*gen*::miniTn7-*lac*)

Lac$^+$ papillation of colonies on MacConkey Lactose indicator plates without antibiotics, at the indicated incubation temperatures is reported as average number of papillae per colony over time. The average number of Lac$^+$ papillae on 10 colonies is plotted (each data point) along with the standard deviation of the average number of Lac$^+$ papillae (bar through data points). Data is from simultaneous plating of the indicated strains on the same batch of plates. The Lac$^-$ donor site of miniTn7-*lac* was the F' plasmid, pOX38*gen*::miniTn7-*lac*, and *tns* functions were as indicated. *tns* functions were supplied by the following plasmids;

*tns*ABCDE = pCW4

*tns*ABC+E = pCW4::miniMuΩ^{107D}

*tns*ABC+D = pCW4::miniMuΩ^{76E}

*tns*ABC = pCW15

(-) = No plasmid

A:

Panel A represents the papillation at 30°C of NLC51 *att*Tn7::miniTn7-Km**R**199 pOX38*gen*::miniTn7-*lac* + *tns* plasmids.

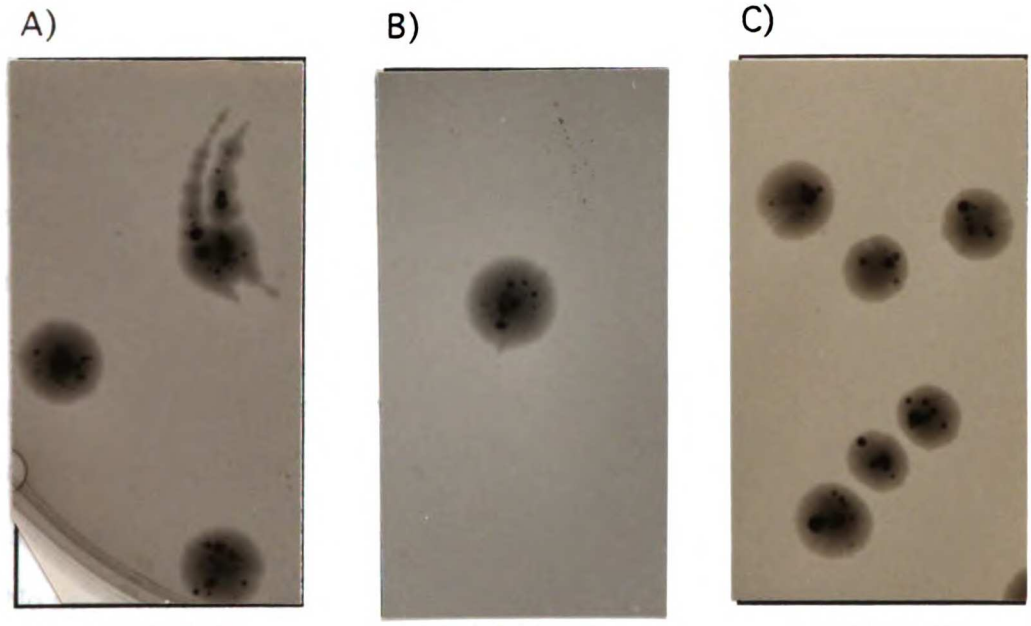
B:

Panel B represents the papillation at 37°C of NLC51 *att*Tn7::miniTn7-Km**R**199 pOX38*gen*::miniTn7-*lac* + *tns* plasmids.

C:

Panel C represents the papillation at 37°C of NLC28*att*Tn7::miniTn7-Km**R**199 pOX38*gen*::miniTn7-*lac* + *tns* plasmids.

Figure 2-4
Papillation of NLC51
With MiniTn7-lac in a F' Donor Site



(NLC51 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* p*tns*)

Figure 2-4 Legend

Papillation of NLC51 With MiniTn7-*lac* in a F' Donor Site

Lac$^+$ papillation of NLC51*att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* +*tns* plasmids as indicated was photographed after incubation at 37°C for 72 hours on MacConkey Lactose indicator plates without antibiotics. Data is from simultaneous plating of the indicated strains on the same batch of plates. NLC51 is *rec*A$^-$. The Lac$^-$ donor site of miniTn7-*lac* was the F' plasmid, pOX38*gen*::miniTn7-*lac*, and *tns* functions were as indicated. *tns* functions were supplied by the following plasmids;

A) *tns*ABCDE = pCW4

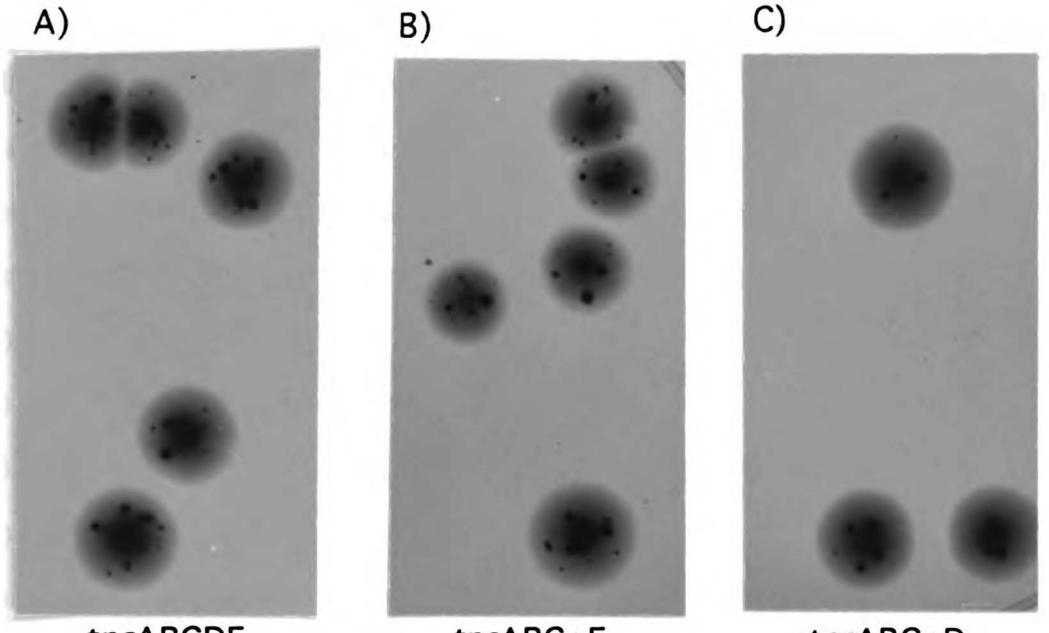
B) *tns*ABC+E = pCW4::miniMuΩ^{107D}

C) *tns*ABC+D = pCW4::miniMuΩ^{76E}

D) *tns*ABC = pCW15

E) No *tns* = No plasmid

Figure 2-5
Papillation of NLC28
With MiniTn7-lac in a F' Donor Site



(NLC28*att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* p*tns*)

Figure 2-5 Legend

Papillation of NLC28 With MiniTn7-*lac* in a F' Donor Site

Lac$^+$ papillation of NLC28*att*Tn7::miniTn7-Km**R**199 pOX38*gen*::miniTn7-*lac* + *tns* plasmids as indicated was photographed after incubation at 37°C for 72 hours on MacConkey Lactose indicator plates without antibiotics. Data is from simultaneous plating of the indicated strains on the same batch of plates. NLC28 is *rec*A$^+$. The Lac$^-$ donor site of miniTn7-*lac* was the F' plasmid, pOX38*gen*::miniTn7-*lac*, and *tns* functions were as indicated and were supplied by the following plasmids;

A) *tns*ABCDE = pCW4

B) *tns*ABC+E = pCW4::miniMuΩ^{107D}

C) *tns*ABC+D = pCW4::miniMuΩ^{76E}

D) *tns*ABC = pCW15

E) No *tns* = No plasmid

Figure 2-6

Papillation With MiniTn7-*lac* in a Chromosomal *att*Tn7 Donor Site

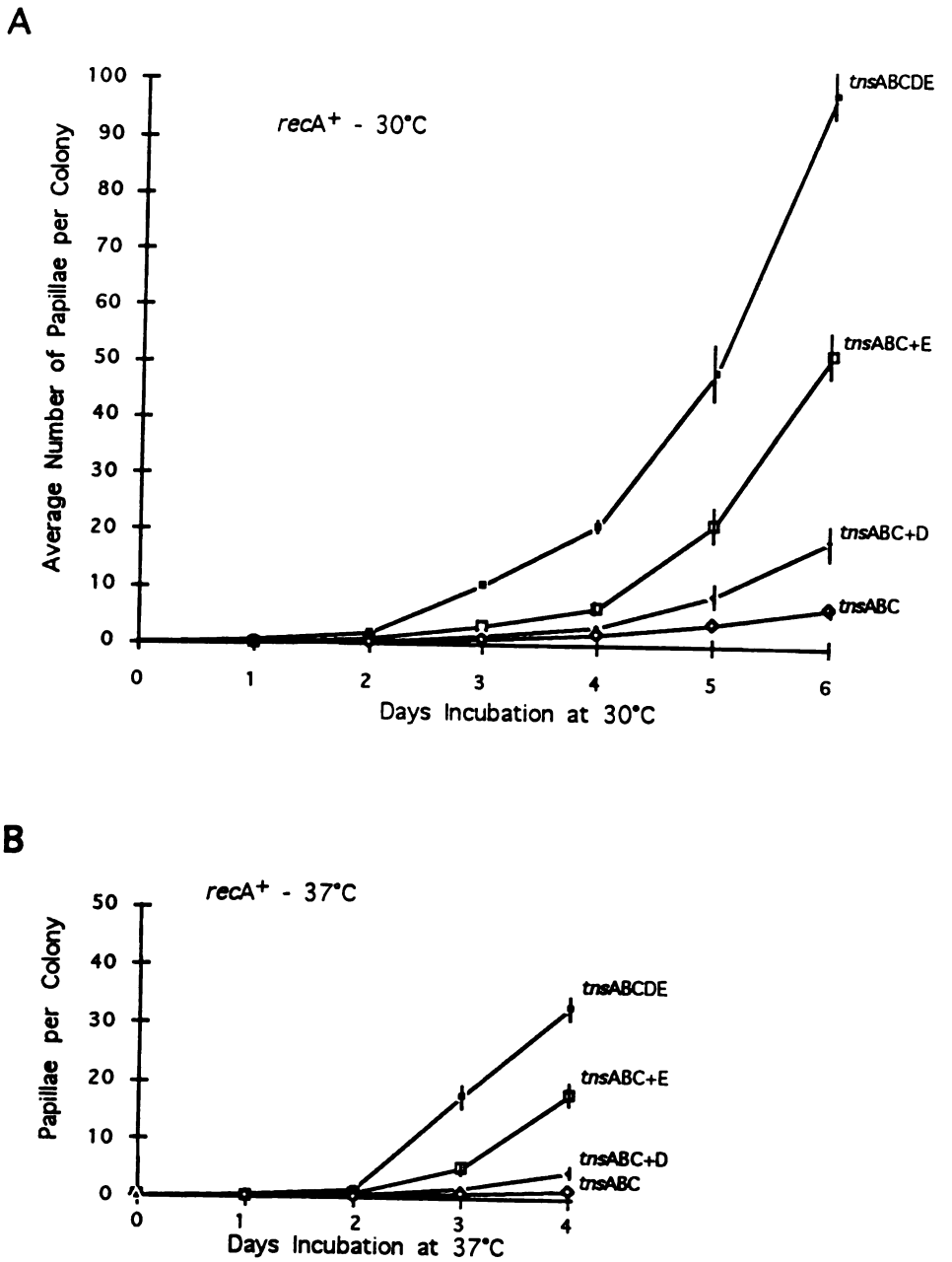


Figure 2-6 Legend

Papillation With MiniTn7-*lac* in a Chromosomal *att*Tn7 Donor Site

Lac^+ papillation of colonies on MacConkey Lactose indicator plates without antibiotics at the indicated incubation temperatures is reported as average number of papillae per colony over time. The average number of Lac^+ papillae on 10 colonies is plotted (each data point) along with the standard deviation of the average number of Lac^+ papillae (bar through data points). The Lac^- donor site of miniTn7-*lac* was chromosomal *att*Tn7::miniTn7-*lac*, and *tns* functions were as indicated. *tns* functions were supplied by the following plasmids;

*tns*ABCDE = pCW4

*tns*ABC+E = pCW4::miniMuΩ^{107D}

*tns*ABC+D = pCW4::miniMuΩ^{76E}

*tns*ABC = pCW15

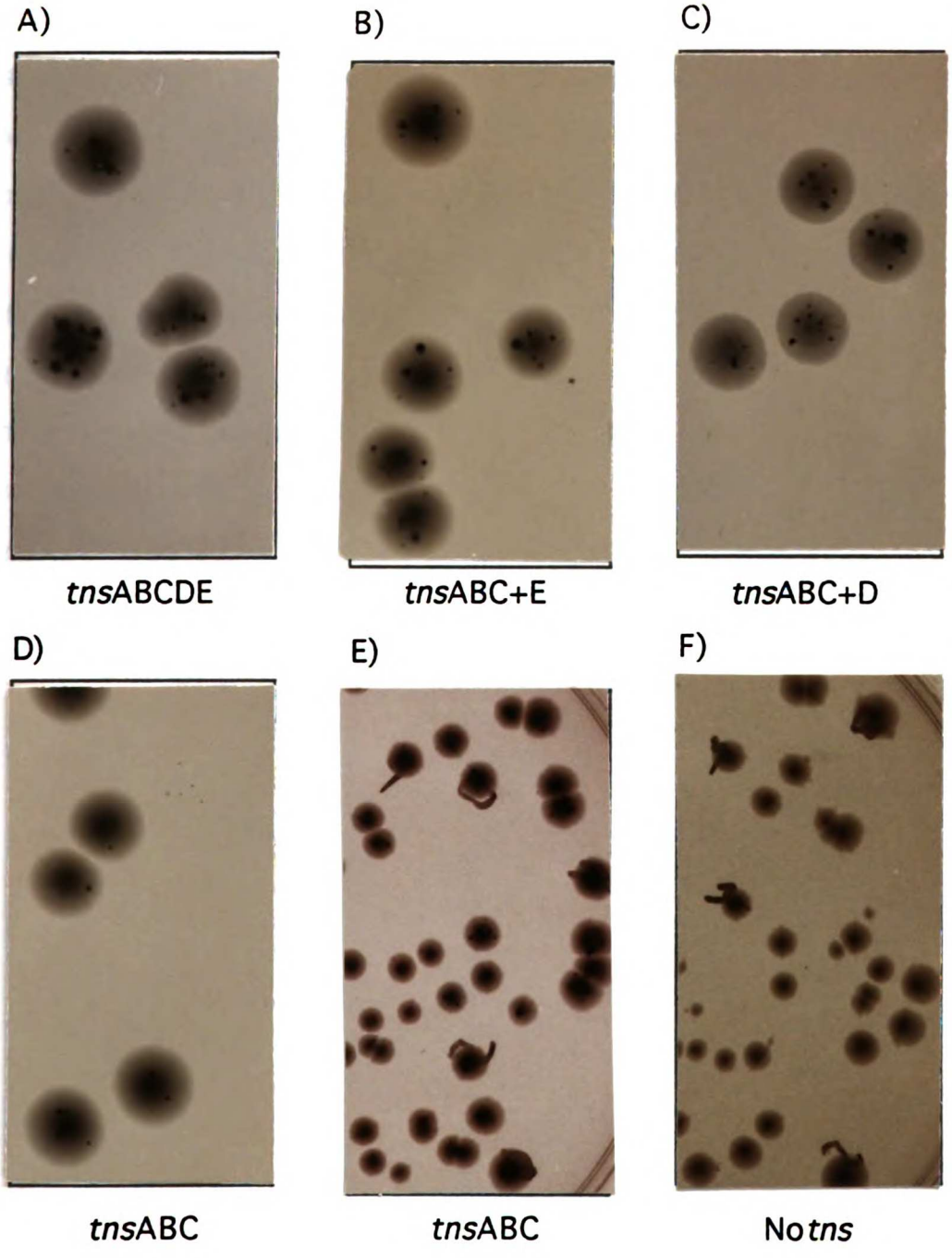
A:

Panel A presents the papillation at 30°C of NLC28*att*Tn7::miniTn7-*lac* + *tns* plasmids.

B:

Panel B presents the papillation at 37°C of NLC28*att*Tn7::miniTn7-*lac* + *tns* plasmids.

Figure 2-7
Papillation of NLC28 With MiniTn7-lac in a Chromosomal *att*Tn7 Donor Site



(NLC28*att*Tn7::miniTn7-*lac* p*tns*)

Figure 2-7 Legend

Papillation of NLC28 With MiniTn7-*lac* in a Chromosomal *att*Tn7 Site Donor Site

Lac$^+$ papillation of NLC28*att*Tn7::miniTn7-*lac* +*tns* plasmids as indicated was photographed after incubation at 37°C on MacConkey Lactose indicator plates. Colonies presented in panels A-D are from simultaneous plating of the indicated strains on the same batch of plates after a 72 hour incubation. Colonies presented in panels E and F are from simultaneous plating of the indicated strains on the same batch of plates after 48 hours. The Lac$^-$ donor site of miniTn7-*lac* was chromosomal *att*Tn7::miniTn7-*lac*, and *tns* functions were as indicated and supplied by the following plasmids;

A) *tns*ABCDE = pCW4

B) *tns*ABC+E = pCW4::miniMuΩ^{107D}

C) *tns*ABC+D = pCW4::miniMuΩ^{76E}

D) *tns*ABC = pCW15

E) *tns*ABC = pCW15

F) No *tns* = No *tns* plasmid

Figure 2-8
Variability of Papillation Within Experiments

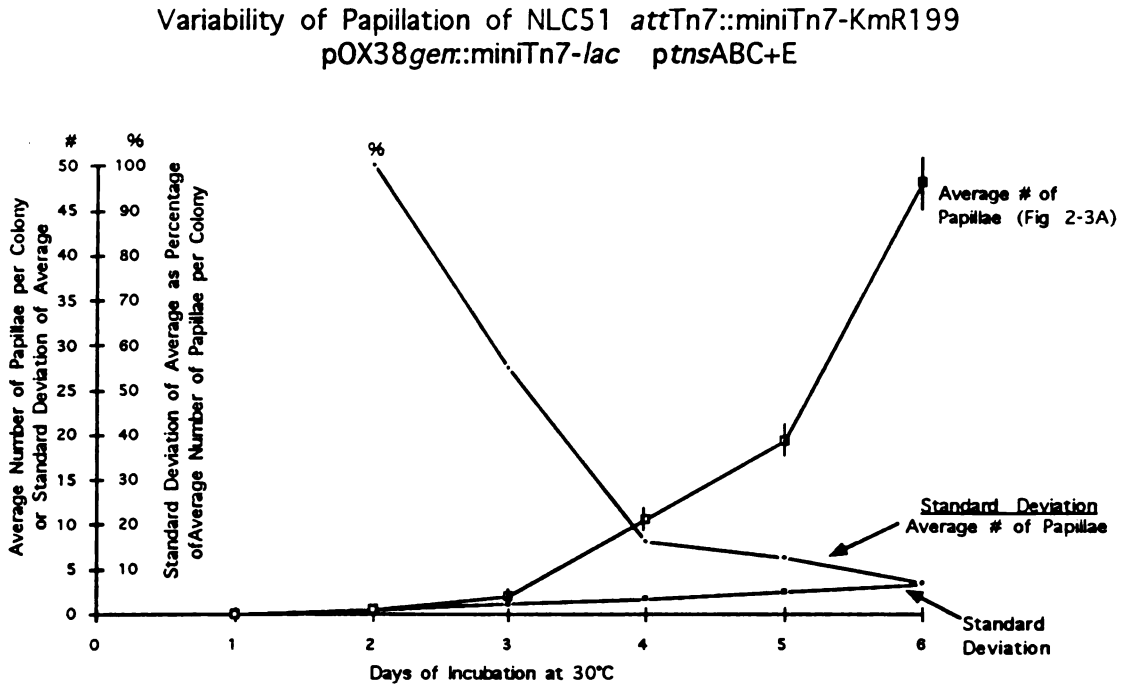


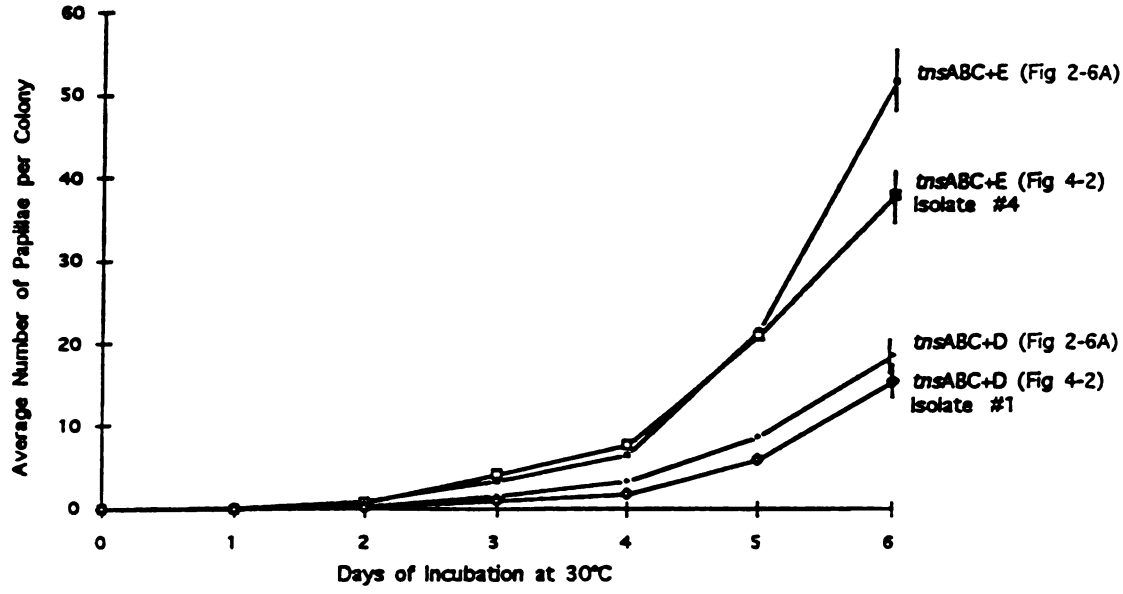
Figure 2-8 Legend

Variability of Papillation Within Experiments

Lac^+ papillation of NLC51*att*Tn7::miniTn7-Km**R**199 pOX38*gen*::miniTn7-*lac* + *tns*ABC+E (pCW4::miniMuΩ^{107D}) after incubation at 30°C on MacConkey Lactose indicator plates without antibiotics as described for Figures 2-3A. The Lac^- donor site of miniTn7-*lac* was the F' plasmid, pOX38*gen*::miniTn7-*lac*, and *tns*ABC+E functions were supplied by pCW4::miniMuΩ^{107D}. The average number of Lac^+ papillae on 10 colonies is plotted (open box) along with the standard deviation of the average number of Lac^+ papillae (bar through open box and black box line). Also plotted is the standard deviation of the mean as a percentage of the mean number of papillae (black diamond).

Figure 2-9
Variability of Papillation Between Experiments

A) Independent Tests of NLC28 *att*Tn7::miniTn7-*lac* p*tns* Papillation



B) Papillation of Independent Isolates of NLC28 *att*Tn7::miniTn7-*lac* p*tns* Incubated Simultaneously

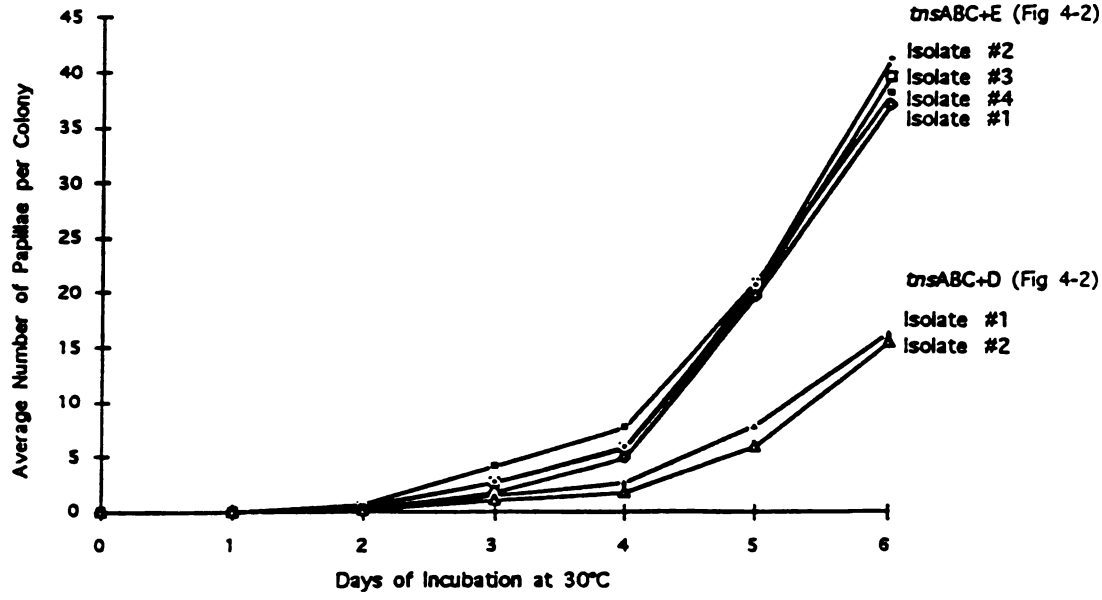


Figure 2-9 Legend

Variability of Papillation Between Experiments

Lac$^+$ papillation of NLC28*att*Tn7::miniTn7-*lac* + *tns*ABC+E (pCW4::miniMuΩ^{107D}) or *tns*ABC+D (pCW4::miniMuΩ^{76E}) after incubation at 30°C on MacConkey Lactose indicator plates as described for Figures 2-6A and 4-2. In both panels, each time course represents the average number of papillae on ten colonies of the indicated strain. Panel A compares papillation time courses from the experiments described in Figures 2-6A and 4-2 (Chapter 4) which were done separately, on separate batches of MacConkey Lactose plates. Time courses derived from Figure 2-6A were done on MacConkey Lactose plated containing no antibiotics. Papillation of isolates #1 and #2 (*tns*ABC+E) from Figure 4-2 and isolate #2 (*tns*ABC+D) was done on MacConkey Lactose plates supplemented with 50 ug/ml spectinomycin. Papillation of isolates #3 and #4 (*tns*ABC+E) from Figure 4-2 and isolate #1 (*tns*ABC+D) was done on MacConkey Lactose plates supplemented with 20 ug/ml tetracycline. Colonies examined in Panel B were incubated simultaneously.

The Major Class of Lac$^+$ Papillae are the Result of Intermolecular Translocation

The nature of the molecular rearrangements that lead to Lac$^+$ papillae was examined by conjugation of the miniTn7-*lac* donor (pOX38*gen*::miniTn7-*lac*) from Lac$^+$ papillae into new Lac$^-$ hosts. Our other assays for Tn7 transposition (mating-out and λ-hop) require intermolecular translocation. Intermolecular translocation is not obviously required for miniTn7-*lac* papillation. We found that papillae generated in the absence of *tns* functions reflect intramolecular rearrangements. In striking contrast, the majority of papillae generated in the presence of *tns*ABCDE reflect intermolecular translocation of the miniTn7-*lac* element, i.e. result from *tns*-dependent transposition.

tns-Independent Papillation is the Result of Intramolecular Events.

Ten pOX38*gen*::miniTn7-*lac* plasmids from rare Lac$^+$ papillae on colonies of a strain containing or lacking *tns* genes were conjugated into new Lac$^-$ hosts and scored for their Lac phenotype. All ten of the F's from these Lac$^+$ papillae generated in the absence of *tns* genes conferred a Lac$^+$ phenotype to the new host cells (Table 2-4, Line 1). The F' location of the Lac$^+$ information implies that *tns*-independent Lac$^+$ papillation is the result of an intramolecular event such as a non-transposition-related intramolecular rearrangement.

tns-Dependent Papillation is Usually the Result of Intermolecular Events.

Sixteen of twenty pOX38*gen*::miniTn7-*lac* plasmids from Lac$^+$ papillae generated in a strain containing *tns*ABCDE, were Lac$^-$ (Line 2). This suggests that a Lac$^+$ miniTn7-*lac* lies in the chromosome, and thus has undergone intermolecular translocation (from the F' to the chromosome). The Lac$^+$ papillae generated in the presence of *tns*ABCDE, for which the Lac$^+$ information resides on the donor F', may represent intramolecular *tns*-dependent transposition or *tns*-independent rearrangements.

*tns*ABC Stimulated Papillation is the Result of Intramolecular Events.

All of the twenty pOX38*gen*::miniTn7-*lac* plasmids from Lac$^+$ papillae generated in a strain containing *tns*ABC, were Lac$^+$ (Line 3). This suggests that these Lac$^+$ papillae result from intramolecular events such as non-transposition related intramolecular rearrangement or intramolecular transposition.

Table 2-4

tns Genes Determine The Inter vs. Intramolecular Nature of Events Resulting in Papillation

	Transposition Genes	Status of F' from Lac$^+$ Papillae Lac+	Lac-
1)	(-)	10	0
2)	*tns*ABCDE	4	16
3)	*tns*ABC	20	0

Table 2-4 Legend

Lac$^+$ papillae were purified from NLC28 attTn7::miniTn7-KmR199 pOX38gen::miniTn7-lac colonies containing the indicated *tns* genes (*tns*ABCDE = pCW4 and *tns*ABC = pCW15). The F' was transferred to CW51 by conjugation and Lac phenotype evaluated by plating on MacConkey Lactose indicator plates.

Discussion

The goal of this work was to develop and characterize a visual assay for Tn7 transposition. The assay developed is based on the ability of a transposition event to move a genetically hidden, or unexpressed, function to an exposed or expressed site. MiniTn7-*lac* was constructed to carry promoterless *lac*ZYA genes between the ends of Tn7. Transposition of miniTn7-*lac* from a non-transcribed to a transcribed region results in a Lac$^+$ phenotype which can be visualized on MacConkey Lactose plates. With the miniTn7-*lac* papillation assay, each colony can easily be visually screened for the rate of transposition. The miniTn7-*lac* papillation assay will be useful for identifying mutations which affect Tn7 transposition.

Validation of MiniTn7-*lac* Papillation Assay:

To demonstrate that papillation of miniTn7-*lac* actually reports transposition, we compared requirements for miniTn7-*lac* papillation and intermolecular miniTn7-*lac* translocation to an F' (as measured by the mating-out assay). Efficient miniTn7-*lac* papillation and miniTn7-*lac* translocation both require *tns*ABC and *tns*D or *tns*E. Additionally, we showed that the majority of Lac$^+$ papillae generated in the presence of *tns*ABCDE are the result of intermolecular translocation of miniTn7-*lac*.

A very low background rate of papillation is seen without transposition function genes. Papillae seen in the absence of *tns* genes were shown to express *lac* from the miniTn7-*lac* donor molecule, suggesting that the Lac$^+$ papillae seen in the absence of *tns* functions result from intramolecular events, possibly unrelated to transposition.

We noted a small stimulation in the rate of papillation in the presence of only *tns*ABC. Examination of miniTn7-*lac* donor molecules from such papillae revealed that the Lac$^+$ phenotype is the result of intramolecular events. Determining whether this papillation is, in fact, dependent on *tns*ABC, and whether it represents intramolecular transposition or some other form intramolecular event will require further examination.

Limitations of the miniTn7-*lac* Papillation Assay:

Lac^+ papillation indirectly reports transposition after the cell in which the Lac^- to Lac^+ transition occurred has overgrown it's neighbors to form a papillae. Thus the rate of papillation is likely to be affected not only by the rate of transposition, but by the growth rate of the strain being examined, and by the growth advantage *lac* transcription gives to the cells. In Figures 2-3 and 2-6 we saw that the rate of papillation is affected by the temperature of incubation (most likely affecting growth rate). Because the strains used in the experiments presented in this chapter were isogenic except for the *tns* supplying plasmids, we expect there to be little difference in growth rates and the relative advantages of *lac* transcription between the strains being compared. Even so, we noted in Figure 2-9 a significant difference in the papillation rate of the same strain tested separately, possibly due to growth rate differences. Comparison of papillation rates between experiments is problematic due to possible differences in assay conditions (temperature, plate media composition, metabolic history of the bacterial strains, etc.), however the variability of the rate of papillation within an experiment was low (see error bars of Figures 2-3 and 2-6). Differences in growth rate and the relative advantages of *lac* transcription will become a concern in Chapters 4 and 5 where the effect of host mutations on Tn7 transposition are examined using the papillation assay.

Papillation and Transposition Target Site Usage:

The nature of transposition target sites is of special interest in the study of Tn7. Though Tn7 transposes with little target site specificity in the *tns*ABC+E transposition pathway, Tn7 is unusual in its ability to transpose to specific sites, *att*Tn7 and pseudo-*att*Tn7, in an orientation-specific manner using *tns*ABC+D (Barth et al., 1978; Hauer and Shapiro, 1984; Krishnapillai et al., 1984; Lichtenstein and Brenner, 1981; McKown et al.,

1988; Moore and Krishnapillai, 1982; Ogawa et al., 1984). Papillation reports transposition when the target site is expressed and the transposon is oriented such that *lac* will be transcribed. Papillation rates can misrepresent transposition rates when transposition target sites or the insertion orientation into target sites is significantly different between two populations being tested.

The rate of miniTn7-*lac* papillation is influenced by the set of transposition functions supplied in *trans*. Other Tn7 transposition assays have shown that transposition via *tns*ABC+E to apparently random sites and transposition via *tns*ABC+D to pseudo-*att*Tn7 sites occur at nearly the same frequency (Kubo and Craig, 1990); however, we observe that miniTn7-*lac* papillation rate is much slower for *tns*ABC+D mediated transposition. This difference between assays may be reflective only of *tns* gene expression level differences from the plasmids carrying the *tns* genes. Alternatively the difference between *tns*ABC+D and *tns*ABC+E mediated papillation may be reflective of target site usage. Transposition via *tns*ABC+D most frequently uses approximately eight pseudo-*att*Tn7 sites in the E. *coli* chromosome, and insertion in these targets is likely to be orientation specific (Kubo and Craig, 1990). Thus, many *tns*D-promoted miniTn7-*lac* transposition events may not be detectable by Lac^+ papillation. By contrast, *tns*E-promoted transposition events are directed to sequence non-specific targets, and so should have no target transcription bias resulting from limited insertion sites.

Use of R70 for the Right End of MiniTn7-*lac*:

We observed that the frequency of miniTn7-*lac* transposition is reduced in comparison to other miniTn7 elements. The use of **R**70 for the right end sequences of miniTn7-*lac* may account for a decrease in the level of miniTn7-*lac* transposition in comparison with intact Tn7 and other "full" ended miniTn7 elements. In previous studies, use of **R**70 for the right end sequences of a miniTn7 element whose ends were separated by 1 Kb was found to decrease transposition slightly (Arciszewska et al.,

1989). The use of **R**70 in combination with increasing the separation of the left and right transposon ends to 6.5 Kb may account for the decreased miniTn7-*lac* transposition. We did observe that the frequency of miniTn7-*lac* transposition increases to that of intact Tn7 when *tns* genes are supplied by a multicopy plasmid. *tns* genes on these multicopy plasmids are overexpressed in comparison to *tns* expression from intact Tn7 (Orle and Craig, 1991). We noted that the increase of miniTn7-*lac* transposition frequency to that of Tn7's may reflect suppression of decreased TnsB binding to the end sequences of miniTn7-*lac* or suppression of decreased miniTn7-*lac* synaptic transposition complex formation through over-expression of TnsB. To test whether over-expression of *tns*B is responsible for the restoration of miniTn-*lac* transposition frequency back to that of intact Tn7's, the effect of over-expression of only *tns*B should be tested. To examine the effect of *tns*B over-expression, the bacterial strain NLC28 *att*Tn7::miniTn7-*lac* ϕ80dII*lac*::Tn7 pOX38*gen* (used for Table 2-2, line 3) could be used in conjunction with either a plasmid over-expressing only *tns*B, or the plasmid vector only control. The transposition of both intact Tn7 and miniTn7-*lac* could be measured in the same experiment. If miniTn7-*lac*'s transposition frequency is decreased due to decreased binding of TnsB binding the ends of the transposon, we would expect that miniTn7-*lac* would be more sensitive to *tns*B over-expression than Tn7. If however, suppression of the decreased miniTn7-*lac* transposition phenotype requires over-expression of the other *tns* genes, decreased miniTn7-*lac* transposition may be due to a defect subsequent to TnsB binding such as complex formation between the two transposon ends and Tns proteins.

The suppressability of miniTn7-*lac*'s decreased transposition by a multicopy source of *tns* functions may provide a useful tool for the understanding of Tn7 transposition. In Chapter Four of this thesis, we examine the effects of specific host functions on Tn7 transposition. The impairment of miniTn7-*lac* by it's **R**70 end may

make it exquisitely sensitive to conditions affecting synaptic transposition complex formation and strand transfer.

A New Assay for Tn7 Transposition:

The development of a genetically tractable visual assay for Tn7 transposition will enable the identification of mutations affecting transposition. We have established here that papillation faithfully reports transposition and large numbers of colony papillation rates are easily assessed. Using the papillation assay, mutants can be screened for an altered rate of transposition by scoring colony papillation phenotype. E. *coli* mutants affecting Tn7 transposition have already been identified using the miniTn7-*lac* papillation assay (see Chapter 5 of this thesis). MiniTn7-*lac* papillation has recently been used to identify *tns*C mutants able to carry out transposition in the absence of *tns*D or *tns*E (A. Stellwagen, personal communication).

MiniTn7-*lac* can also be used as a reporter of transposition orientation. Tn7 has been shown to transpose in an orientation specific manner to some plasmids and to chromosomal *att*Tn7 (Barth et al., 1978; Hauer and Shapiro, 1984; Krishnapillai et al., 1984; Lichtenstein and Brenner, 1981; McKown et al., 1988; Moore and Krishnapillai, 1982; Ogawa et al., 1984). Because of the orientation-specific manner of *lac* transcription, miniTn7-*lac* can be used as a sensitive assay for transposition orientation. Chapter 3 of this thesis employs miniTn7-*lac* to examine transposition orientation to *att*Tn7.

Chapter 3: Orientation Specificity of Tn7 Transposition to *att*Tn7

Abstract:

Transposon Tn7 is extraordinary in its ability to transpose at high efficiency to a single site, *att*Tn7, in addition to transposing at low frequency to apparently random sites. Transposition to *att*Tn7 has previously been characterized as extremely orientation-specific, with the right end of Tn7 inserting adjacent to *glm*S. We demonstrate here that insertions of Tn7 in *att*Tn7 can occur in the opposite orientation, with the left transposon end adjacent to *glm*S. We identified opposite orientation insertions by taking advantage of miniTn7-*lac*. Insertion of miniTn7-*lac* into *att*Tn7 in the standard orientation results in a Lac^- phenotype. By contrast, insertion of miniTn7-*lac* into *att*Tn7 in the opposite orientation results in a Lac^+ phenotype, allowing us to identify these rare opposite orientation insertions into *att*Tn7. Opposite-orientation insertion of miniTn7-*lac* into *att*Tn7, with its left end adjacent to *glm*S, was confirmed through Southern blot analysis. The frequency of opposite orientation insertion is 1% the frequency of the standard (right end adjacent to *glm*S) insertion orientation. Implications of opposite-orientation insertion are considered.

Introduction

Tn7 transposition to *att*Tn7 is highly orientation specific (Barth et al., 1978; Hauer and Shapiro, 1984; Krishnapillai et al., 1984; Lichtenstein and Brenner, 1981; McKown et al., 1988; Moore and Krishnapillai, 1982; Ogawa et al., 1984). To begin dissecting how the transposition machinery specifies orientation, we use miniTn7-*lac* as a sensitive probe to assay the degree of insertion orientation specificity.

To achieve and detect transpositional orientation specificity, target and donor orientation must be distinguished during the process of integration. Sequence complementary of homologous sequences determines orientation for homologous recombination. The mechanism of homologous recombination involves the homologous pairing of DNA strands followed by reciprocal strand exchange (R. Holliday, 1964; DasGupta et al., 1981; and reviewed by G. M. Weinstock, 1987; and Eggleston and Kowalczykowski, 1991). Orientation of recombination is defined (for non-palindromic sequences) by homologous pairing of the two DNA segments undergoing recombination. Homologous recombination will always connect the Crick strand of one DNA segment to the Crick strand of the other.

Transposition does not involve homologous sequences or pairing, and since many transposons have little target sequence specificity, there are few cues by which to achieve orientation specificity. In transposition, a segment of DNA moves from one genetic location to another, requiring no homology between the two locations (J. A. Shapiro, 1979; and reviewed in Craig and Kleckner, 1987). A "transposase" brings two related ends of a transposon together and catalyzes DNA strand transfer to a third DNA segment, the target site. Orientation of transposon insertion need not be inherently defined by the mechanism of transposition as in homologous recombination. The Crick-strand of a transposon may by ligated to either Crick or Watson strands of the insertion site, i.e. insertion can occur in either orientation.

Tn7 is an extraordinary transposon in its ability to transpose at high efficiency to a single site, *att*Tn7, as well as transpose at low frequency to apparently random sites (Barth and Grinter, 1977; Barth et al., 1978; Hauer and Shapiro, 1984; Rogers et al., 1986; Waddell and Craig, 1988; Kubo and Craig, 1990). Tn7 transposition to *att*Tn7 targets (promoted by the Tn7 encoded genes *tns*ABC+D) is orientation specific to both the chromosomal *att*Tn7 site (Gay et al., 1986; Hauer and Shapiro, 1984; Lichtenstein and Brenner, 1981; Lichtenstein and Brenner, 1982) and *att*Tn7 sites cloned into plasmids (Lichtenstein and Brenner, 1981; Rogers et al., 1986; Gringauz et al., 1988; McKown et al., 1988). Tn7 inserts in *att*Tn7 with the right end of Tn7 adjacent to the TnsD binding sequences of *att*Tn7 (Bainton et al., 1993).

Tn7 transposition via *tns*ABC+E to sequence non-specific targets can also be orientation-specific. Physical analysis of *tns*ABC+E directed Tn7 insertions into many different sites, apparently unrelated in sequence, on Inc plasmids such as InP1, IncP10 and IncM, has shown a startling orientation specificity (Barth and Datta, 1977; Barth et al., 1978; Cowan and Krishnapillai, 1982; Krishnapillai et al., 1984; Moore and Krishnapillai, 1982; Ogawa et al., 1984). Nearly all of these insertions were found to lie in the same orientation with respect to the plasmid.

Models of the mechanism of transposition can be constrained with an understanding of the degree of transposition orientation specificity. The degree of Tn7's transposition orientation specificity is unknown; however in E. *coli* no opposite orientation insertions have been observed among about 25 examined chromosomal *att*Tn7 insertions and approximately another one hundred in *att*Tn7 sites cloned on plasmids (Gay et al., 1986; Hauer and Shapiro, 1984; Lichtenstein and Brenner, 1981; Lichtenstein and Brenner, 1982; Gringauz et al., 1988; McKown et al., 1988). Of the Tn7 insertions in *att*Tn7 examined for orientation, relatively few chromosomal *att*Tn7 site insertions have been examined compared to insertions in *att*Tn7 sites cloned into plasmids.

How does Tn7 transpose in an orientation specific manner? To achieve transposition orientation specificity, Tn7 must distinguish the orientation of the insertion site relative to its ends. Transposition to an *att*Tn7 site cloned on plasmids is orientation-specific, with the right end of Tn7 inserting adjacent to the TnsD binding sequences (Lichtenstein and Brenner, 1981; McKown et al., 1988; Gringauz et al., 1988). TnsD binds a non-palindromic sequence in *att*Tn7 displaced from the point of insertion, giving the target an inherent asymmetry which likely orients the *att*Tn7 target relative to Tn7 (Bainton et al., 1993). Thus, target orientation may be partially defined by the *att*Tn7 sequence recognized and bound by TnsD. The target site cues orienting TnsE-directed transposition are unknown.

Tn7 must also be able to distinguish between its right and left ends to achieve insertion orientation-specificity. The left and right *cis*-acting end sequences of Tn7 are nonequivalent in sequence, as well as in their ability to promote transposition and confer transposition immunity (Arciszewska et al., 1989). The *cis*-acting sequences at the left end of Tn7 (Tn7**L**) contain three 30 base pair TnsB binding repeats separated by 30 and 40 bp. The *cis*-acting sequences at the right end of Tn7 (Tn7**R**) contain four head-to-tail TnsB binding repeats. This sequence difference between Tn7**R** and Tn7**L** is reflected by their ability to participate in transposition. MiniTn7 constructs flanked on each side by the left end sequences (Tn7**L**-Tn7**L**) do not transpose or confer transposition immunity. However, Tn7**R**-Tn7**R** constructs do transpose and are sufficient to confer transposition immunity (Arciszewska et al., 1989).

The non-equivalence of Tn7 right and left ends is likely to be the donor site cue used to orient transposition, in that all mini-Tn7 transposable elements that contain both left and right ends capable of promoting transposition, transpose in an orientation-specific manner (Arciszewska et al., 1989). Of particular interest to this work, a mini-Tn7 element composed only of an antibiotic resistance gene flanked by Tn7**L**166-Tn7**R**70

(the Tn7 ends flanking miniTn7-*lac*) transposes in an orientation-specific manner to *att*Tn7, analogous to an intact Tn7 element (Arciszewska et al., 1989).

The orientation information contained in the target site and transposon ends must be translated through the structure of the synaptic transposition complex to specify which transposon DNA strand is ligated to which target DNA strand. Transposition orientation specificity might be achieved if the synaptic complex is functional in only the structure generated by correct alignment of asymmetric components. If the synaptic complex is functional in only one form, opposite orientation transposition would be mechanistically excluded. The extent of transposition orientation specificity directly reports the free energy barrier to opposite orientation insertion, and thus to an extent, the asymmetry of the synaptic transposition complex.

Prior to the work reported here, our understanding of the degree of Tn7 transposition orientation specificity stemmed from the observation that no opposite orientation insertions were observed in a total of approximately 25 insertions in E. *coli* chromosomal *att*Tn7 (Gay et al., 1986; Hauer and Shapiro, 1984; Lichtenstein and Brenner, 1981; Lichtenstein and Brenner, 1982) and possibly 100 insertions in *att*Tn7 sites cloned into plasmids (Gay et al., 1986; Hauer and Shapiro, 1984; Lichtenstein and Brenner, 1981; Lichtenstein and Brenner, 1982; Gringauz et al., 1988; McKown et al., 1988).

To examine the orientation of large numbers of Tn7 insertions in *att*Tn7, we have taken advantage of the conjunction of unidirectional transcription of miniTn7-*lac* to express *lac*, and, as we demonstrate here, unidirectional transcription across *att*Tn7. The *lac*ZYA genes within miniTn7-*lac* must be transcribed from the left end of miniTn7-*lac* to generate a Lac^+ phenotype. Transcription across *att*Tn7 is shown here to be unidirectional, proceeding counter-clockwise across the point of Tn7 insertion from *glm*S (see Figure 3-1), as expected from our understanding of the DNA sequence of genes surrounding *att*Tn7 (Walker et al., 1984). We postulate that insertion of miniTn7-*lac* in

*att*Tn7 disrupts the transcription terminator of *glm*S (Gay et al., 1986; Gringauz et al., 1988), and transcription from *glm*S continues across miniTn7-*lac*. In the standard orientation of miniTn7-*lac* insertion in *att*Tn7, with the right end of the transposon inserted adjacent to *glm*S, *lac* is not transcribed. If insertion were to occur in the opposite orientation, with the left end of miniTn7-*lac* adjacent to *glm*S, *lac* is transcribed and the cells should become Lac^+. This chapter reports unidirectional transcription across *att*Tn7, and the utilization of miniTn7-*lac*'s ability to report insertion orientation in *att*Tn7 to expose a low frequency of opposite orientation miniTn7-*lac* insertion in *att*Tn7.

Figure 3-1

Transcription Across *att*Tn7

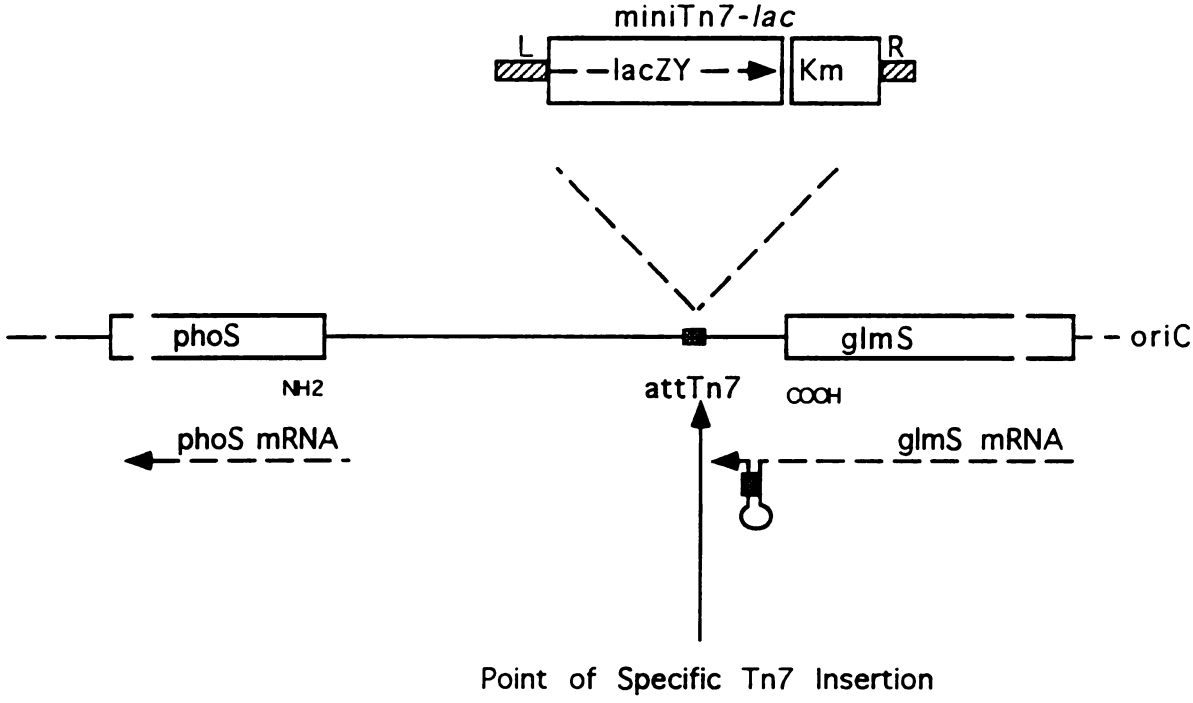


Figure 3-1 Legend

MiniTn7-*lac* and Transcription Across *att*Tn7

Transcription of *glm*S, *pho*S, and the *lac* genes on miniTn7-*lac* is depicted (not to scale). MiniTn7-*lac* is composed of the left 166 bp (indicated as L), and the right 70 bp (indicated as R) of intact Tn7, flanking the *lac*ZYA/Km genes. Promoterless *lac* is oriented in miniTn7-*lac* such that its transcription must originate from outside the left end of the element. *att*Tn7, the site of specific Tn7 insertion, is transcribed from *glm*S. *att*Tn7 is located approximately 20 bp from the DNA encoding the carboxy terminus of GlmS, within the DNA encoding the stem of a stem-loop transcription termination structure (Gay et al., 1986; Gringauz et al., 1988). The *pho*S gene begins nearly 300 bp from *att*Tn7, and is transcribed away from *att*Tn7.

Results

In this chapter, we analyze miniTn7-*lac* transposition to the chromosomal *att*Tn7 target and show that, although standard orientation miniTn7-*lac* insertion in *att*Tn7 predominates, opposite orientation insertion does occur at a low frequency.

MiniTn7-*lac* Can Transpose to *att*Tn7 in Either Insertion Orientation.

In order to examine miniTn7-*lac* transposition to the chromosomal *att*Tn7 site, miniTn7-*lac* was introduced on a F' plasmid (pOX38*gen*::miniTn7-*lac*) into NLC28 cells containing pCW4 (a plasmid which expresses *tns*ABCDE). These cells were then plated on MacConkey lactose indicator plates supplemented with tetracycline (20 μg/ml) and the resulting colonies were examined for Lac^+ papillation. Chromosomal DNA was isolated from cells of either Lac^+ or Lac^- phenotype isolated from the colonies on MacConkey lactose indicator plates. Southern blot analysis of this chromosomal DNA revealed that miniTn7-*lac* can transpose to *att*Tn7 with either its left or right end adjacent to *glm*S (see below).

Transcription Across *att*Tn7 Occurs Only From the Direction of *glm*S.

Insertion of miniTn7-*lac* in *att*Tn7 in the standard orientation with the right end of the transposon adjacent to *glm*S results in a Lac^- phenotype, while opposite orientation insertion in *att*Tn7 results in a Lac^+ phenotype (see Figure 3-2 and 3-3). The Lac^- phenotype of standard orientation insertions in *att*Tn7 implies that transcription across *att*Tn7 does not occur from the direction of *pho*S.

MiniTn7-*lac* Insertion in *att*Tn7 in the Standard (Right End Adjacent to *glm*S) Orientation:

Figure 3-3 presents Southern analysis of Lac$^-$ and Lac$^+$ miniTn7-*lac* insertions in *att*Tn7. Panels D and E show that Lac$^-$ miniTn7-*lac* insertions in *att*Tn7 display junction fragments consistent with miniTn7-*lac* insertion in *att*Tn7 in the standard, right-end-adjacent-to-*pho*S orientation. The 1.2 Kb band seen in lanes D2 and E2 is diagnostic of standard orientation insertion, being the result of HpaI+EcoRI digestion within miniTn7-*lac* and *pho*S.

In lane C2, we also see that 1-10% of cells in a culture whose founder cell had a vacant *att*Tn7 display the 1.2 Kb HpaI+EcoRI band, and thus have miniTn7-*lac* in *att*Tn7 in the standard orientation. This 1-10% occupancy of *att*Tn7 is consistent with previous observations of *att*Tn7 occupancy by Southern analysis (Hauer and Shapiro, 1984), and with the 1.2×10^{-2} frequency of miniTn7-*lac* transposition to *att*Tn7 as measured by the mating-out assay (see Chapter 2, Table 2-3).

MiniTn7-*lac* Insertion in *att*Tn7 in the Opposite (Left End Adjacent to *glm*S) Orientation:

Lanes F1 and F2 of Figure 3-3 show that Lac$^+$ miniTn7-*lac* insertions in *att*Tn7 display junction fragments consistent with miniTn7-*lac* insertion in *att*Tn7 in the opposite, left-end-adjacent-to-*glm*S, orientation. The 7.2 Kb band seen in lane F1 and the 1.6 Kb band seen in lanes F2 are diagnostic of opposite orientation insertion in *att*Tn7.

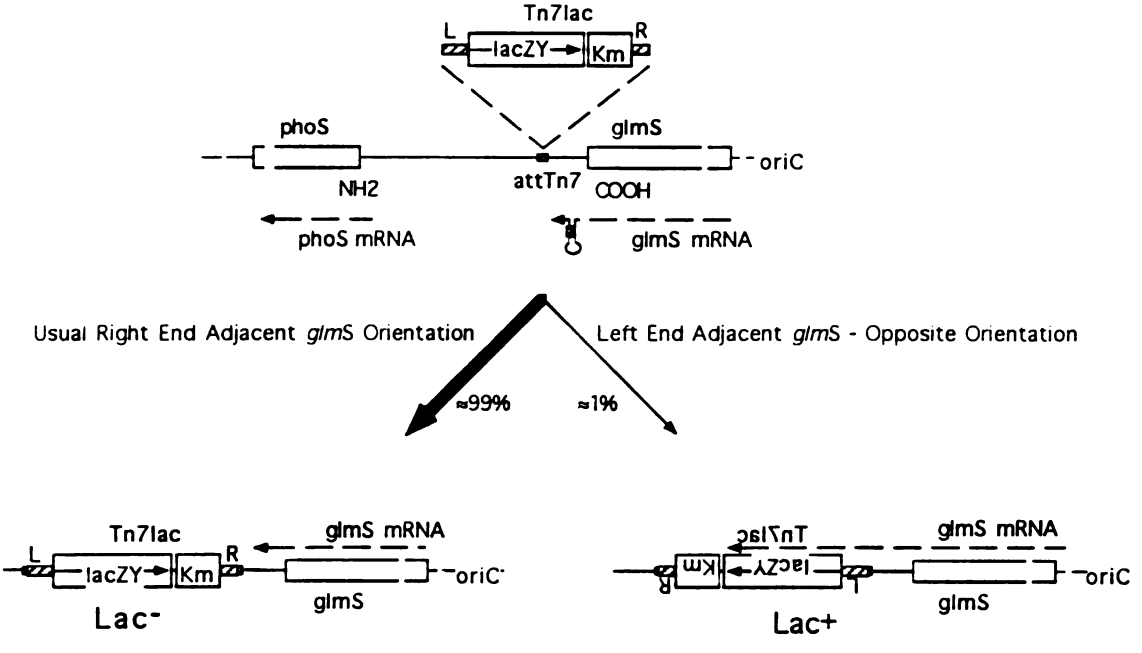
In lanes C1 and C2, where fractional occupancy of *att*Tn7 is examined in a culture whose founder cell began with *att*Tn7 vacant, we see that 1-10% of cells in a culture with miniTn7-*lac* in *att*Tn7 in the standard orientation (the 1.2 Kb band in lane C2). It is notable, however, that we see little evidence of insertion in the opposite orientation which would produce a 7.2 Kb band in C1, and a 1.6 Kb band in C2. The absence of bands diagnostic of opposite orientation in Panel C may be due to a lack of sensitivity, in that it seems likely that an opposite orientation insertion frequency of at least 10% of standard

orientation would be necessary to observe bands on this blot (noting again that only 1-10% of cells in a culture have undergone standard orientation insertion in *att*Tn7).

MiniTn7-*lac* Left-end-adjacent-to-*glm*S insertion in *att*Tn7 is opposite to all insertions in E. *coli att*Tn7 previously characterized. This is the first observation in E. *coli* of Tn7 transposition to *att*Tn7 in the orientation in which the left end of Tn7 is adjacent to *glm*S.

Figure 3-2

Insertion Orientation in *att*Tn7 Determines Lac Phenotype



Lac⁻ Insertions in *att*Tn7
Standard Orientation

Lac⁺ Insertions in *att*Tn7
Opposite Orientation

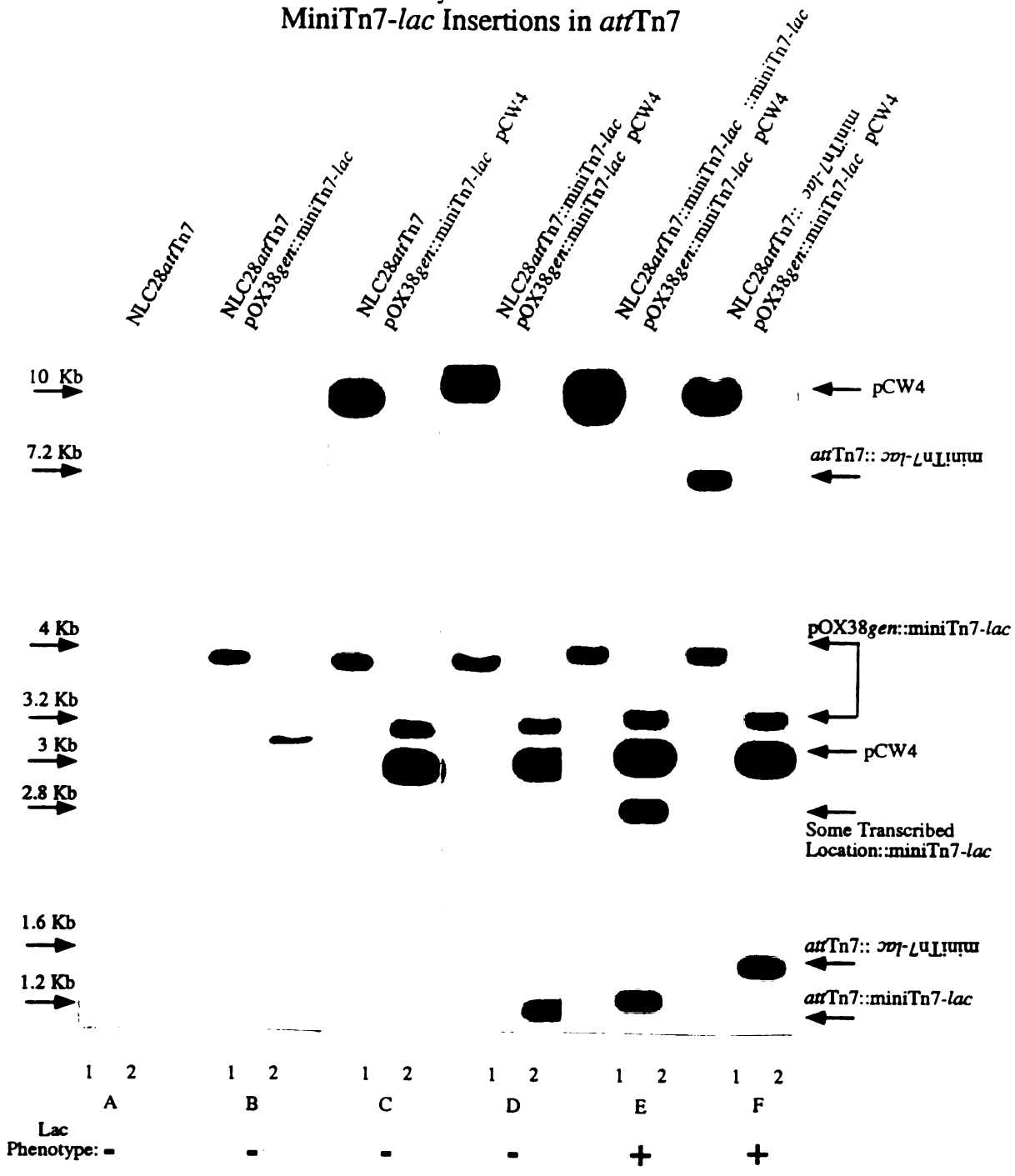
Figure 3-2 Legend

MiniTn7-*lac* Insertion Orientation in *att*Tn7 Determines Lac Phenotype

Transcription of the *lac* genes of miniTn7-*lac* upon insertion *att*Tn7 in either the standard or opposite orientations is depicted (not to scale). Transcription across *att*Tn7 occurs only from the direction of *glm*S. Promoterless *lac* is oriented within miniTn7-*lac* such that its transcription must originate from outside the left end of the element. Thus, miniTn7-*lac* insertion in *att*Tn7 in the standard (right end adjacent to *glm*S) results in a Lac^- phenotype. MiniTn7-*lac* insertion in *att*Tn7 in the opposite (left end adjacent to *glm*S) leads to transcription of *lac* and results in a Lac^+ phenotype. The majority (≈99%; see Figure 3-3 and text) of miniTn7-*lac* transposition events to *att*Tn7 are in the standard orientation, with the right end of the transposon adjacent to *glm*S. However, at a low frequency (≈1%), miniTn7-*lac* transposes to *att*Tn7 with its left end adjacent to *glm*S, rather than in the standard orientation.

Figure 3-3

Southern Analysis of Lac$^+$ and Lac$^-$
MiniTn7-*lac* Insertions in *att*Tn7



*att*Tn7::miniTn7-*lac* = Standard Orientation Insertion
*att*Tn7:: miniTn7-*lac* = Opposite Orientation Insertion

Figure 3-3 Legend

Southern Analysis of Lac$^+$ and Lac$^-$ MiniTn7-*lac* Insertions in *att*Tn7

A culture of NLC28 pCW4 (*tns*ABCDE) pOX38*gen*::*mini*Tn7-*lac* cells in early log-phase growth was plated on MacConkey Lactose supplemented with 20 ug/ml tetracycline. Colonies were examined for papillation phenotypes. Cells from Lac$^+$ papillae and Lac$^-$ colony backgrounds were isolated and purified. Chromosomal DNA from these Lac$^-$ and Lac$^+$ cells was analyzed for novel junction fragments created by transposition. Chromosomal DNA was digested with either HindIII+EcoRI for all lanes labeled "1", or digested with HpaI+EcoRI for all lanes labeled "2". Southern blots were probed with an oligonucleotide complementary to bases L31 through L60 of the top strand at the left end of miniTn7-*lac* (see Materials and Methods). Insertion of miniTn7-*lac* in *att*Tn7 with its left end proximal to *pho*S produces a diagnostic 1.2 Kb HpaI+EcoRI band; insertion in *att*Tn7 with miniTn7-*lac* left end proximal to *glm*S produces a diagnostic 1.6 Kb HpaI+EcoRI band, and a 7.2 Kb HindIII+EcoRI band. MiniTn7-*lac* in the pOX38*gen* donor site generates a 3.2 Kb HpaI+EcoRI band, and a 4.0 Kb HindIII+EcoRI band. The plasmid supplying transposition functions (pCW4) was not expected to hybridize with the L31-60 oligonucleotide probe; however pCW4-dependent 3 Kb HpaI+EcoRI and 10 Kb HindIII+EcoRI bands were observed which apparently result from nonspecific hybridization to the high copy plasmid DNA.

Lanes A1, A2: Chromosomal DNA from a liquid culture of NLC28 (A1 and A2) shows no hybridization to the L31-60 oligo.

Lanes B1, B2: Chromosomal DNA from a liquid culture of NLC28 pOX38*gen*::*mini*Tn7-*lac* displays a 4.0 Kb HindIII+EcoRI band in lane B1 and 3.2 Kb HpaI+EcoRI band in B2 - diagnostic of miniTn7-*lac* on pOX38*gen* and no insertion in *att*Tn7.

Lanes C1 and C2: Chromosomal DNA from a liquid culture of NLC28 pCW4 (*tns*ABCDE) pOX38*gen*::*mini*Tn7-*lac*, whose single cell progenitor did not contain *att*Tn7::miniTn7-*lac*, was digested with HindIII+EcoRI in lane C1 and HpaI+EcoRI in C2. A 1.2 Kb HpaI+EcoRI band in C2 is the result of miniTn7-*lac* transposition to *att*Tn7 in the standard orientation (conferring a Lac$^-$ phenotype) in a small percentage (1-10%) of the cells. pCW4 hybridizes with the L31-60 oligo probe, and exhibits a 10 Kb HindIII+EcoRI band in C1, and a 3.0 Kb HpaI+EcoRI band in C2.

Lanes D1 and D2: Chromosomal DNA from Lac$^-$ cells which exhibit a "Slow Papillation" phenotype (NLC28 *att*Tn7::miniTn7-*lac* pCW4 (*tns*ABCDE) pOX38*gen*::*mini*Tn7-*lac* - see Figure 3-5 and text) exhibit the 1.2 Kb HpaI+EcoRI band indicative of miniTn7-*lac* insertion in *att*Tn7 in the usual Lac$^-$ orientation (with the element's left end adjacent to *pho*S). In these Lac$^-$ cells, we also see the 3.2 Kb HpaI+EcoRI band and the 4.0 Kb HindIII+EcoRI band diagnostic of miniTn7-*lac* in the pOX38*gen* donor site.

Lanes E1 and E2: Chromosomal DNA from Lac$^+$ papillae from "Slow Papillation" colonies (NLC28 *att*Tn7::miniTn7-*lac* pCW4 (*tns*ABCDE) pOX38*gen*::*mini*Tn7-*lac* X::miniTn7-*lac* - see Figure 3-5 and text) exhibit both the 1.2 Kb HpaI+EcoRI band indicative of miniTn7-*lac* insertion in *att*Tn7 in the usual Lac$^-$ orientation, and an additional band (indicated at 2.8 Kb HpaI+EcoRI in lane E2) indicative of a another insertion in an expressed location producing the Lac$^+$ papillae.

Lanes F1 and F2: Chromosomal DNA from Lac$^+$ papillae from "Fast Papillation" colonies (NLC28 *att*Tn7::miniTn7-*lac*(opposite orientation) pCW4 (*tns*ABCDE) pOX38*gen*::*mini*Tn7-*lac* - see Figure 3-5 and text) exhibit the 1.6 Kb HpaI+EcoRI band and a 7.2 Kb HindIII+EcoRI band consistent with miniTn7-*lac* insertion into *att*Tn7 with the element's left end adjacent to *glm*S. MiniTn7-*lac* in the pOX38*gen* donor site again generates the 3.2 Kb HpaI+EcoRI band (Lane F2), and 4.0 Kb HindIII+EcoRI band (Lane F1).

Colony Papillation Type Determined by State of *att*Tn7 in the Single Cell Progenitor.

In the previous section, we showed that opposite orientation miniTn7-*lac* insertion into *att*Tn7 can occur and that different phenotypes result: standard orientation insertion results in a Lac$^-$ phenotype, whereas opposite orientation insertion results in a Lac$^+$ phenotype. Here, we examine the papillation phenotype of cells which begin with a vacant *att*Tn7 site, and follow miniTn7-*lac* transposition from an F' donor site.

Three Papillation Phenotypes Types Are Observed For MiniTn7-*lac* Transposition to *att*Tn7.

In the absence of *tns* genes necessary to promote transposition to *att*Tn7 (*tns*ABC+D), no effect of a vacant *att*Tn7 site is observed on miniTn7-*lac* papillation. Thus, papillation in the presence of miniTn7-*lac*, a vacant *att*Tn7 site, and either no *tns* genes, only *tns*ABC, or *tns*ABCE, results in papillation phenotypes similar to those described in Chapter 2 for a blocked *att*Tn7 site (data not shown).

By contrast in the presence of an available *att*Tn7 transposition target, and *tns* functions sufficient to promote transposition to *att*Tn7, miniTn7-*lac* papillation results in three papillation phenotypes types : Slow Papillation , Entirely Lac$^+$, and Fast Papillation colony phenotypes (see Figures 3-4 and 3-5). The three different papillation types reflect the state of *att*Tn7 in the plated progenitor cell.

A "Slow Papillation" Colony Type Results from MiniTn7-*lac* Transposition to *att*Tn7 in Lac- (Standard Orientation) Before Colony Formation Begins.

If, before plating, miniTn7-*lac* transposes to *att*Tn7 in the usual Lac$^-$ orientation, the colony starts Lac$^-$, and Lac$^+$ papillae slowly arise at a rate similar to that described in the previous chapter for papillation in the presence of a blocked *att*Tn7 site (Case 1,

Figure 3-5). We observed that the Slow Papillation colony type constitutes approximately 7% of the total colonies in these papillation experiments (see Table 3-1). Southern analysis of chromosomal DNA purified from Lac$^-$ cells of these Slow Papillation colonies is shown in Panel D of Figure 3-3. We see that in the Lac$^-$ colony background, miniTn7-*lac* resides in both the Lac$^-$ pOX38*gen* donor site (3.2 Kb HpaI+EcoRI band in Lane D2 and the 4.0 Kb HindIII+EcoRI band in Lane D1), and the standard, Lac$^-$, orientation in *att*Tn7 (1.2 Kb HpaI+EcoRI band in Lane D2).

Southern analysis of chromosomal DNA purified from Lac$^+$ papillae that arise on the Slow Papillation colonies (Lanes E1 and E2 of Figure 3-3), shows that these Lac$^+$ papillae are the result of a second miniTn7-*lac* transposition event. Panel E shows that miniTn7-*lac* in the Lac$^+$ papillae on the Slow Papillation colonies is at three locations: the Lac$^-$ donor site (3.2 Kb HpaI+EcoRI band in Lane E2 and the 4.0 Kb HindIII+EcoRI band in Lane E1), the Lac$^-$ insertion in standard orientation in *att*Tn7 (1.2 Kb HpaI+EcoRI band in Lane E2), and a Lac$^+$ insertion at a third site (2.8 Kb HpaI+EcoRI band in Lane E2), which is presumably transcribed

A Lac+ (Red) Colony Type is the Result of MiniTn7-*lac* Transposition to *att*Tn7 in Lac+ (Opposite Orientation) Before Colony Formation Begins.

If miniTn7-*lac* transposes to *att*Tn7 in the Lac$^+$ (opposite orientation) before plating, then the entire colony is Lac$^+$ and further Lac$^+$ papillation is impossible (large red colony in Figure 3-4 and Case 2, Figure 3-5). Though subsequent transposition of miniTn7-*lac* is expected to occur, because entire colony is Lac$^+$, transposition of miniTn7-*lac* as reported by Lac$^+$ papillation is hidden. This colony class is very rare ($\approx$1/1000 of the total number of colonies - see next section on Frequency of Opposite Orientation Insertion). In these entirely Lac$^+$ colonies, we expect miniTn7-*lac* resides in both the Lac$^-$ pOX38*gen* donor site, and the Lac$^+$ (opposite) orientation in *att*Tn7. Although we did not examine chromosomal DNA from "entirely red" colonies, as shown

above, we have determined that certain Lac$^+$ cells do contain opposite orientation insertions of miniTn7-*lac* in *att*Tn7.

A "Fast Papillation" Colony Type is the Result of MiniTn7-*lac* No Transposition to *att*Tn7 Before Colony Formation Begins.

If miniTn7-*lac* has not transposed to *att*Tn7 by the time the colony progenitor is plated, a high rate of papillation ensues producing the Fast Papillation colony type (Figure 3-4 and Case 3, Figure 3-5 and Panel B, Figure 3-5). Papillae appear on the Fast Papillation colony type at a much greater rate than the rate of papillation observed in the presence of a blocked *att*Tn7 site (see Chapter 2, Figure 2-5B and compare to Figure 3-4). The Fast Papillation colony type constitutes the majority (≈93%) of the colonies plated in these vacant *att*Tn7 papillation experiments. Because rate of papillation in the presence of *tns*ABC+D and a vacant *att*Tn7 site is dramatically higher than papillation in presence of a blocked *att*Tn7 site and either *tns*ABCDE, *tns*ABC+D, or *tns*ABC+E (see Chapter 2, Figure 2-5A, B, or C and compare to Figure 3-4), we expect that most of the Lac$^+$ papillae on the Fast Papillation colony type contain miniTn7-*lac* the Lac$^-$ F' donor site and the opposite (Lac$^+$) orientation in *att*Tn7. Southern analysis of the Lac$^+$ papillae in these quickly papillating colonies indeed demonstrated that Lac$^+$ papillae can result from opposite (Lac$^+$) orientation insertion in *att*Tn7 (Figure 3-3, Panel F). We see in that miniTn7-*lac* in the Lac$^+$ papillae resides at both the Lac$^-$ pOX38*gen* donor site (3.2 Kb HpaI+EcoRI band in Lane F2 and the 4.0 Kb HindIII+EcoRI band in Lane F1), and in the Lac$^+$ opposite orientation in *att*Tn7 (as indicated by the 1.6 Kb HpaI+EcoRI band in Lane F1, and a 7.2 Kb HindIII+EcoRI band in Lane F2). We expect that small percentage the Lac$^+$ papillae in these Fast Papillation cells are the result of transposition to some non-*att*Tn7 location, from which *lac* is expressed (analogous to papillation in the presence of a blocked *att*Tn7 site).

Most of the Lac$^-$ cells from the Fast Papillation colony type in the colonies contain miniTn7-*lac* in only the Lac$^-$ F' donor site (i.e. no transposition has occurred - see Figure 3-3, Lanes C1 and C2). However, approximately 7% of the cells should contain miniTn7-*lac* in both the Lac$^-$ F' donor site and the standard Lac$^-$ orientation in *att*Tn7 (i.e. transposition to *att*Tn7 in the standard orientation has occurred). Figure 3-3 shows that miniTn7-*lac* in a culture of the cells from the Lac$^-$ colony background resides unexpressed in the pOX38*gen* donor site (3.2 Kb HpaI+EcoRI band in Lane C2 and the 4.0 Kb HindIII+EcoRI band in Lane C1), and a small percentage of the Lac$^-$ background also contains a standard orientation (Lac$^-$) insertion in *att*Tn7 (indicated by the weak 1.2 Kb HpaI+EcoRI band in Lane C2 and seen as Slow Papillation sectors on the Fast Papillating colonies in Figure 3-4).

A small fraction of the papillae on the Fast Papillation colony type may result from a second miniTn7-*lac* transposition event after *att*Tn7 is occupied by miniTn7-*lac* in the standard, Lac$^-$ orientation in the first transposition event from the pOX38*gen*::miniTn7-*lac* donor site. This situation is equivalent to the papillae observed on the Slow Papillation colony type (see above).

"Circulo de Feugo" - Ring of Papillation in Fast Papillation Colonies

Fast Papillation colonies appear to contain a ring of very high papillation activity (see Figure 3-4). Outside of this ring of papillation, very little papillation occurs. Papillation does occur inside the ring of papillation, though greatly reduced in comparison to the papillation ring. This papillation ring may not be *tns*ABC+D specific in that faint evidence of papillation rings has been observed in strains carrying only *tns*ABC+E (see Figure 3-6), however ring patterns in these strains are far less striking. In Figure 3-6, the papillation of a*dam*-13 mutant carrying only *tns*ABC+E appears to generate rings of papillation. The lack of striking rings generated by *tns*ABC+E promoted papillation may be due to the far lower rate of papillation in the the absence of

transposition to *att*Tn7, or may in fact reflect a *tns*ABC+D biased phenomenon. It is possible that the papillation rings observed here may be indicative of regulation of Tn7 transposition in response to the cellular environment (see discussion in Chapter 5).

Figure 3-4
Colony Papillation with *tns*ABC+D
and a Vacant *att*Tn7 Site

NLC28 pCW4::miniMuΩ^{76E} (*tns*ABC+D) pOX38*gen*::miniTn7-*lac*

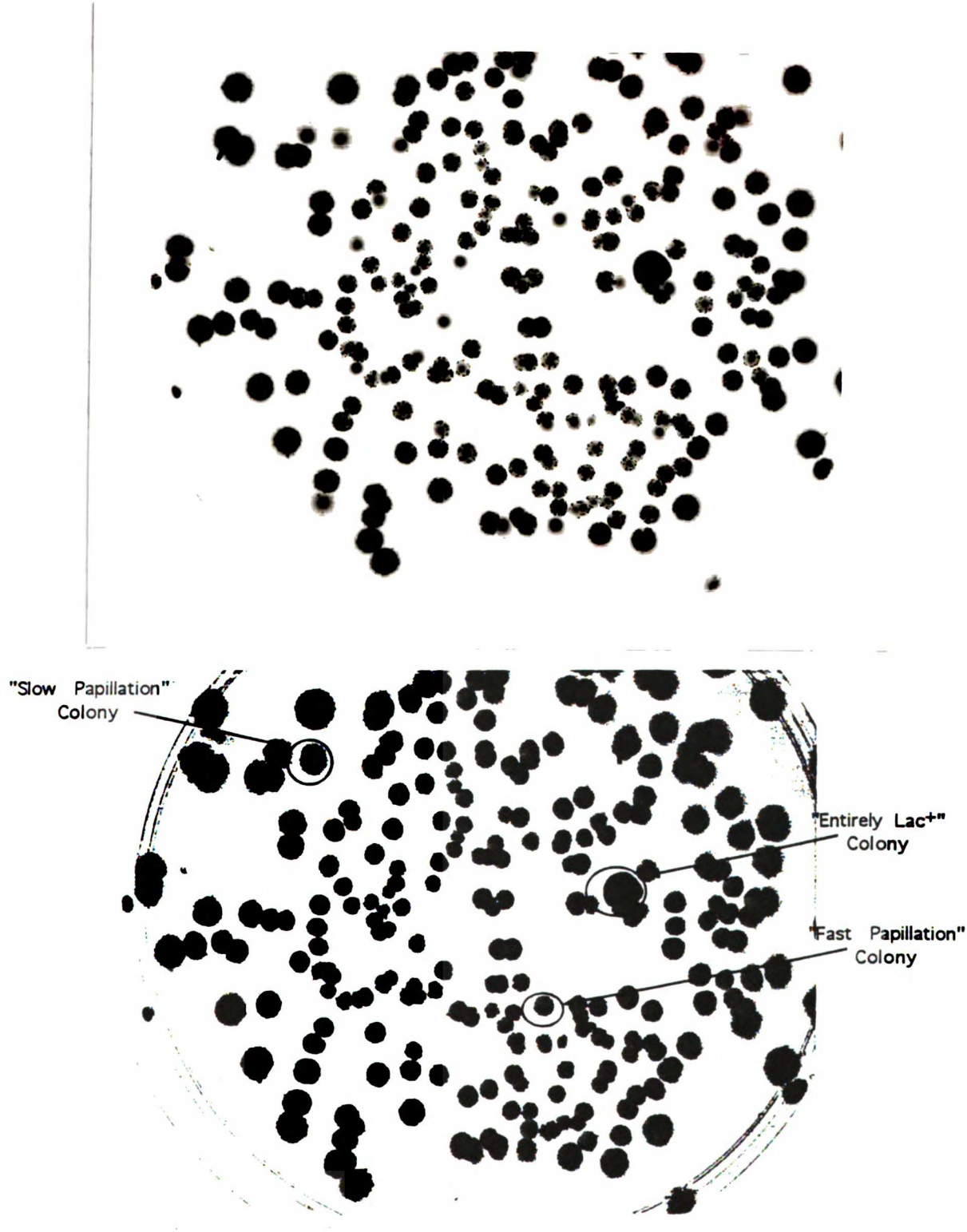


Figure 3-4 Legend

Colony Papillation with *tns*ABC+D and a Vacant *att*Tn7 Site

Colony papillation of NLC28 pCW4::miniMuΩ^{76E} (*tns*ABC+D) pOX38*gen*::miniTn7-*lac* in which *att*Tn7 begins vacant, and *tns* functions sufficient to promote transposition to *att*Tn7 are present, generates three basic colony papillation types - Slow Papillation colonies, Entirely Lac$^+$ colonies, and Fast Papillation colonies (as indicated on photocopy below photograph). After growing colonies on LB + tetracycline for 8 hours, colonies were used to inoculate liquid cultures in LB + tetracycline. After 5 hours of shaking incubation at 37°C, liquid cultures were diluted (10^{-4}) in LB and plated on MacConkey Lactose indicator plates supplemented with 20 ug/ml tetracycline. Plates were incubated 12 hours at 37°C and 72 hours at 30°C before photographing.

Figure 3-5

State of *att*Tn7 Determines Colony Papillation

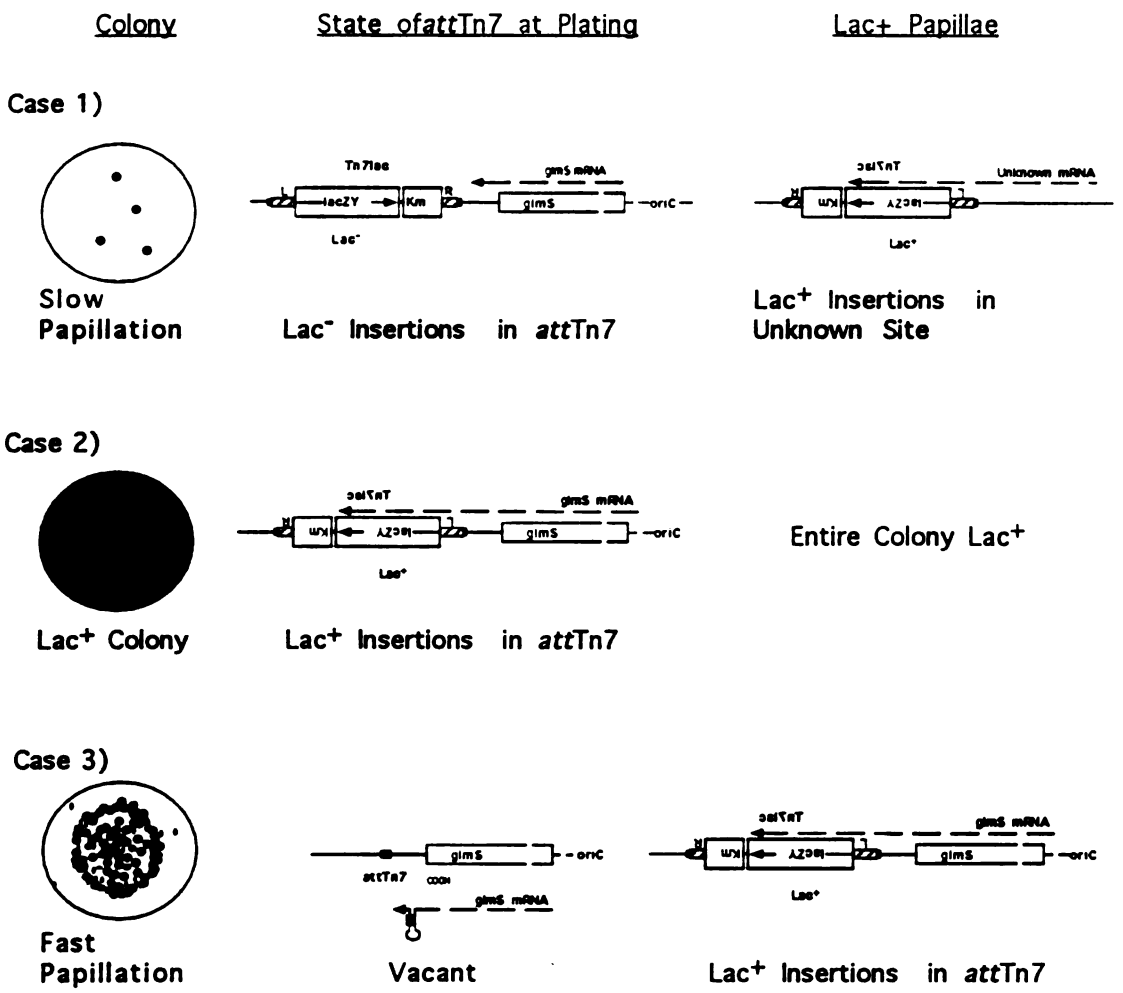


Figure 3-5 Legend

State of *att*Tn7 Determines Colony Papillation

Colony papillation of strains in which *att*Tn7 begins vacant, and *tns* functions sufficient to promote transposition to *att*Tn7 are present, generates three colony papillation types - Slow Papillation (Case 1), Entirely Lac$^+$ colonies (Case 2), and Fast Papillation (Case 3) for papillation of miniTn7-*lac* from a phenotypically Lac$^-$ donor site on an F' (pOX38*gen*::miniTn7-*lac*). The different colony types report the state of the *att*Tn7 in the plated colony progenitor.

Case 1: *att*Tn7 Filled by MiniTn7-*lac* in Lac- Orientation.

Case 1 reflects miniTn7-*lac* transposition to *att*Tn7 in the Lac$^-$ (standard orientation) before the colony progenitor was plated. Lac$^+$ papillae on these colonies are the result of miniTn7-*lac* transposition to some other location, from which *lac* is expressed.

Case 2: *att*Tn7 Filled by MiniTn7-*lac* in Lac+ Orientation.

Case 2 depicts the result of miniTn7-*lac* transposition to *att*Tn7 in the Lac$^+$ (opposite orientation) before the colony progenitor was plated. The entire colony is Lac$^+$, and thus further miniTn7-*lac* transposition can-not be reported by papillation.

Case 3: *att*Tn7 Vacant.

Case 3 depicts the result of no transposition to *att*Tn7 in the colony progenitor. *att*Tn7 is vacant in most of the Lac$^-$ portion of the colony (as depicted), and occupied in the Lac$^-$/standard orientation in a small portion of the cells (shown only for case 1). The Lac$^+$ papillae in these Fast Papillation are also a mix of mostly miniTn7-*lac* transposition to *att*Tn7 in the Lac$^+$/opposite orientation (as depicted), and a small percentage are the result of transposition to some other location, from which *lac* is expressed (shown only for case 1).

Figure 3-6
Colony Papillation via *tns*ABC+E May Form Rings

NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107E}

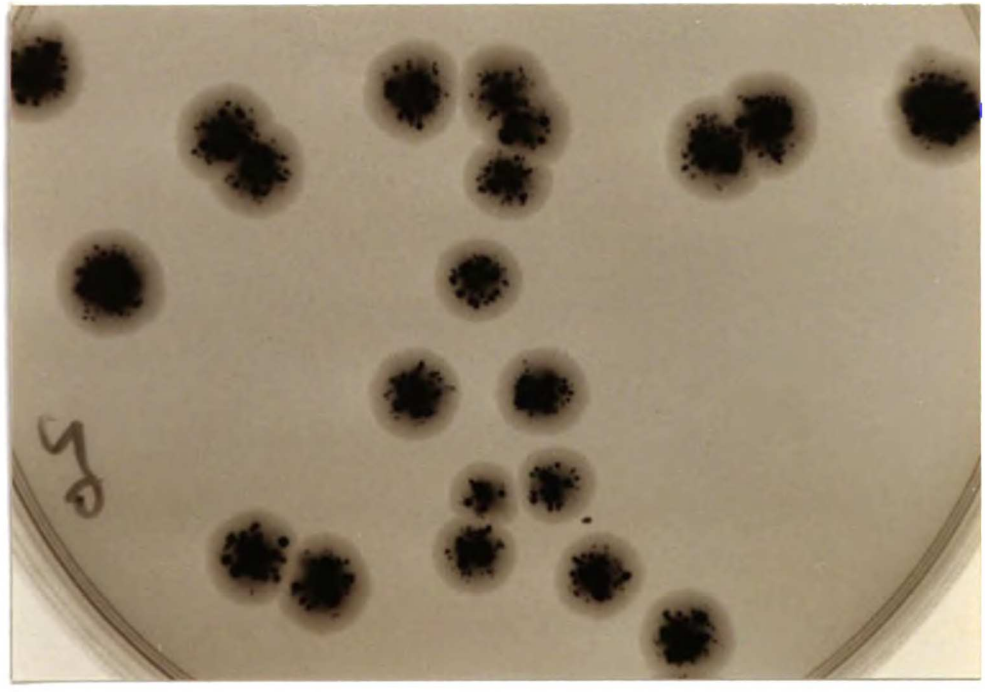


Figure 3-6 Legend

Colony Papillation via *tns*ABC+E May Form Rings

Lac$^+$ papillation of NLC28 *att*Tn7::miniTn7-*lac* *dam*-13 pCW4::miniMuΩ^{107D} (*tns*ABC+E). MiniTn7-*lac* transposes from *att*Tn7 via *tns*ABC+E to non-specific target sites generating colonies with a high degree of papillation. NLC28 *att*Tn7::miniTn7-*lac* *dam*-13 pCW4::miniMuΩ^{107D} was grown overnight in LB + tetracycline (20 ug/ml). Culture was diluted in LB and plated MacConkey Lactose indicator plates without antibiotics. Photograph taken after incubation at 37°C for 72 hours.

Frequency of Opposite Orientation Insertion in *att*Tn7 is Approximately 1% of the Frequency of Standard Orientation Insertion.

Genetic and physical analysis of miniTn7-*lac* insertions in *att*Tn7 suggest that opposite (Lac$^+$; left end adjacent to *glm*S) orientation insertion occurs at a frequency of approximately 1% that of standard (Lac$^-$; right end adjacent to *glm*S) orientation insertion

Comparison of the Frequency of Slow Papillation vs. Entirely Red Colony Types:

The relative frequency of miniTn7-*lac* standard (right end adjacent to *glm*S) vs. opposite (left end adjacent to *glm*S) insertion in *att*Tn7 is reflected in the relative frequency of two colony types. As described above, early transposition to *att*Tn7 in the standard orientation with miniTn7-*lac* right end adjacent to *glm*S results in a Slow Papillation colony type. Early transposition to *att*Tn7 in the opposite orientation with miniTn7-*lac* left end adjacent to *glm*S results in entirely Lac$^+$ colonies. The relative frequency of transposition in the two orientations can be measured by the ratio of entirely Lac$^+$ to Slow Papillation colony types.

Frequency of Slow Papillation Colony Type (Standard Orientation Insertion in *att*Tn7):

From four independent trials, examining a total of 6,529 colonies from two early log-phase cultures and 8,863 colonies from two late stationary-phase cultures of NLC28 pOX38*gen*::miniTn7-*lac* pCW4::miniMuΩ^{76E} (*tns*ABC+D), we observed that quickly papillating colonies (colonies in which *att*Tn7 was empty when the colony progenitor was plated) constitute the ≈93% of colonies (see Table 3-1). Approximately 7% of the total colonies plated are the slowly papillating colonies (colonies in which miniTn7-*lac* transposed to *att*Tn7 in the standard orientation before the colony progenitor was plated). A figure for miniTn7-*lac* transposition to the chromosomal *att*Tn7 of 7% (occupancy of chromosomal *att*Tn7 per cell) agrees well with the observed frequency of 1.2×10^{-2} (insertions in *att*Tn7 per F') for miniTn7-*lac* transposition to pOX38*gen*::*att*Tn7 as

measured by mating out (see Chapter 2, Table 3-3) and with the 1-10% occupancy of chromosomal *att*Tn7 as measured by Southern analysis (see Figure 3-3, Lane C2).

Frequency of Entirely Lac+ Colony Type (Opposite Orientation Insertion in *att*Tn7):

Entirely Lac$^+$ colonies occur at a much lower frequency than the Slow Papillation colony type. Four independent screenings for Lac$^+$ colonies were made with the same strain used above. Of the 6,529 colonies from two independent early log-phase cultures of NLC28 pOX38*gen*::miniTn7-*lac* pCW4::miniMuΩ^{76E} (*tns*ABC+D), 7 colonies were found to be of the Entirely Lac$^+$ colony type (Table 3-1). This means that 1.4% of the insertions in *att*Tn7 were in the opposite (Lac$^+$) orientation (7 Entirely Lac$^+$ divided by 489 Slow Papillation Colony types - Table 3-1). A 1.4% frequency of opposite orientation insertion may however be an overestimate if the resulting Lac$^+$ phenotype gives the this population a comparative growth advantage to the rest of the cells in the early log-phase cultures.

To test whether the 1.4% frequency of opposite orientation insertion in *att*Tn7 is an overestimate due to growth advantage of the Lac$^+$ cells, we examined the frequency of Entirely Lac$^+$ colonies from two independent late stationary-phase cultures of NLC28 pOX38*gen*::miniTn7-*lac* pCW4::miniMuΩ^{76E} (*tns*ABC+D) were we would expect that a growth advantage difference to have an exaggerated effect. We found that of 8,863 colonies examined, 638 were of the Slow Papillation colony type and 11 were of the Entirely Lac$^+$ colony type (Table 3-1). This translates to a 1.7% frequency of opposite (Lac$^+$) orientation insertion in *att*Tn7 (11 Entirely Lac$^+$ divided by 638 Slow Papillation Colony types - Table 3-1). Thus it seems unlikely that the 1.4% frequency of opposite orientation insertion in *att*Tn7 is a gross overestimate due to growth advantage of the Lac$^+$ cells.

Physical analysis of the occupancy of the chromosomal *att*Tn7 site in a culture that begins with *att*Tn7 vacant indicates that in agreement with the above genetic analysis

of the frequencies of standard and opposite orientation insertions in *att*Tn7, opposite orientation insertion occurs at a frequency significantly below standard orientation insertion. Figure 3-3, lane C shows a low percentage (approximately 1-10%) occupancy of *att*Tn7 by miniTn7-*lac* in the usual (left end adjacent to *pho*S) orientation. However, no opposite orientation insertion is seen. From the above genetic observations we would expect the band intensity from opposite orientation insertion to be about 1% the of the signal from standard orientation insertion, and only 0.1% of the donor site band intensity. Thus it is not surprising that bands indicative of opposite orientation are not observed in the Panel C of Figure 3-3.

Table 3-1

Frequency of Opposite Orientation Insertion of MiniTn7-*lac* in *att*Tn7

A)	**Entirely Lac$^+$**	**Slow Papillation**	**Total Number of Colonies**
Colonies From a Culture in Early Log-Phase Growth:			
Trial #1	4	218	3349
Trial #2	3	271	3180
Totals	7	489	3529
Colony Type as % of Total Colonies	0.1%	**7.5%**	
Entirely Lac$^+$ as % of Slow Papillation Colony Type		**1.4%**	

B)	**Entirely Lac$^+$**	**Slow Papillation**	**Total Number of Colonies**
Colonies From a Culture in Stationary-Phase Growth:			
Trial #1	5	357	4080
Trial #2	6	281	4783
Totals	11	638	8863
Colony Type as % of Total Colonies	0.1%	**7.2%**	
Entirely Lac$^+$ as % of Slow Papillation Colony Type		**1.7%**	

Table 3-1 Legend

Frequency of three basic colony papillation types - Slow Papillation colonies, Entirely Lac$^+$ colonies, and Fast Papillation generated by NLC28 pCW4::miniMuΩ^{76D} (*tns*ABC+D) as if Figure 3-4. After growing colonies on LB + tetracycline for 8 hours, colonies were used to inoculate liquid cultures in LB + tetracycline. After 5 hours (Panel A for data on colonies from a culture in early log-phase growth) or after 12 hours (Panel B for data on colonies from a culture in stationary-phase growth) of shaking incubation at 37°C, liquid cultures were diluted (10^{-4} Panel A and 10^{-6} Panel B) in LB and plated on MacConkey Lactose indicator plates supplemented with 20 ug/ml tetracycline. Plates were incubated 12 hours at 37°C and counted for Entirely Lac$^+$ colonies, the further incubated 72 hours at 30°C before counting Slow Papillation colonies.

Discussion

Previous studies of Tn7's *tns*ABC+D promoted transposition to the bacterial chromosome at *att*Tn7 and to *att*Tn7 sites cloned on plasmids have found that insertion is orientation specific: Tn7 inserts into *att*Tn7 with the left end of Tn7 proximal to *pho*S and the right end adjacent to the TnsD binding sequences of *att*Tn7 in *glm*S (Gay et al., 1986; Hauer and Shapiro, 1984; Lichtenstein and Brenner, 1981; Lichtenstein and Brenner, 1982; Rogers et al., 1986; Gringauz et al., 1988; McKown et al., 1988; Quadri et al., 1989). The extent of insertion orientation specificity was unknown, however no opposite orientation insertions in E.*coli*'s *att*Tn7 had been observed among roughly one hundred *att*Tn7 insertions in E. *coli* examined (Gay et al., 1986; Gringauz et al., 1988; Hauer and Shapiro, 1984; Lichtenstein, 1982; Lichtenstein and Brenner, 1981; McKown et al., 1988). The work described in this chapter has directly demonstrated that miniTn7-*lac* can transpose to the chromosomal *att*Tn7 site with either miniTn7-*lac*'s right or left end adjacent to *glm*S.

Unidirectional transcription across *att*Tn7 from *glm*S, and unidirectional transcription of *lac* in from the left end of miniTn7-*lac* combine to form a sensitive assay of miniTn7-*lac* orientation in *att*Tn7. Transposition of miniTn7-*lac* to *att*Tn7 with it's right end adjacent to *glm*S results in a Lac^- phenotype, while insertion with the left end of miniTn7-*lac* adjacent to *glm*S results in a Lac^+ phenotype.

Why has opposite orientation insertion of Tn7 not been previously observed? The answer most likely lies in the low frequency of opposite orientation insertion, combined with the inability heretofore to bias observation to those insertions likely to be in the opposite orientation. MiniTn7-*lac* presents the opportunity to screen for or select opposite orientation insertion by means of their Lac^+ phenotype.

In addition to exposing the ability of miniTn7-*lac* to insert in *att*Tn7 in the opposite orientation, this work allows us to estimate the frequency of opposite orientation insertion relative to standard orientation insertion. We found that opposite orientation

insertion must occur at a frequency significantly below that of the usual right end adjacent to *glm*S insertion frequency. Entirely Lac$^+$ colonies (the result of early transposition of miniTn7-*lac* to *att*Tn7 in the opposite orientation) occur much less frequently than the slowly papillating colony class that are the result of early transposition of miniTn7-*lac* to *att*Tn7 in the standard orientation. Additionally previous analysis of approximately 25 insertions in chromosomal *att*Tn7, and possibly 100 insertions in *att*Tn7 sites cloned into plasmid, saw no opposite orientation insertions (Gringauz et al., 1988; McKown et al., 1988). We also observed that opposite orientation insertion (resulting in "Fast Papillation") occurs more frequently than transposition to pseudo-*att*Tn7 and random sites (comparing Chapter 2 Figure 2-5 to Figure 3-4 of this chapter).

One issue that needs to be addressed is the possibility that miniTn7-*lac* itself has lost some of the orientation specificity of Tn7. The *cis*-acting right end of miniTn7-*lac* consists of only 70 base pairs of the right end of Tn7 and thus is missing one of the four right end TnsB binding sites (see Chapter 2). The R70 end of miniTn7-*lac* is of concern because previous studies have shown that though miniTn7 elements containing R70 can transpose, their frequency of transposition is reduced and the ability to confer transposition immunity to the replicon in which they reside is reduced (Arciszewska et al., 1989). However, the ability demonstrated here of miniTn7-*lac* to transpose to *att*Tn7 in either orientation, *in vivo*, has recently been reflected, *in vitro*, by Tn7 elements with all four TnsB binding sites (Bainton and Craig, personal communication). Transposition of Tn7 elements with full right ends, *in vitro*, appears to result in insertion of the transposon left end adjacent to *glm*S TnsD binding sequences at 1-5% the frequency as right end adjacent to *glm*S TnsD recognition sequences. Further investigation of the orientation specificity *in vivo* of Tn7 elements with complete ends will be necessary to resolve this question.

Orientation specificity is an important component to the understanding of Tn7 transposition. The mechanism of transposition does not involve homologous pairing between the transposon and target and thus has no DNA inherent mechanism of determining relative orientation. The ability of Tn7 to transpose to *att*Tn7 with high orientation specificity implies that Tn7 insertion site orientation relative to the Tn7 ends is distinguished.

Tn7 transposition to *att*Tn7 involves the conjunction of two non-equivalent transposon ends, an asymmetric target site, and four transposon-encoded proteins. The synaptic complex built of these asymmetric components must form a structure competent to perform transposition. If the synaptic complex was functional in only one form, opposite orientation transposition would be mechanistically excluded. This work has shown that at a low frequency miniTn7-*lac* does insert in *att*Tn7 with its left end adjacent to *glm*S, and thus must be allowed by the mechanism of transposition.

Given that the frequency of opposite orientation insertion into *att*Tn7 is 1% of the frequency of standard orientation insertion, we can estimate the energy barrier between achieving productive synaptic complexes for the two different orientations if the transposition reaction does not utilize external energy (ATP). This may be a valid assumption since Tn7 transposition can be accomplished *in vitro* in the presence of non-hydrolizable analogs of ATP (Bainton et al., 1993). The energy barrier, or difference in the free energy of activation ($\Delta\Delta G^{\dagger}$), between opposite orientation (left end adjacent to *glm*S) verses standard orientation insertion (right end adjacent to *glm*S) can be expressed as:

$$\Delta\Delta G^{\dagger} = -RT\ln\frac{\text{Frequency of Opposite Orientation Insertion}}{\text{Frequency of Standard Orientation Insertion}}$$

$$= -RT\ln(0.01)$$

$$= -(1.987)(310)\ln(0.01)$$

$$= 2.8 \text{ to } 4.2 \text{ kcal/mole} \qquad \text{at } 37^{\circ}\text{C}$$

Thus the energy barrier between the two insertion orientation reaction paths is surprisingly low, the equivalent of one or two hydrogen bonds. A low energy difference between insertion orientation paths constrains models for the structure of the synaptic complex.

The limited models of Tn7's synaptic complex that we can build with the current information are based on the known roles of the Tns proteins, and the structure of the DNA segments involved (ends and target), and analogies with other transposons. TnsA, TnsB, TnsC and TnsD are directly involved in transposition of Tn7 to *att*Tn7 (Bainton et al., 1993). Previous work has shown that TnsD binds specifically to *att*Tn7, and through interaction with TnsC, localizes TnsC (which is a non-specific DNA binding protein) to *att*Tn7 (Bainton et al., 1993). TnsB binds three sites in the left end and four sites in the right end of the transposon (McKown et al., 1987; Arciszewska and Craig, 1991; Arciszewska et al., 1991; Tang et al., 1991). Interactions between TnsB and TnsD+C, perhaps though TnsA, bring the transposon ends and *att*Tn7 together in a nearly orientation specific manner.

If we assume that for low energy differences, specific protein-DNA interactions cannot invert orientation on non-palindromic DNA binding sites, then inversion of transposition orientation must be due to an alteration of protein/protein interactions. Orientation preference must lie in either the energy differences between two protein-protein interactions or a relative mechanistic inefficiency of one of the transpososome forms.

A path describing the three points at which orientation discrimination could occur can be formalized:

Ends Recognition and Synapsis => Ends-Target Synapsis => Strand Transfer

The first two steps where orientation discrimination may occur are protein-protein interactions and the last one is an enzymatic differentiation. The potential protein-protein

interactions which distinguish transposon end orientation relative to the target are: 1) the interactions of TnsB (+ TnsA?) to bring the ends together in a synaptic complex, and 2) the interactions of synaptic complex with TnsD+C on *att*Tn7. In both cases, an asymmetric TnsB (+ TnsA?) synaptic complex is likely formed since TnsB is distributed asymmetrically on the left and right ends of Tn7.

TnsB binds three sequence repeats in the left end of Tn7 separated by 30 and 40 bases. TnsB binds four head-to-tail sequence repeats in Tn7's right end. It seems likely that in the synaptic complex, the superstructure of each end will be specific and different. If orientation specificity is lost in recognition of the ends during formation of the synaptic complex, the confusion will be manifest though formation of inappropriate superstructures.

Transposition orientation may also be lost through the inappropriate interaction of an appropriately formed synaptic complex. For orientation to be lost in communication of the synaptic complex with the target, the synaptic complex must have sufficiently similar surfaces that inversion results in an energy penalty of only 2.8 kcal, and the inverted structure is still transposition competent.

Orientation specificity may also come through enzymatic differentiation rather than protein-protein interaction differentiation. Upon strand transfer, insertion orientation is determined. If the transposition complex is more "active" - better able to perform strand transfer in one orientation than the other, insertion orientation bias would result. An asymmetric transposition complex which holds the transposon terminal hydroxyl closer to the target phosphate in one orientation than the other would be an example of orientation bias for strand transfer.

The actual mechanism by which Tn7's transposition orientation bias is generated is not yet clear. It seems remarkable that transposition is possible in an opposite orientation. The identification of mutants affecting transposition orientation will aid our understanding of transposition. Orientation mutants will provide clue's to how the

synaptic complex is formed and indicate how transposition orientation is maintained. MiniTn7-*lac* is specifically suited to the identification of transposition mutants, and miniTn7-*lac*'s ability to report transposition orientation can be gainfully employed for the identification of transposition orientation mutants.

Chapter 4: Effects of DAM, FIS, IHF and H-NS Mutations on Tn7

Abstract:

To examine the potential involvement of E. *coli* components in Tn7 transposition, we have analyzed Tn7 transposition in the presence of mutations in genes encoding proteins known to affect other bacterial transposons: DAM (DNA adenine methylase), and the histone-like proteins FIS (Factor for Inversion Stimulation), H-NS (Histone-like protein H1), and IHF (Integration Host Factor). We found that miniTn7-*lac* transposition is increased in strains containing *dam*-13, a loss of function allele. Similar stimulation of transposition by *dam* inactivation has been observed for other bacterial transposons (Tn10 and Tn5) which, like Tn7, move *via* a conservative transposition mechanism. Work on Tn10 suggests DAM methylation links transposition to DNA replication through DAM sites (GATC) in the transposase binding *cis*-acting transposon end sequences. We speculate that DAM methylation may play a similar role in the control of miniTn7-*lac* transposition. We also observed that miniTn7-*lac* transposition is generally decreased by null alleles of *fis*, *hns*, and *hip* or *him*A (the genes encoding the subunits of IHF), suggesting that these proteins may also participate in Tn7 transposition.

Introduction

The goal of the experiments described in this chapter was to examine the effects on Tn7 transposition of mutations in some of the genes known to affect other transposons and recombination systems. This work specifically documents effects of loss-of-function alleles of the gene encoding the DAM methylase, and the genes encoding histone-like proteins (FIS, H-NS, and IHF) on the transposition of Tn7. In general, transposition involves the synapsis of two transposon ends and an insertion site. Synapses of these three DNA segments is followed by strand transfer recombination which, in the case of conservative transposition, removes both transposon DNA strands from the donor site, leaving a double-strand break in the donor molecule. Transposon-encoded transposase generally performs these functions; however, host factors often participate in and regulate the process of transposition (for reviews see Berg and Howe, 1989; and Kleckner, 1990 - specific cases referenced below).

DNA Adenine Methylation

The bacterial DNA adenine methylation (DAM) system is one of the best understood examples of host regulation of transposition. Transposition of IS10, IS903, and IS50 (Roberts et al., 1985; Yin et al., 1988) is linked to DNA replication through DAM.

E. *coli* employs DNA adenine methylation to distinguish newly synthesized DNA (Bale et al., 1979; Campbell and Kleckner, 1990; reviewed in Barras and Marinus, 1989). The product of the *dam* gene, DNA adenine methyltransferase, methylates the N-6 position of adenine on both strands of the duplex sequence GATC. The control of replication origins (Campbell and Kleckner, 1990), replication mismatch repair bias (Grilley et al., 1989; Lahue and Modrich, 1988; Lahue et al., 1987), and gene expression (Braun and Wright, 1986; Seiler et al., 1986; Plumbridge and Soll, 1987; Plumbridge,

1987; Bolker and Kahmann, 1989) all use DNA adenine methylation as an indicator of newly synthesized DNA. After semiconservative replication, the DNA is hemimethylated, with the newly synthesized strand not yet methylated.

DAM regulation is thought to be an important method of linking conservative transposition to replication (Roberts et al., 1985). Hemimethylated DNA generally persists after replication fork passage for less than five minutes, about 10% of the cell cycle (Campbell and Kleckner, 1990). Although conservative transposition moves both strands of the donor element into the target site by transposing after replication fork passage, conservative transposons can effectively leave a copy at the donor site. Activation of only one of the hemimethylated transposons after replication fork passage acts to ensure that an intact copy of the donor replicon with a copy of the transposon in the donor site remains. As described above, conservative transposition leaves a double-strand break at the donor site. The intact arm of the replication fork may be also be used to direct double-strand gap repair of the arm in which transposition has left a double stranded break. Tn7, Tc1 and P element transposition have been shown to stimulate double strand gap repair at the donor site (Hagemann and Craig, 1993; Plasterk and Groen, 1992; Engels et al., 1990). Failure to repair the donor molecule after replication fork passage will result in loss of just one arm of the replication fork rather than loss of the entire replicon.

An illustrative example of DAM regulation of transposition is seen with IS10. DAM has been shown to regulate IS10 transposition by controlling both expression and activity of transposase (Roberts et al., 1985). DNA adenine methyltransferase acts at two GATC sequences in IS10. One DAM site is near the outside end in the -10 region of the pIN transposase promoter. DAM methylation at this site decreases transposase expression by decreasing pIN transcription. The other DAM site lies within the inside end transposase binding site. DAM methylation of the transposase binding site presumably decreases transposase activity by inhibiting end binding (Roberts et al.,

1985). In *dam*⁻ bacterial strains, IS10 transposition activity increases about 100-fold. IS50 transposition is regulated by DAM in the same way as IS10, with DAM sites repressing transposase transcription (McCommas and Syvanen, 1988; Yin et al., 1988) and blocking transposase end binding activity (Dodson and Berg, 1991; Makris et al., 1988).

Activation of transposition after replication by hemimethylation also has an important role in horizontal transfer. Horizontal entry of a DAM-regulated transposon by conjugal transfer of a single DNA strand, followed by complementary strand synthesis, results in a burst of transposition activity from the hemimethylated duplex DNA. A similar burst of transposition is seen from undermethylated phage infection (McCommas and Syvanen, 1988).

We report here that miniTn7-*lac* transposition is increased in strains containing *dam*-13, a loss of function allele. *dam*-13 was found to stimulate miniTn7-*lac* papillation and both miniTn7-*lac* and Tn7 transposition as measured with the mating-out assay. Continuing work by Bob DeBoy in Dr. Craig's lab has not been able to reproduce the increase of Tn7 transposition as measured by mating-out, though it has reproduced the effect on miniTn7-*lac* papillation (Bob DeBoy and N. Craig, personal communication). Due to this inability to reproduce the mating-out results, the significance of these results is unclear.

Histone-Like Proteins

The work described in this chapter also examines the possible involvement of the histone-like proteins FIS, H-NS, and IHF in Tn7 transposition. Histone-like proteins are small, basic, usually heat-stable, DNA-binding proteins that are highly conserved between prokaryotes and eukaryotes at the primary sequence level (Drlica and Rouviere, 1987; Pettijohn, 1988). Histone-like proteins wrap or bend DNA to form higher-order structures (Broyles and Pettijohn, 1986; Rouviere-Yaniv et al., 1979; Moitoso de Vargas

et al., 1989; Snyder et al., 1989). A synaptic transposition complex is a highly ordered structure, with three DNA segments held in close proximity, and in an appropriate fashion to promote the strand transfer recombination. Histone-like proteins are known to be involved in the transposition of Mu, IS1, γδ, Tn5, and Tn10 (Berg and Howe, 1989). Histone-like proteins have been shown to bind and bend transposon DNA to facilitate transposase binding, and possibly synaptic complex formation (Surette et al., 1987; Wiater and Grindley, 1988; Surette et al., 1989; Hubner et al., 1989; Lavoie and Chaconas, 1990). We will examine mutations in the genes encoding three histone-like proteins, FIS, H-NS, and IHF, for affects on Tn7 transposition.

FIS

Factor for inversion stimulation (FIS) is a small, heat-stable DNA-binding protein. Though FIS is a sequence-specific DNA-binding protein, the sequence determinants of a FIS-binding site are difficult to define due to the lack of an obvious consensus sequence (Hubner and Arber, 1989; Finkel and Johnson, 1992). When bound to DNA, FIS has been shown to induce a substantial bend (40-90°) (Thompson and Landy, 1988; Gille et al., 1991). Like the histone-like proteins, FIS is thought to facilitate the assembly of DNA-protein complexes through bending DNA. Though FIS has many of the physical characteristics of histone-like proteins, it is often considered separately because it is unrelated at the primary sequence level.

FIS was identified by its ability to stimulate Hin and Gin site-specific DNA inversion reactions (Johnson et al., 1986; Johnson and Simon, 1985; Kahmann et al., 1985). FIS was later shown also to be involved Cin, Pin and phage λ site-specific DNA recombination reactions (Ball and Johnson, 1991a; Thompson et al., 1987). FIS stimulates phage λ excision over 200-fold. FIS bound adjacent to *att* directly enhances Xis binding and may alter *att* topology to favor excision (Ball and Johnson, 1991a; Thompson et al., 1987). FIS is also involved in the regulation of Tn5. FIS stimulates

Tn5 transposition approximately 10-fold during exponential E. *coli* growth, and can inhibit IS50 transposition (Weinreich and Reznikoff, 1992).

HNS

H-NS is a small, moderately abundant, DNA-binding, histone-like protein. H-NS is thought to bind preferentially to bent DNA, stabilizing the bend (Yamada et al., 1991). H-NS is formed as a homo-dimer. H-NS mutants have pleiotropic effects on gene expression and DNA supercoiling, reflected in the number of names by which it is known: H1, *pro*U, *osm*Z, *pil*G, *bgl*Y, *vir*R and *drd*X (Hulton et al., 1990; Owen-Hughes et al., 1992).

hns mutants affect transposition of Mu. Mu transposition in *hns*⁻ mutants is elevated (Falconi et al., 1991). Purified H-NS stabilizes Mu repressor-DNA complexes *in vitro*, suggesting that *in vivo*, H-NS contributes to the Mu transposition repression via Mu repressor (Gama et al., 1992).

IHF

Integration host factor (IHF) was identified through the isolation of E. *coli* mutants that fail to support phage λ site-specific recombination (Kikuchi and Nash, 1978; Miller et al., 1979). IHF is know to bind sequence-specifically to DNA, inducing a bend. Many recombination systems have since been shown to utilize IHF. IHF is composed of two non-equivalent protein subunits, both small and heat stable, encoded by *him*A and *hip* (Mechulam et al., 1985; Miller et al., 1984).

IHF has been shown to be involved in the transposition of IS1, γδ, Tn5, IS10 and Mu (Gamas et al., 1987; Gamas et al., 1985; Morisato and Kleckner, 1987; Krause and Higgins, 1986; Surette and Chaconas, 1989; Surette et al., 1989; Wiater and Grindley, 1988; Wiater and Grindley, 1990a; Makris et al., 1990). IHF binding to the transposon ends has been show to cooperatively facilitate transposase binding for γδ (Wiater and

Grindley, 1988). IHF is required for IS10 outside end activity *in vivo* and *in vitro* (Morisato and Kleckner, 1987; Roberts et al., 1987; Huisman et al., 1989; Kleckner, 1989), and as with γδ, IHF binds next to the transposase binding site. Efficient Mu transposition *in vitro* at *in vivo* levels of supercoiling requires IHF as well as HU (see Chapter 1). IHF's participation is mediated through an IHF binding site in the Mu early promoter region (Goosen and van de Putte, 1984; Goosen et al., 1984). IHF binding generates a specific geometrical configuration, which includes a sharp bend in the DNA, and is required for optimal induction of synapsis of the Mu transposon ends (Surette and Chaconas, 1989; Surette et al., 1989).

Regulation of Transposition by Histone-like Proteins:

In addition to direct facilitation of transpososome formation, regulation of the levels of the histone-like proteins can act to regulate transposition. Through host-regulated expression of the histone-like proteins, transposition can by constrained to certain growth conditions during which the host histone-like proteins are available. FIS is highly expressed only in early exponential growth, and so can link recombination to growth (Ball and Johnson, 1991a; Ball and Johnson, 1991b; Thompson et al., 1987). With this burst of FIS expression, a FIS dependent increase in Tn5 transposition frequency has been observed in early exponential growth of Tn5's host (Weinreich and Reznikoff, 1992).

The histone-like proteins examined in this chapter have also been implicated in the regulation of transposon-encoded gene expression. Binding of IHF between O1 and O2 in the Mu operator dramatically alters the balance between divergently transcribed P_E and P_C expression (Goosen and van de Putte, 1984; Krause and Higgins, 1986). IHF mutants have been shown to reduce transcription from the Mu early promoter (Krause and Higgins, 1986). IHF has also been implicated in multicopy inhibition control of IS10, via transposase promoter pIN (Kleckner, 1989). H-NS is known to regulate many

host genes in respond to environmental cues, though its regulation of transposition has not been well-examined.

Tn7

When the work described in this chapter was begun, no host components of Tn7 transposition had yet been defined. The object of this work was to test for the involvement of DAM, FIS, H-NS, and IHF in the transposition of Tn7 through *in vivo* analysis of transposition in the presence of mutations in the genes encoding these proteins.

Results:

DAM: DNA Adenine Methylation

We found that a loss-of-function mutation in the host E. *coli dam* gene can stimulate Tn7 transposition as demonstrated by evaluation in isogenic *dam*$^+$ and *dam*$^-$ strains of the rate of miniTn7-*lac* papillation and the frequency of intermolecular translocation of miniTn7-*lac* and Tn7 using the mating-out assay.

Inactivation of DAM Can Stimulate MiniTn7-*lac* Transposition.

The effect of DAM on Tn7 transposition was examined using the *dam*-13 allele. *dam*-13 is a loss of DAM function allele generated by Tn9 insertion (Marinus et al., 1983; Parker and Marinus, 1988). *dam* mutation is not expected to significantly affect cell growth (Bale et al., 1979). *dam*-13 had little to no effect on the growth of the strains tested (see Figure 4-2).

Papillation:

*tns*ABC+E promoted miniTn7-*lac* papillation from *att*Tn7 to sequence non-specific targets promoted by a plasmid-borne *tns* source was substantially stimulated in *dam*-13 NLC28 (graphic summary in Figure 4-1, and photographs in Figure 4-2). *tns*ABC+D promoted papillation from the *att*Tn7 donor site to pseudo-*att*Tn7 target sites is decreased in *dam*-13 NLC28. Figure 4-2 shows the papillation of both colonies and patches of *dam*$^+$ and *dam*-13 NLC28 *att*Tn7::miniTn7-*lac* + either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. Note that the difficulty of patching the same number of cells and the likelihood of patching cells initially Lac$^+$ due to transposition prior to patching makes quantitative analysis of patches problematic. The graphic summaries presented in this chapter represent the average number of papillae in colonies.

Mating-Out:

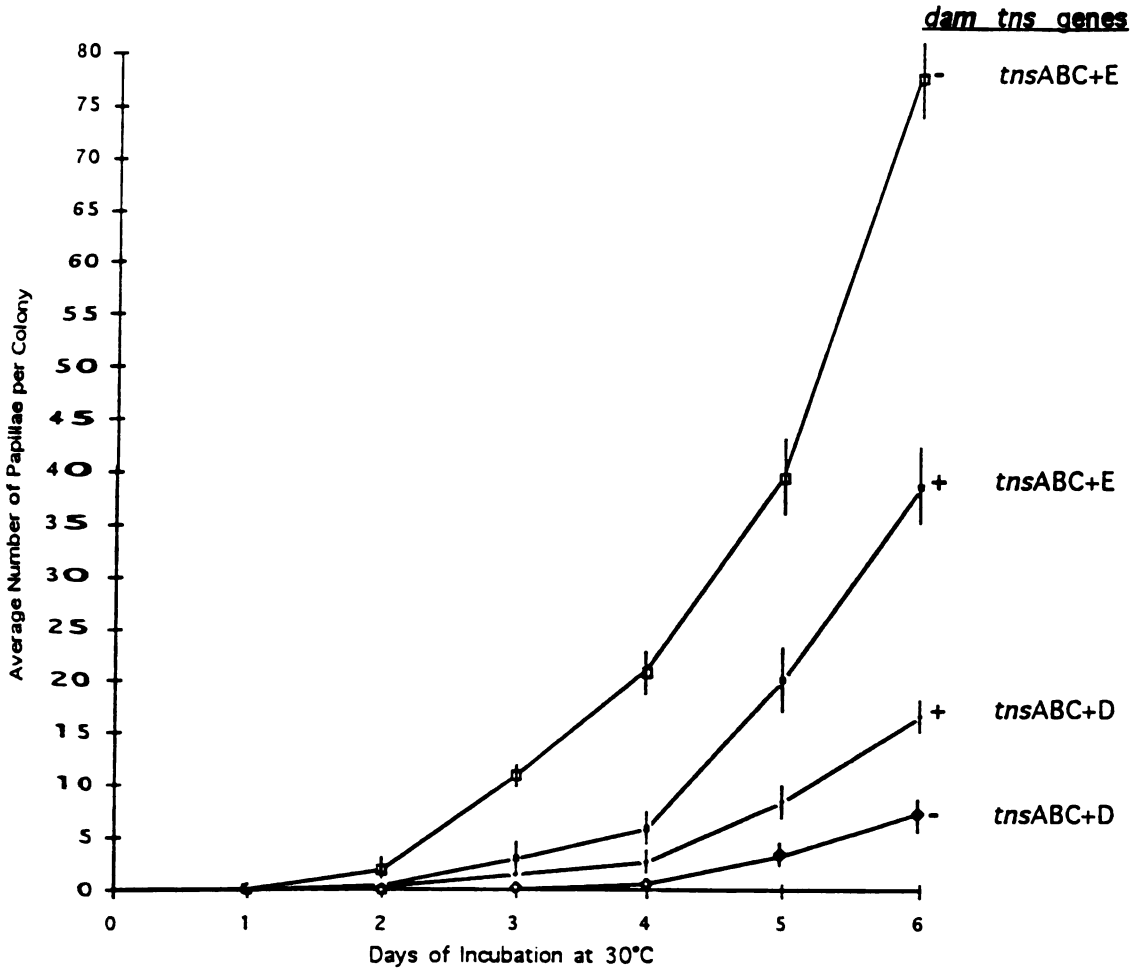
Table 4-1 summarizes the effects of *dam*-13, a loss-of-function allele generated by Tn9 insertion (Marinus et al., 1983; Parker and Marinus, 1988), on *tns*E-dependent transposition from chromosomal *att*Tn7 to the conjugal target plasmid pOX38*gen* in a variety of strain backgrounds in comparison to isogenic *dam*$^+$ strains. Tn7 transposition was consistently increased by *dam* -13, though the magnitude of this effect varied between strain backgrounds. In a MC4100 derivative (NLC28), Tn7 transposition was stimulated about 175-fold, whereas a much more modest stimulation (4-5 fold) was observed in the RZ (from CSH26), and NK strain backgrounds (see Materials and Methods). The particular difference(s) in these strains critical to the dramatic differential effects of *dam* inactivation remains to be established.

These results suggest that a change in GATC methylation state can have an effect on Tn7 transposition, resulting in substantially increased transposition frequency under some conditions, however continuing work by Bob DeBoy in Dr. Craig's lab has not been able to reproduce the increase of Tn7 transposition as measured by mating-out, though has reproduced the effect on miniTn7-*lac* papillation (N. Craig, personal communication). Due to this inability to reproduce the mating-out results, the significance of these results is unclear.

It is also noted that the conjugation based ("mating-out") assay used in this thesis to examine transposition frequencies probably measures both translocation of the transposable element to the conjugal plasmid followed by conjugation of that plasmid to CW51, and a background frequency of F' donor strain mutation to nalidixic acid resistance (see Materials and Methods). Thus, numbers reported as transposition frequencies as measured by the mating-out assay in this thesis may not reflect only transposition events. This caveat is expected to be significant for transposition frequencies below 10^{-7} (Bob DeBoy and Nancy Craig, personal communication).

Figure 4-1

Effect of *dam*-13 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site



(NLC28*att*Tn7::miniTn7-*lac* p*tns*)

Figure 4-1 Legend

The Effect of *dam*-13 on MiniTn7-*lac* Papillation

From an *att*Tn7::miniTn7-*lac* Donor Site

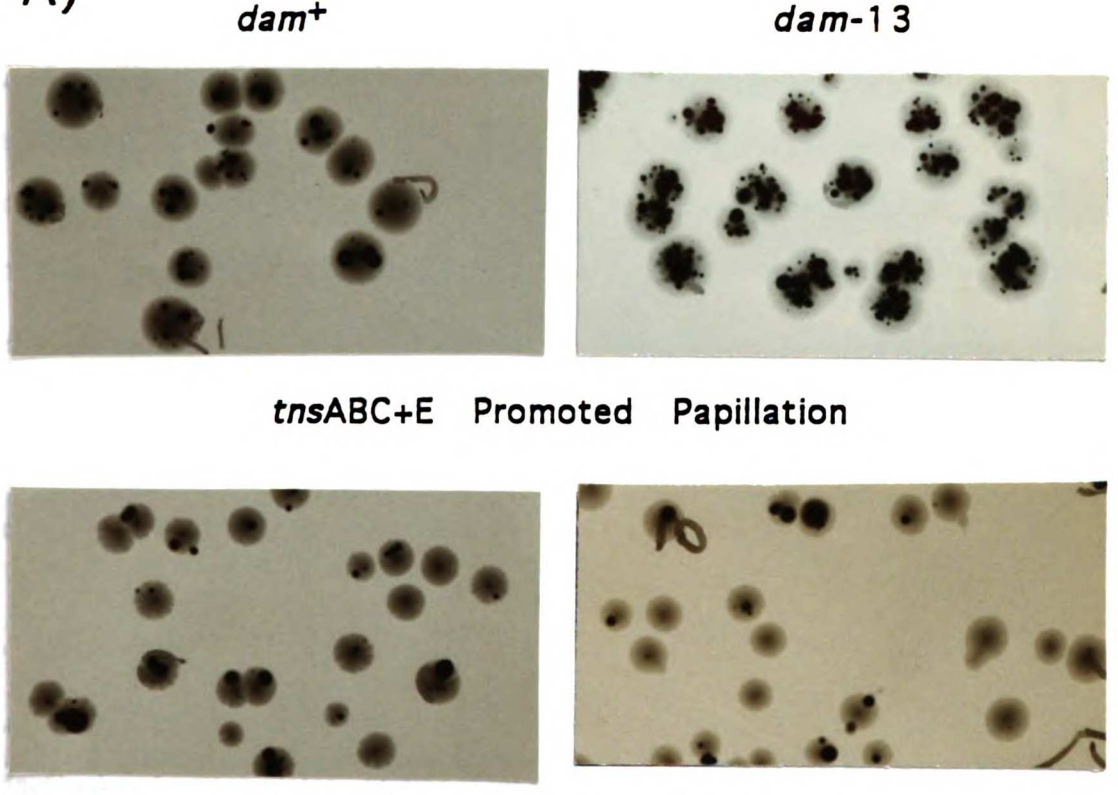
A time course of Lac$^+$ papillation of isogenic *dam*$^+$ and *dam*-13 bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids) on the MacConkey Lactose indicator plates (shown directly in Figure 4-2), was generated by counting Lac$^+$ papillae every 24 hours during incubation at 30°C. *dam*$^+$ and *dam*-13 bacterial cultures were inoculated in LB + 20 ug/ml tetracycline and grown overnight with shaking incubation at 37°C. Liquid cultures were diluted in LB and plated on MacConkey Lactose plates. The *tns* plasmids were maintained by tetracycline at a concentration of 20 ug/ml in the MacConkey agar. The average number of Lac$^+$ papillae on 10 colonies is plotted (each data point) along with the standard deviation of the average number of Lac$^+$ papillae (bar through data points). Data is from simultaneous plating of the indicated strains on the same batch of plates. Lac$^+$ papillae report miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. Status of *dam* and the *tns* genes present is indicated to the right of the day 6 endpoints.

Figure 4-2

The Effect of *dam*-13 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site

NLC28*att*Tn7::miniTn7-*lac* p*tns*

A)



B)

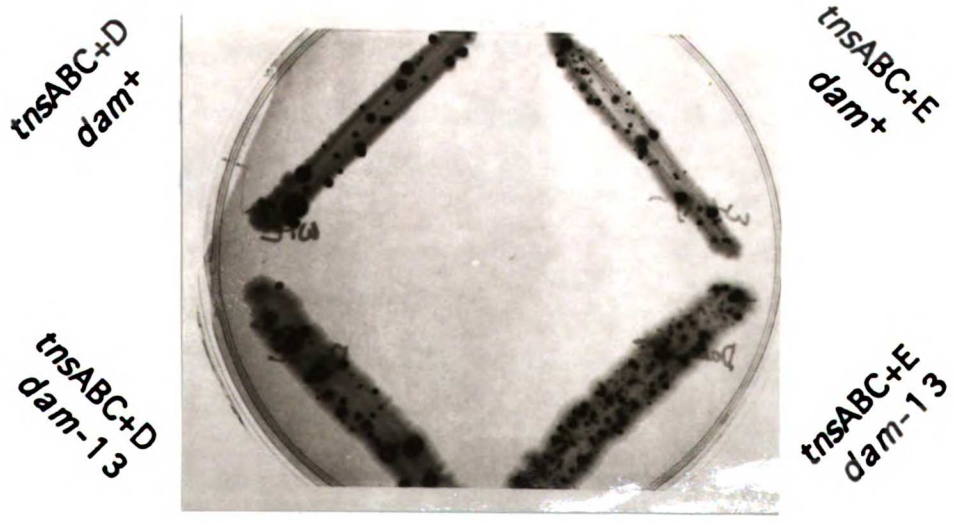


Figure 4-2 Legend

The Effect of *dam*-13 on MiniTn7-*lac* Papillation

From an *att*Tn7::miniTn7-*lac* Donor Site

Panel A:

Lac$^+$ papillation of isogenic *dam*$^+$ and *dam*-13 bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids) on MacConkey Lactose indicator plates was photographed after a 4 day incubation at 30°C. Data is from simultaneous plating of the indicated strains on the same batch of plates, and are the colonies used for Figure 4-1. Lac$^+$ papillae report miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. The *tns* plasmids were maintained by tetracycline at a concentration of 20 ug/ml in the MacConkey agar.

Panel B:

Lac$^+$ papillation of the isogenic *dam*$^+$ and *dam*-13 strains from Panel A are patched on MacConkey Lactose plates. Strains were as indicated above, with *dam*$^+$ at the top of the plate to be compared to the isogenic *dam*-13 strain below it. *tns*ABC+D promoted papillation is presented on the left side of the plate while *tns*ABC+E promoted papillation is presented on the right side of the plate. Patches were photographed after a 4 day incubation at 30°C. MacConkey agar was supplemented with tetracycline at a concentration of 20 ug/ml.

Table 4-1

Tn7 Transposition in *dam*-13 vs. *dam*$^+$

Donor Site	Mobile Element	Transposition Frequency *dam*$^+$	*dam*-13	Ratio *dam*$^-$/*dam*$^+$
NLC28*att*Tn7	Tn7	$2.0 \pm 0.5 \times 10^{-6}$	$3.5 \pm 4.0 \times 10^{-4}$	176
RZ201*att*Tn7	Tn7	$5.1 \pm 3.2 \times 10^{-6}$	$2.3 \pm 2.1 \times 10^{-5}$	4.5
NK7419*att*Tn7	Tn7	$9.0 \pm 1.0 \times 10^{-6}$	$3.5 \pm 0.9 \times 10^{-5}$	3.9

Table 4-1 Legend

Transposition frequency of Tn7 from the chromosomal *att*Tn7 site to pOX38*gen* in isogenic *dam*$^{+/-}$ strains was measured using the mating-out assay. The mean and standard deviation of 5 separate measurements is reported. F$^+$ strain background indicated under the "Donor Site" category. Status of *dam* as indicated, with *dam*-13 being a previously characterized and published loss-of-function allele of *dam* (Marinus et al., 1983; Parker and Marinus, 1988). The F$^-$ recipient strain was CW51. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

Effects of *dam* Mutation When Two Tn7 Elements are Present:

In the experiments described above, the effects of *dam*-13 were evaluated in strains which contained a single Tn7 element, either intact Tn7 or miniTn7-*lac* in *att*Tn7 to which *tns* functions were supplied from a plasmid source. The effect of *dam*-13 was also tested in the two mobile Tn7 elements configuration (see Table 4-2): with miniTn7-*lac* in *att*Tn7 and Tn7 at a second chromosomal location (ϕ80dIII*lac*). Intact Tn7 at ϕ80dIII*lac* supplies *tns* functions to the miniTn7 element. This strain configuration, with two Tn7 elements within the same cell, was used to evaluate the effects of newly generated host mutants (see Chapter 5).

*tns*E-Dependent Transposition:

In contrast to the dramatic stimulation of *tns*E-dependent Tn7 transposition from *att*Tn7 to pOX38*gen* by *dam*-13 (175-fold), only a modest stimulation (3-fold) of Tn7 transposition from ϕ80dIII*lac* was observed with *dam*-13. Conversely, miniTn7-*lac* transposition from *att*Tn7 decreased 3-fold as measured by mating-out and was down by papillation (not shown).

*tns*D-Dependent Transposition:

The effects of *dam* on *tns*D-dependent transposition are different from *dam*'s effect of *tns*E-dependent transposition. *tns*D-dependent transposition to pOX38*gen*-*att*Tn7 was found to be modestly decreased 2-fold by *dam*-13 for intact Tn7 from ϕ80dIII*lac*, and increased 2-fold for miniTn7-*lac* from *att*Tn7.

The reason for the opposite responses of two elements (miniTn7-*lac* and Tn7) in the same cell to *dam*-13 in Table 4-2 remains unresolved. The opposite effect of *dam*-13 on miniTn7-*lac* and Tn7 may reflect competition between two Tn7 element within the same strain, as was seen in Chapter 2 of this thesis. If DAM differentially affects one of

the elements, then it may have the opposite effect of the other transposable element. One possibility is that intact Tn7, which transposes with higher intrinsic activity than miniTn7-*lac* in DAM$^+$ strains, is actually a more effective competitor for *tns* functions under the *dam*$^-$ conditions, leading to the effective decrease in miniTn7-*lac* transposition.

Table 4-2

Tn7 and miniTn7-*lac* Transposition in *dam*$^-$13 vs. *dam*$^+$

Transposition to pOX38*gen*

Donor Site	Mobile Element	Transposition Frequency *dam*$^+$	*dam*-13	Ratio *dam*$^-$/*dam*$^+$
*att*Tn7	miniTn7-*lac*	$3.5 \pm 1.2 \times 10^{-7}$	$9.6 \pm 3.8 \times 10^{-8}$	0.3
ϕ80dIIlac	Tn7	$3.2 \pm 0.6 \times 10^{-6}$	$8.8 \pm 2.9 \times 10^{-6}$	2.8

Transposition to pOX38*gen-attTn7*

Donor Site	Mobile Element	Transposition Frequency *dam*$^+$	*dam*-13	Ratio *dam*$^-$/*dam*$^+$
*att*Tn7	miniTn7-*lac*	$1.6 \pm 1.3 \times 10^{-3}$	$3.0 \pm 2.1 \times 10^{-3}$	1.9
ϕ80dIIlac	Tn7	$4.2 \pm 0.3 \times 10^{-2}$	$2.6 \pm 1.8 \times 10^{-2}$	0.6

Table 4-2 Legend

Transposition frequency in isogenic *dam*$^{+/-}$ strains of miniTn7-*lac* from the chromosomal *att*Tn7 site of NLC28 and Tn7 from a second chromosomal site - ϕ80dII*lac* was measured using the mating-out assay. The mean and standard deviation of 5 separate measurements is reported. Transposition of miniTn7-*lac* is promoted by *tns* genes from Tn7. The F' transposition targets were as indicated. The F$^-$ recipient strain was CW51. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

FIS: Factor for Inversion Stimulation

Evaluation of transposition using miniTn7-*lac* papillation and measurement of intermolecular translocation to a conjugal target plasmid revealed that inactivation of the host E. *coli fis* gene can result in decreased miniTn7-*lac* transposition.

MiniTn7-*lac* Transposition Decreased by *fis* Null Mutation.

Papillation:

MiniTn7-*lac* papillation was evaluated in strains containing either of two different *fis* null alleles *fis*-767 (Johnson et al., 1988) or *fis*-985 (Weinreich and Reznikoff, 1992), which are replacements of nucleotides +68 to +215 of the *fis* coding sequence with Km^R for *fis*-767 and Sp^R for *fis*-985. Though *fis* mutations lack a dramatic growth impairment phenotype, they may show slightly increased lag times when stationary cells are re-inoculated into rich media (K. Hughes and R. Johnson, unpublished), and *fis* mutants are reported to have slower growth in very rich media (Nilsson et al., 1992). *fis*-767 and *fis*-985 had little to no effect on the growth under the conditions tested (see Figure 4-4).

MiniTn7-*lac* papillation from chromosomal *att*Tn7 promoted by *tns*-containing plasmids is reduced by *fis*$^-$ mutation in both *tns*D-dependent and *tns*E-dependent transposition pathways (graphic summary in Figure 4-3, photographs in Figure 4-4). MiniTn7-*lac* papillation from a plasmid donor site (on pOX38*gen*) promoted by *tns*-containing plasmids is also reduced by *fis* mutation (Figure 4-5). In the bacterial strains depicted in Figure 4-5 *att*Tn7 is occupied by a second Tn7 element (miniTn7-KmR199 (Arciszewska, et al., 1989)), so all miniTn7-*lac* papillation events examined here are to either pseudo-*att*Tn7 sites *via tns*ABC+D promoted transposition, or sequence non-specific sites *via tns*ABC+E promoted transposition (Kubo and Craig, 1990).

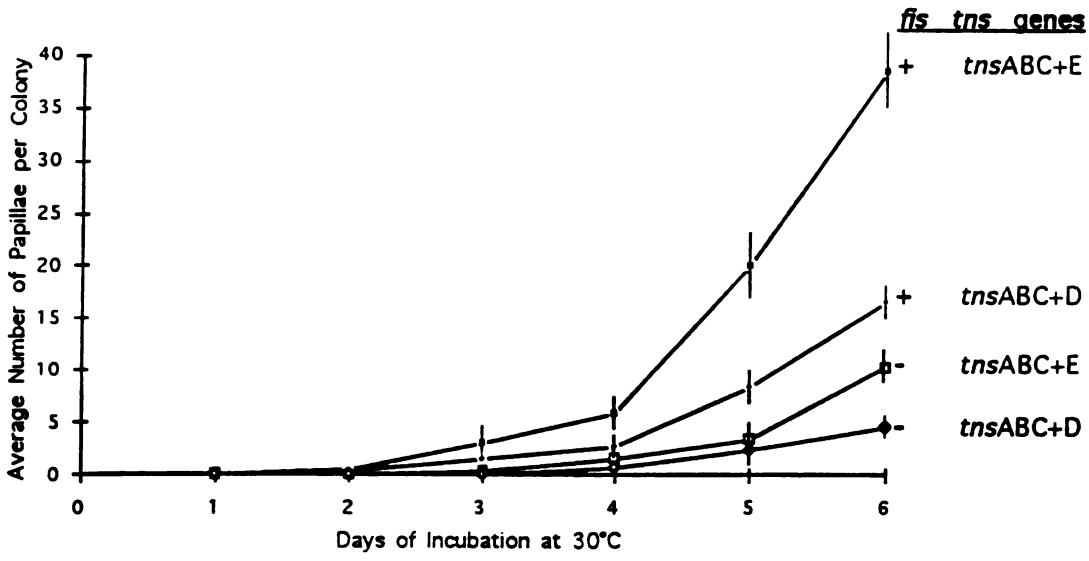
Mating-Out:

Table 4-3 shows that the translocation of miniTn7-*lac* from *att*Tn7 to a conjugal plasmid in *fis* null strains was decreased 10-fold for *tns*D-dependent transposition to pOX38*gen*-*att*Tn7 and 5-fold for *tns*E-dependent events. In these mating-out assays, miniTn7-*lac* was complemented with *tns* genes from a Tn7 element elsewhere in the chromosome (ϕ80dIII*lac*).

These results suggest that the transposition of miniTn7-*lac* is decreased by inactivation of the E.*coli fis* gene.

Figure 4-3

Effect of *fis*-985 on MiniTn*7*-*lac* Papillation From an *att*Tn*7*::miniTn*7*-*lac* Donor Site



(NLC28*att*Tn7::miniTn7-*lac* p*tns*)

Figure 4-3 Legend

The Effect of *fis*-985 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site

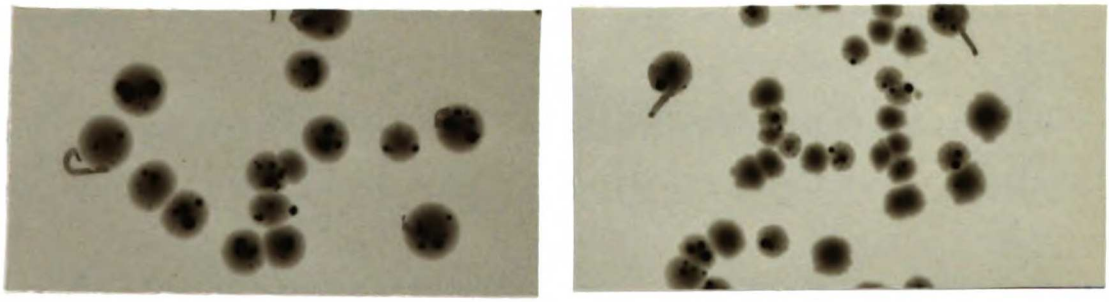
A time course of Lac$^+$ papillation of isogenic *fis*$^+$ and *fis*-985 (Weinreich and Reznikoff, 1992) bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids) on the MacConkey Lactose indicator plates (shown in Figure 4-4), was generated by counting Lac$^+$ papillae every 24 hours during incubation at 30°C. *fis*$^+$ and *fis*-985 bacterial cultures were inoculated in LB + 20 ug/ml tetracycline and grown overnight with shaking incubation at 37°C. Liquid cultures were diluted in LB and plated on MacConkey Lactose plates. The *tns* plasmids were maintained by tetracycline at a concentration of 20 ug/ml in the MacConkey agar. The average number of Lac$^+$ papillae on 10 colonies is plotted (each data point) along with the standard deviation of the average number of Lac$^+$ papillae (bar through data points). Data is from simultaneous plating of the indicated strains on the same batch of plates. Lac$^+$ papillae report miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. Status of *fis* and the *tns* genes present is indicated to the right of the day 6 endpoints.

Figure 4-4

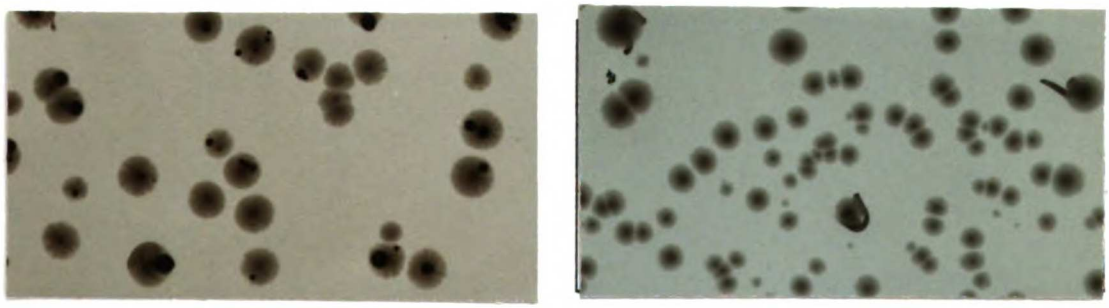
The Effect of *fis*-985 on miniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site

NLC28*att*Tn7::miniTn7-*lac* p*tns*

fis$^+$ *fis*-985



***tns*ABC+E Promoted Papillation**



***tns*ABC+D Promoted Papillation**

Figure 4-4 Legend

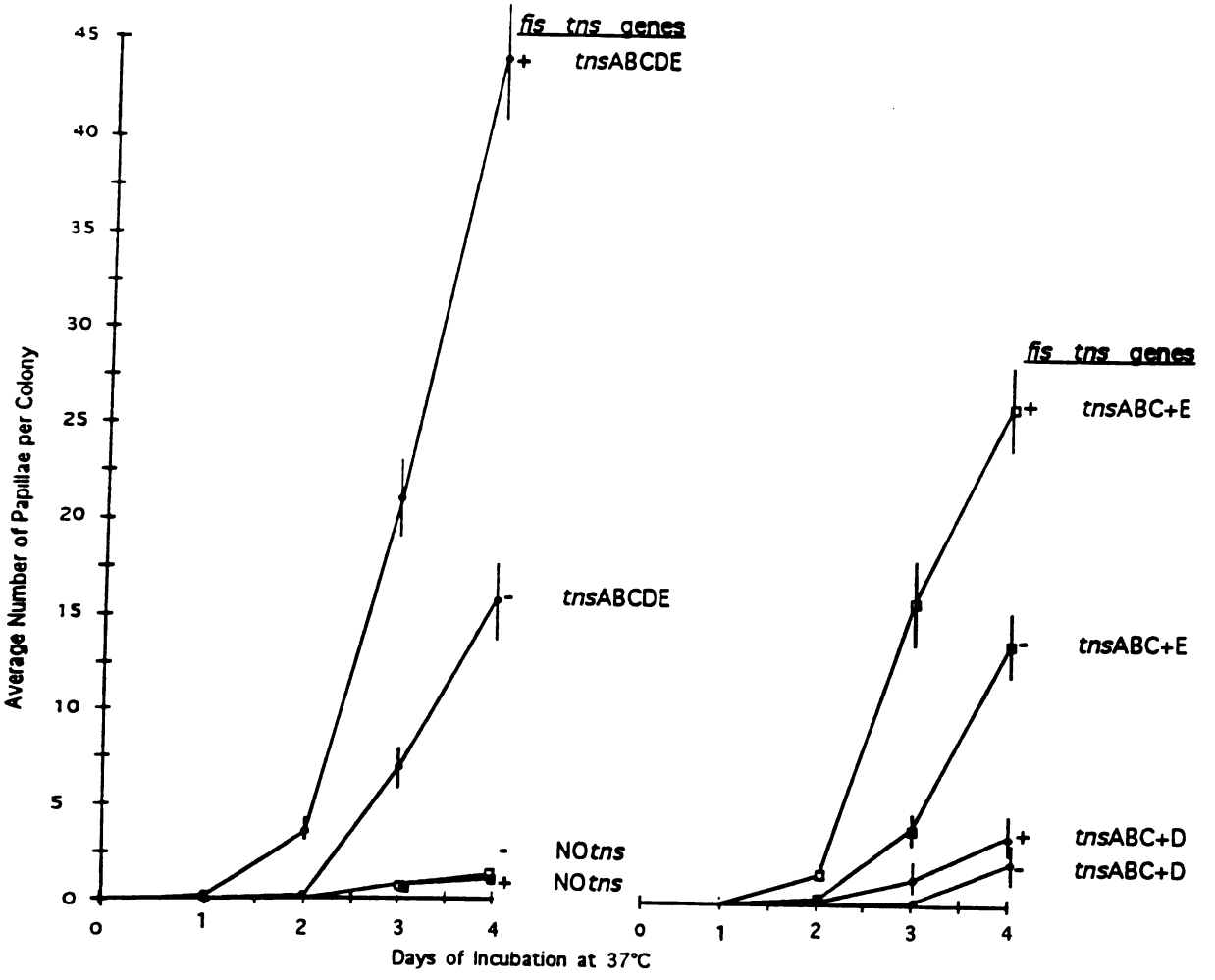
The Effect of *fis*-985 on MiniTn7-*lac* Papillation

From an *att*Tn7::miniTn7-*lac* Donor Site

Lac$^+$ papillation of isogenic *fis*$^+$ and *fis*-985 (Weinreich and Reznikoff, 1992) bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids) on MacConkey Lactose indicator plates, was photographed after a 4 day incubation at 30°C. Data is from simultaneous plating of the indicated strains on the same batch of plates, and are the colonies used for Figure 4-3. Lac$^+$ papillae report miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. The *tns* plasmids were maintained by tetracycline at a concentration of 20 ug/ml in the MacConkey agar.

Figure 4-5

Effect of *fis*-767 on MiniTn*7*-*lac* Papillation From a pOX38*gen*::miniTn*7*-*lac* Donor Site



(NLC28*att*Tn7::miniTn7-Cm pOX38*gen*::miniTn7-*lac* p*tns*)

Figure 4-5 Legend

The Effect of *fis*-767 on MiniTn7-*lac* Papillation

From a pOX38*gen*::miniTn7-*lac* Donor Site

A time course of Lac$^+$ papillation of isogenic *fis*$^+$ and *fis*-767 (Johnson et al., 1988) bacterial colonies (NLC28 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* + *tns* plasmids) on MacConkey Lactose indicator plates was generated by counting Lac$^+$ papillae every 24 hours during incubation at 37°C. The average number of Lac$^+$ papillae on 5-10 colonies is plotted (each data point) along with the standard deviation of the average number of Lac$^+$ papillae (bar through data points). Data is from simultaneous plating of the indicated strains on the same batch of plates. Lac$^+$ papillae report miniTn7-*lac* transposition from an F' (pOX38*gen*) donor site in NLC28, promoted as indicated by *tns*ABCDE from pCW4; *tns*ABC+E from pCW4::miniMuΩ^{107D}; *tns*ABC+D from pCW4::miniMuΩ^{76E}; and "NO *tns*" by pACYC (the plasmid used to generate pCW4). *att*Tn7 was occupied by miniTn7-Cm preventing miniTn7-*lac* transposition to *att*Tn7. Plasmids were maintained by tetracycline at a concentration of 20 ug/ml in the MacConkey agar. Status of *fis* and the *tns* genes present is indicated to the right of each day 4 endpoint.

Table 4-3
Tn7 and miniTn7-*lac* Transposition in *fis*⁻ vs. *fis*⁺

Tn7 and miniTn7-*lac* Transposition to pOX38*gen*

Donor Site	Mobile Element	Transposition Frequency *fis*⁺	*fis*-985	Ratio *fis*⁻/*fis*⁺
NLC28*att*Tn7	miniTn7-*lac*	$1.5 \pm 0.5 \times 10^{-7}$	$2.6 \pm 1.6 \times 10^{-8}$	0.2
ϕ80dIIlac	Tn7	$4.2 \pm 0.3 \times 10^{-6}$	$2.7 \pm 0.6 \times 10^{-6}$	0.6

Tn7 and miniTn7-*lac* Transposition to pOX38*gen*-*att*Tn7

Donor Site	Mobile Element	Transposition Frequency *fis*⁺	*fis*-985	Ratio *fis*⁻/*fis*⁺
NLC28*att*Tn7	miniTn7-*lac*	$1.6 \pm 0.3 \times 10^{-3}$	$1.4 \pm 0.7 \times 10^{-4}$	0.1
ϕ80dIIlac	Tn7	$4.1 \pm 0.4 \times 10^{-2}$	$3.7 \pm 0.6 \times 10^{-2}$	0.9

Table 4-3 Legend

Transposition frequency, in isogenic *fis*$^{+/-}$ strains, of miniTn7-*lac* from the chromosomal *att*Tn7 site of NLC28 and Tn7 from a second chromosomal site (ϕ80dII*lac*) was measured using the mating-out assay. The mean and standard deviation of 5 separate measurements is reported. Transposition of miniTn7-*lac* is promoted by *tns* functions from Tn7. The F' transposition targets were as indicated. Status of *fis* was as indicated with *fis*-985 being a null allele of *fis* generated by replacement of the *fis* gene with a gene conferring spectinomyocin resistance (R. Johnson, personal communication, and Weinreich and Reznikoff, 1992). The F⁻ recipient strain was CW51. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

Inactivation of *fis* has Little Effect on Transposition of Intact Tn7 .

As described above, we observed that miniTn7-*lac* transposition is decreased in strains containing *fis*-null alleles as measured by either papillation or translocation to an F' in the mating-out assay. However we observed little effect of *fis* null mutations (either *fis*-767 or *fis*-985) on the transposition of intact Tn7. As shown in Table 4-3, although miniTn7-*lac* transposition is decreased by *fis*-985, Tn7 transposition is nearly unaffected. As shown in Table 4-4, little effect on Tn7 transposition by *fis*-767 is observed on either *tns*E-dependent transposition to pOX38*gen*, or *tns*D-dependent transposition to pOX38*gen*-*att*Tn7.

One possible explanation for the different effects of *fis* mutation on miniTn7-*lac* vs. intact Tn7 is that the transposition of miniTn7-*lac* may be more sensitive to changes in transposition conditions - specifically TnsB binding to the *cis*-acting ends of the transposon. In Chapter Two of this thesis we saw that miniTn7-*lac* transposition is impaired compared to that of intact Tn7, and this impairment can be overcome when *tns* functions are supplied by a multicopy plasmid (overexpressing the *tns* genes). Thus the observed decrease of miniTn7-*lac* transposition in *fis*$^-$ strains may be a synthetic effect reflecting both the intrinsic fault in of the miniTn7 element and an effect of FIS on recombination (see Discussion).

Table 4-4

Tn7 Transposition in *fis*⁻ vs. *fis*⁺

Tn7 Transposition to pOX38*gen*

Donor Site	Mobile Element	Transposition Frequency *fis*⁺	*fis*-767	Ratio *fis*⁻/*fis*⁺
NLC28*att*Tn7	Tn7	$9.0 \pm 3.8 \times 10^{-6}$	$4.9 \pm 1.7 \times 10^{-6}$	0.5
RJ366*att*Tn7	Tn7	$4.5 \pm 0.5 \times 10^{-6}$	$5.4 \pm 2.5 \times 10^{-6}$	1.2

Tn7 Transposition to pOX38*gen*-*att*Tn7

Donor Site	Mobile Element	Transposition Frequency *fis*⁺	*fis*-767	Ratio *fis*⁻/*fis*⁺
NLC28*att*Tn7	Tn7	$6.4 \pm 1.0 \times 10^{-2}$	$4.2 \pm 1.3 \times 10^{-2}$	0.7

Table 4-4 Legend

Transposition frequency of Tn7 from the chromosomal *att*Tn7 site to pOX38*gen* in isogenic *fis*$^{+/-}$ strains was measured using the mating-out assay. The mean and standard deviation of 5 separate measurements is reported. The F' transposition targets were as indicated. F^+ strain background indicated under the "Donor Site" category. Status of *fis* was as indicated, with *fis*-767 being a previously characterized and published null allele of *fis* (Johnson et al., 1988). The F^- recipient strain was CW51. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

H-NS: Histone-Like Protein H1

hns Null Mutation Can Decrease miniTn7-*lac* and Tn7 Transposition.

Evaluation of Tn7 transposition using miniTn7-*lac* papillation and measurement of intermolecular translocation to a conjugal target plasmid revealed that inactivation of the host E. *coli hns* gene can result in decreased Tn7 transposition.

A Null Allele of *hns* Reduces MiniTn7-*lac* Papillation:

The graphic summary shown in Figure 4-6 and photographs shown in Figure 4-7 demonstrate the reduced papillation observed with *hns*-205 for both *tns*ABC+D and *tns*ABC+E promoted miniTn7-*lac* transposition from *att*Tn7.

hns-205 was originally described as *osm*Z-205 and is a null allele of *hns* generated by Tn10 insertion (Higgins et al., 1988; Hulton et al., 1990). Though *hns* mutations have pleiotropic effects, they lack a dramatic growth impairment phenotype (K. Yasuzawa, 1992). *hns*-205 and *hns*::miniTn10-*tet*^{43} had little effect on the growth of the strains tested (Figures 4-7 and 5-4.43).

*tns*ABC+D promoted miniTn7-*lac* papillation to pseudo-*att*Tn7 sites is very clearly reduced by *hns*-205. *tns*ABC+E promoted miniTn7-*lac* papillation may also be reduced; however, in the *hns*-205 strain, cells spontaneously become mucoid. This mucoidy quickly covers a colony and makes measurement of papillation difficult. The mucoidy is clear and Lac$^+$ papillae can be observed within it, however in the photographs the mucoidy is dark (possibly due to its refractive characteristics) and the underlying papillae are obscured (see Figure 4-7). Data portrayed in Figure 4-6 was generated by counting observable papilla (whether-or-not overlain by mucoidy), a measure that may not accurately reflect the true rate of transposition in a mucoid colony.

It is unknown whether the mucoidy is a direct effect or of *hns*-205. *hns* mutations have not been reported to have a mucoid phenotype, and the *hns* mutation reported in

Chapter 5 does not exhibit a mucoid phenotype (see Chapter 5, Figure 5-4.43). Rare spontaneous mucoidy of presumably *hns*$^+$ NLC28 has been observed, and may be associated with Tn7 transposition from *att*Tn7 affecting neighboring *glm*S (O. Hughes and N. Craig, unpublished observation). It is possible that *hns*-205 increases the frequency of affecting *glm*S upon transposition of miniTn7-*lac* (note that mucoidy occurs more frequently in the *tns*ABC+E promoted papillation where papillation is more frequent - Figure 4-7). To test this hypothesis the experiment portrayed in Figure 4-7 could be done on MacConkey lactose plates supplemented with glucosoimine to bypass *glm*S function.

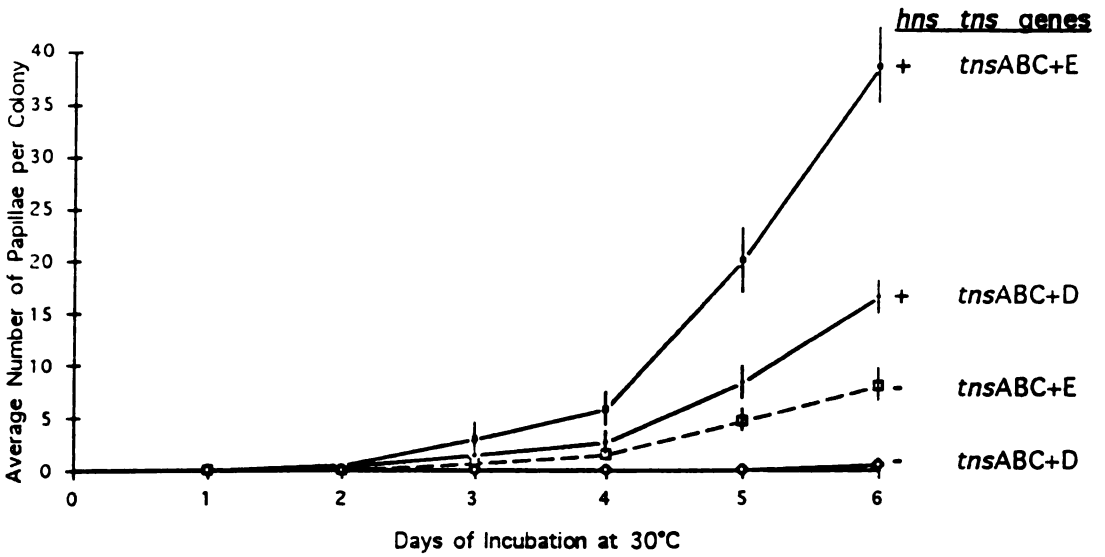
A Null Allele of *hns* Reduces Both Tn7 and MiniTn7-*lac* Translocation:

Reduced miniTn7-*lac* papillation rates in *hns*-205 were again reflected by reduced *tns*ABC+E dependent transposition as measured by the mating-out assay. Table 4-5 shows that transposition to pOX38*gen* by intact Tn7 is reduced 5-fold. When miniTn7-*lac* and intact Tn7 were tested together, a 50-fold reduction of Tn7 transposition was observed while only a 3-fold reduction of miniTn7-*lac* is seen (also in Table 4-5).

These results suggest that H-NS can have a profound effect on Tn7 transposition (especially for *tns*ABC+E promoted transposition), with loss of H-NS substantially decreasing Tn7 transposition.

Figure 4-6

Effect of *hns*-205 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site



NLC28*att*Tn7::miniTn7-*lac* p*tns*

Figure 4-6 Legend

The Effect of *hns*-205 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site

A time course of Lac$^+$ papillation of isogenic *hns*$^+$ and *hns*-205 bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids) on the MacConkey Lactose indicator plates (shown in Figure 4-7), was generated by counting Lac$^+$ papillae every 24 hours during incubation at 30°C. *hns*$^+$ and *hns*-205 bacterial cultures were inoculated in LB + 50 ug/ml spectinomycin and grown overnight with shaking incubation at 37°C. Liquid cultures were diluted in LB and plated on MacConkey Lactose plates. The *tns* plasmids were maintained by spectinomycin at a concentration of 50 ug/ml in the MacConkey agar. The average number of Lac$^+$ papillae on 10 colonies is plotted (each data point) along with the standard deviation of the average number of Lac$^+$ papillae (bar through data points). Data is from simultaneous plating of the indicated strains on the same batch of plates. Lac$^+$ papillae report miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. Status of *hns* and the *tns* genes present is indicated to the right of the day 6 endpoints. Examination of *tns*ABC+E promoted miniTn7-*lac* papillation in the *hns*-205 strain (dashed line) past four days of incubation at 30°C is difficult because some cells become mucoid and overwhelm the colony Due to this mucoidy the validity of the data for papillation promoted by *tns*ABC+E is questionable (see text).

Figure 4-7

The Effect of *hns*-205 on MiniTn*7*-*lac* Papillation From an *att*Tn7::miniTn*7*-*lac* Donor Site

NLC28*att*Tn7::miniTn*7*-*lac* p*tns*

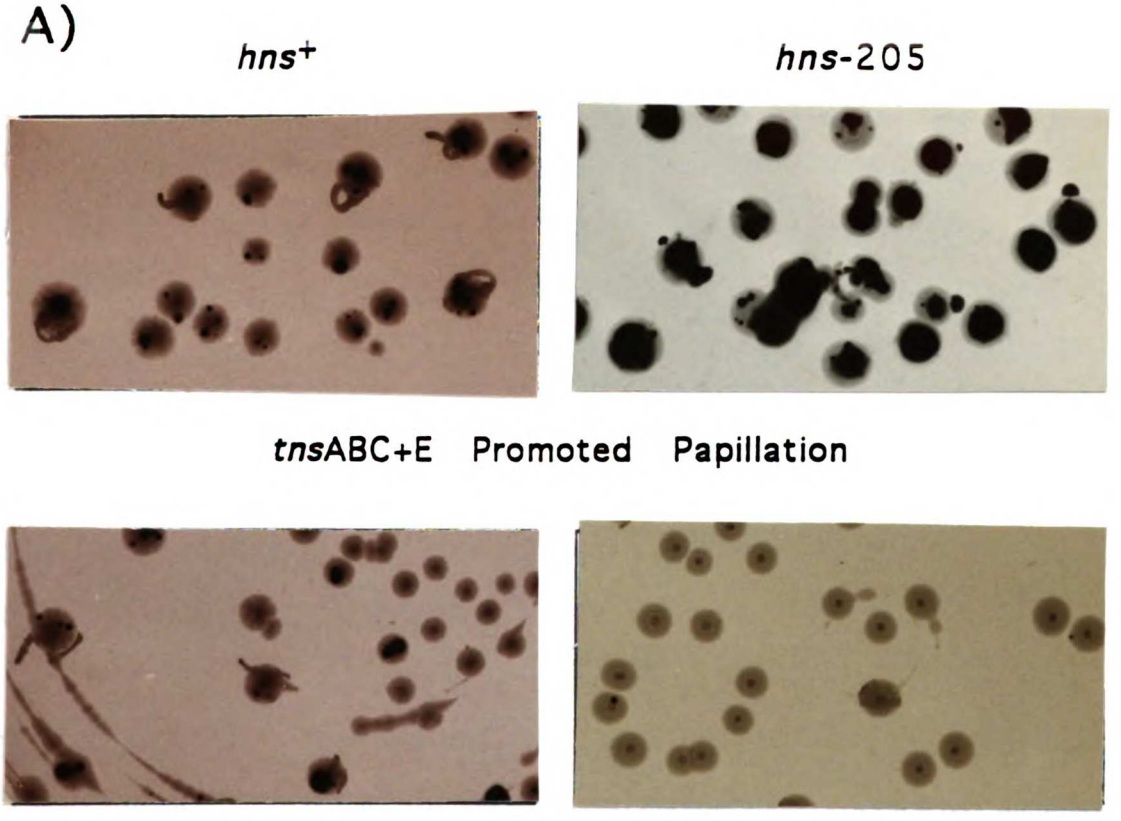


Figure 4-7 Legend

The Effect of *hns*-205 on MiniTn7-*lac* Papillation

From an *att*Tn7::miniTn7-*lac* Donor Site

Panel A presents Lac$^+$ papillation of isogenic *hns*$^+$ and *hns*-205 bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids), on MacConkey Lactose indicator plates, photographed after a 4 day incubation at 30°C. Data is from simultaneous plating of the indicated strains on the same batch of plates, and are the colonies used for Figure 4-6. Lac$^+$ papillae reflect miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. MacConkey agar was supplemented with spectinomycin at a concentration of 50 ug/ml. Examination of *tns*ABC+E promoted miniTn7-*lac* papillation in the *hns*-205 strain past four days of incubation at 30°C becomes difficult because some cells become mucoid and overwhelm the colony.

Panel B presents Lac$^+$ papillation of the isogenic *hns*$^+$ and *hns*-205 from Panel A patched on MacConkey Lactose plates. Patches were also photographed after a 4 day incubation at 30°C. MacConkey agar was supplemented with tetracycline at a concentration of 20 ug/ml.

Table 4-5

Tn7 and miniTn7-*lac* Transposition in *hns*⁻ vs. *hns*⁺

Tn7 Transposition to pOX38*gen*

Donor Site	Mobile Element	Transposition Frequency *hns*⁺	*hns*-205	Ratio *hns*⁻/*hns*⁺
RZ201*att*Tn7	Tn7	$5.1 \pm 3.2 \times 10^{-6}$	$1.2 \pm 0.4 \times 10^{-6}$	0.2

Tn7 and miniTn7-*lac* Transposition to pOX38*gen*

Donor Site	Mobile Element	Transposition Frequency *hns*⁺	*hns*-43*	Ratio *hns*⁻/*hns*⁺
NLC28*att*Tn7	miniTn7-*lac*	$3.5 \pm 1.2 \times 10^{-7}$	$1.1 \pm 1.3 \times 10^{-7}$	0.3
φ80dIIlac	Tn7	$3.2 \pm 0.6 \times 10^{-6}$	$7.7 \pm 13 \times 10^{-8}$	0.02

Table 4-5 Legend

Transposition frequency of Tn7 from the chromosomal *att*Tn7 site to pOX38*gen* in isogenic *hns*$^{+/-}$ strains was measured using the mating-out assay. The mean and standard deviation of 3 separate measurements is reported for the effect of *hns*-210, while values for the effect of *hns*-43* are the averages of 5 separate measurements. The F' transposition targets were as indicated. Strain background is indicated under "Donor Site" category. Alleles of *hns* tested were as indicated, with *hns*-205 being a previously characterized and published null allele of *hns* (Higgins et al., 1988; Hulton et al., 1990). *hns*-43* is miniTn10-*tet* insertion #43, which interrupts the *hns* coding sequence, as described in Chapter 5 of this thesis. The F⁻ recipient strain was CW51. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

IHF: Integration Host Factor

Mutations of either IHF subunit, *hip*-306 or *him*A-825, reduce both *tns*ABC+D and *tns*ABC+E dependent Tn7 transposition as measured by miniTn7-*lac* papillation in comparison to isogenic *hip*$^+$ or *him*A$^+$ strains.

Decreased Papillation From the Chromosomal *att*Tn7 MiniTn7-*lac* Donor Site:

The graphic summary shown in Figure 4-8 and the photographs shown in Figure 4-9 demonstrate the decreased papillation observed with *hip*-306 for both *tns*ABC+D and *tns*ABC+E promoted miniTn7-*lac* transposition from *att*Tn7. The graphic summary shown in Figure 4-10 and the photographs shown in Figure 4-11 detail a similar decrease of miniTn7-*lac* papillation from *att*Tn7 observed with *him*A-825 for both *tns*ABC+D and *tns*ABC+E promoted transposition. *hip* and *him*A mutants do not affect cell growth (K. Yasuzawa, 1992). *hip*-306 and *him*A-825 and had little effect on the growth of the strains tested when measured by the growth of colonies or liquid cultures, however in patches both IHF mutants appear to grow faster and have higher "background" redness on MacConkey plates (Figures 4-9 and 4-11). This may only reflect the initial number of cells plated since "patches" of equal volumes of a liquid cultures did not show a growth or background difference between IHF$^+$ and IHF$^-$ strains.

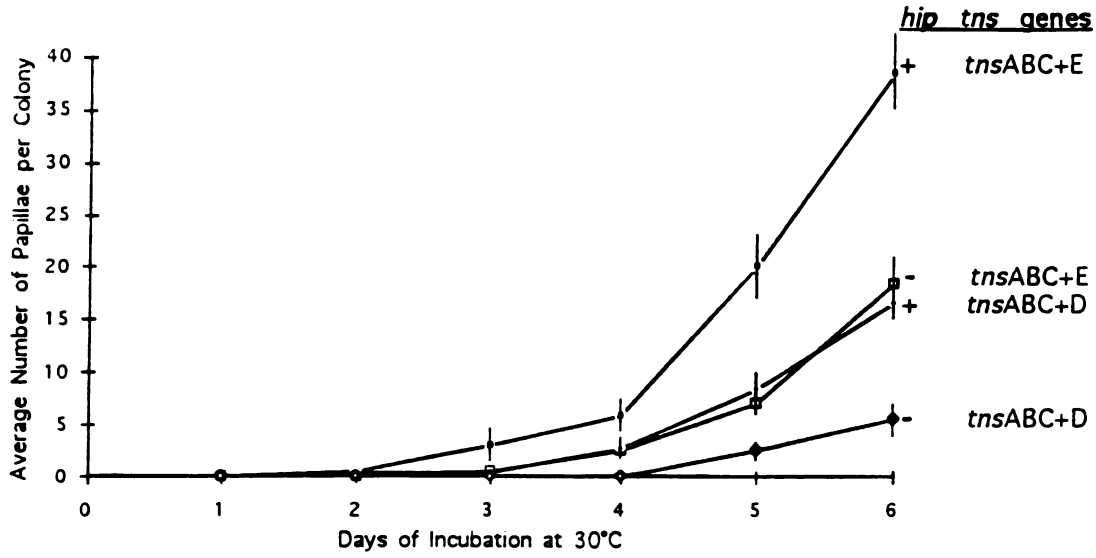
Decreased Papillation From a pOX38*gen*::miniTn7-*lac* Donor Site:

The graphic summary shown in Figure 4-12 demonstrates that papillation is also decreased with *hip*-306 for miniTn7-*lac* transposition from an F' (pOX38*gen*) miniTn7-*lac* donor site. In the bacterial strains depicted in Figure 4-12 *att*Tn7 is occupied by a second Tn7 element (miniTn7-KmR199 (Arciszewska, et al., 1989)), so all miniTn7-*lac* papillation events examined here are to either pseudo-*att*Tn7 sites *via tns*ABC+D promoted transposition, or sequence non-specific sites *via tns*ABC+E promoted transposition (Kubo and Craig, 1990).

Reduced miniTn7-*lac* papillation suggest the histone-like protein, IHF, may play a role in Tn7 transposition. Effects of IHF mutants on Tn7 and miniTn7-*lac* were not measured by the mating-out assay due to the pleiotropic effects IHF mutations have on conjugation. IHF mutants reduce transfer of F and R100 conjugal plasmids (Dempsey, 1987; Gamas et al., 1987).

Figure 4-8

Effect of *hip*-306 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7 Site



(NLC28*att*Tn7::miniTn7-*lac* p*tns*)

Figure 4-8 Legend

The Effect of *hip*-306 on MiniTn7-*lac* Papillation

From an *att*Tn7::miniTn7-*lac* Donor Site

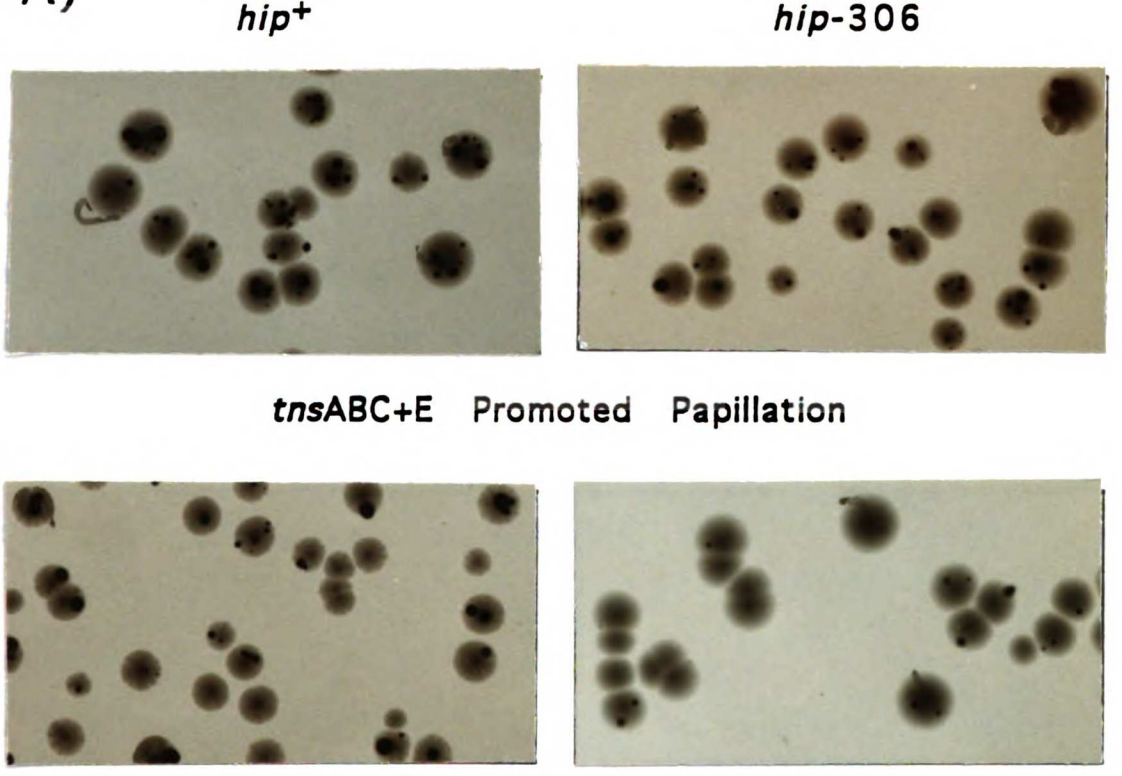
Time courses for the Lac$^+$ papillation of isogenic *hip*$^+$ and *hip*-306 bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids) on the MacConkey Lactose indicator plates (shown in Figures 9), were generated by counting Lac$^+$ papillae every 24 hours during incubation at 30°C. Data is from simultaneous plating of the indicated strains on the same batch of plates. *hip*$^+$ and *hip*-306 bacterial cultures were inoculated in LB + 20 ug/ml tetracycline and grown overnight with shaking incubation at 37°C. Liquid cultures were diluted in LB and plated on MacConkey Lactose plates. The *tns* plasmids were maintained by tetracycline at a concentration of 20 ug/ml in the MacConkey agar. The average number of Lac$^+$ papillae on 10 colonies is plotted (each data point) along with the standard deviation of the average number of Lac$^+$ papillae (bar through data points). Lac$^+$ papillae reflect miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. The *tns* plasmids were maintained by tetracycline at a concentration of 20 ug/ml in the MacConkey agar. Status of *hip* and the *tns* genes present is indicated to the right of the day 6 endpoints.

Figure 4-9

The Effect of *hip*-306 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site

NLC28*att*Tn7::miniTn7-*lac* p*tns*

A)



B)

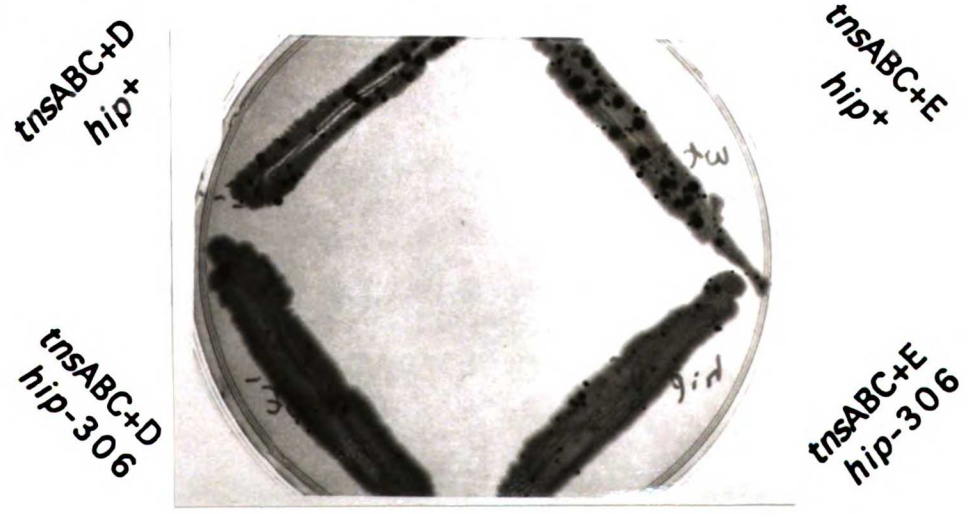


Figure 4-9 Legend

The Effect of *hip*-306 on MiniTn7-*lac* Papillation

From an *att*Tn7::miniTn7-*lac* Donor Site

Panel A:

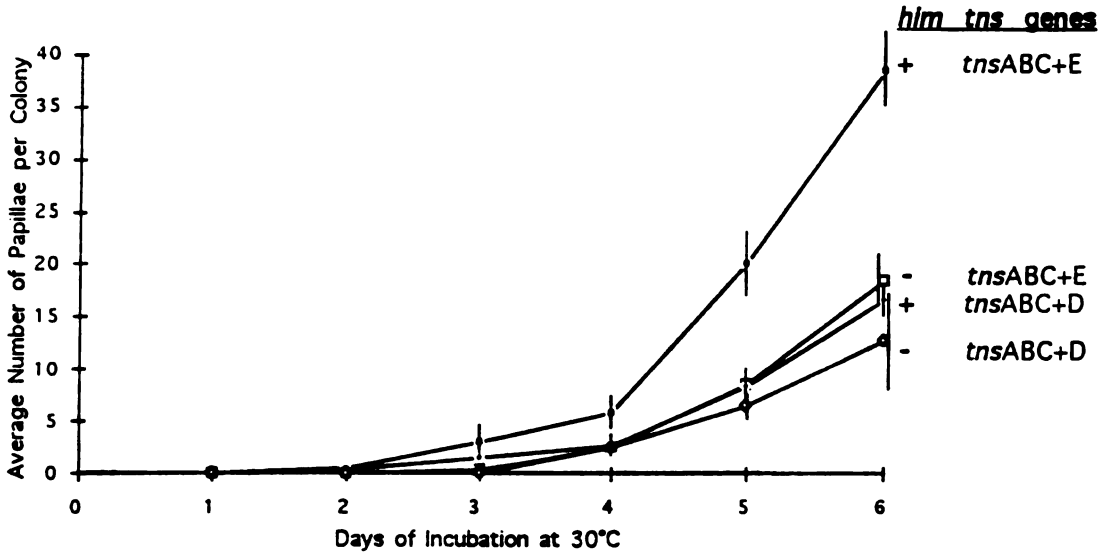
Lac$^+$ papillation of isogenic *hip*$^+$ and *hip*-306 bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids) on MacConkey Lactose indicator plates was photographed after a 4 day incubation at 30°C. Data is from simultaneous plating of the indicated strains on the same batch of plates, and are the colonies used for Figure 4-8. Lac$^+$ papillae report miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. The *tns* plasmids were maintained by tetracycline at a concentration of 20 ug/ml in the MacConkey agar.

Panel B:

Lac$^+$ papillation of the isogenic *hip*$^+$ and *hip*-306 strains from Panel A are patched on MacConkey Lactose plates. Strains were as indicated above, with the *hip*$^+$ strains at the top of the plate to be compared to the isogenic *hip*-306 strain below it. *tns*ABC+D promoted papillation is presented on the left side of the plate while *tns*ABC+E promoted papillation is presented on the right side of the plate. The *hip*$^-$ strains appear in patches to grow faster and have higher "background" redness on MacConkey plates, though this effect was not seen for colonies and may reflect the initial number of cells plated. Patches were photographed after a 4 day incubation at 30°C. MacConkey agar was supplemented with tetracycline at a concentration of 20 ug/ml.

Figure 4-10

Effect of *him*A-825 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site



(NLC28*att*Tn7::miniTn7-*lac* p*tns*)

Figure 4-10 Legend

The Effect of *him*A-825 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site

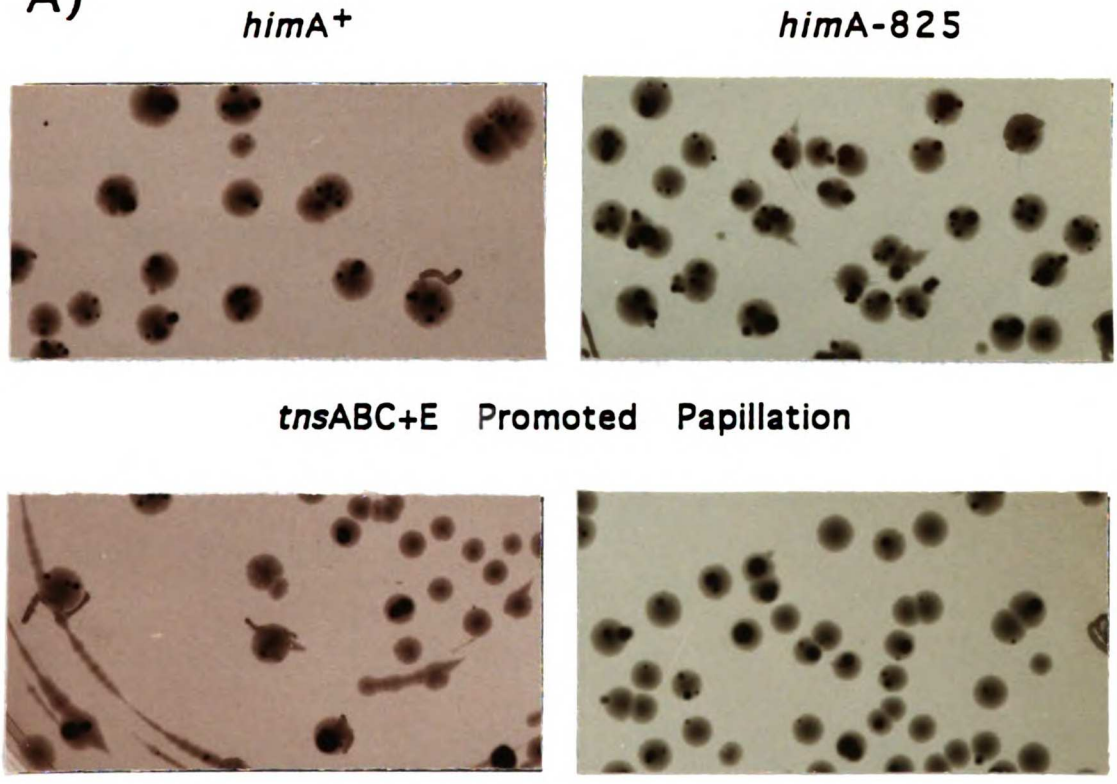
Time courses for the Lac$^+$ papillation of isogenic *him*A$^+$ and *him*A-825 bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids) on the MacConkey Lactose indicator plates (shown in Figure 4-11), were generated by counting Lac$^+$ papillae every 24 hours during incubation at 30°C. Data is from simultaneous plating of the indicated strains on the same batch of plates. *him*A$^+$ and *him*A-825 bacterial cultures were inoculated in LB + 50 ug/ml spectinomycin and grown overnight with shaking incubation at 37°C. Liquid cultures were diluted in LB and plated on MacConkey Lactose plates. The *tns* plasmids were maintained by spectinomycin at a concentration of 50 ug/ml in the MacConkey agar. The average number of Lac$^+$ papillae on 10 colonies is plotted (each data point) along with the standard deviation of the average number of Lac$^+$ papillae (bar through data points). Lac$^+$ papillae reflect miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tns*ABC+D from pCW4::miniMuΩ^{76E}. MacConkey Lactose indicator plates were supplemented with tetracycline at concentration of 20 ug/ml. Status of *him*A and the *tns* genes present is indicated to the right of the day 6 endpoints.

Figure 4-11

The Effect of *him*A-825 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site

NLC28*att*Tn7::miniTn7-*lac* p*tns*

A)



B)

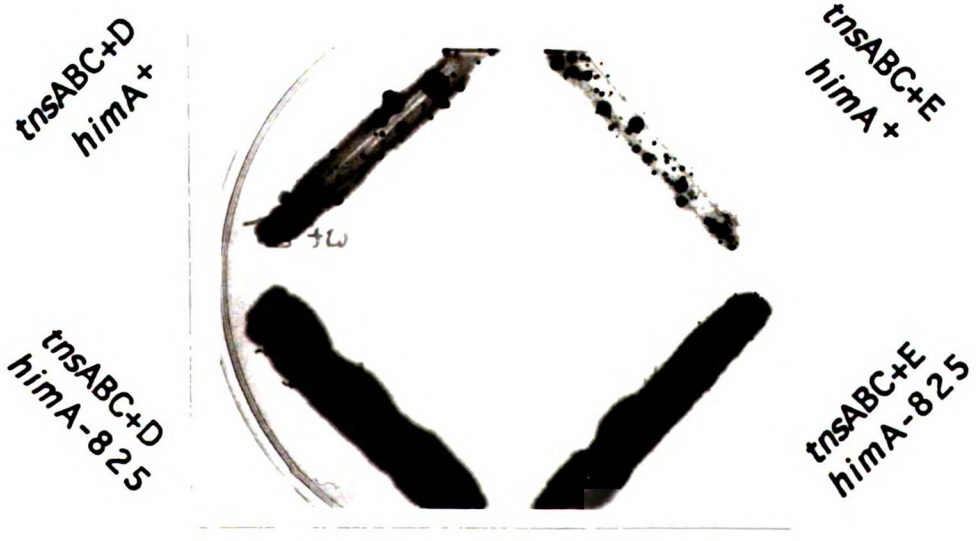


Figure 4-11 Legend

The Effect of *him*A-825 on MiniTn7-*lac* Papillation From an *att*Tn7::miniTn7-*lac* Donor Site

Panel A:

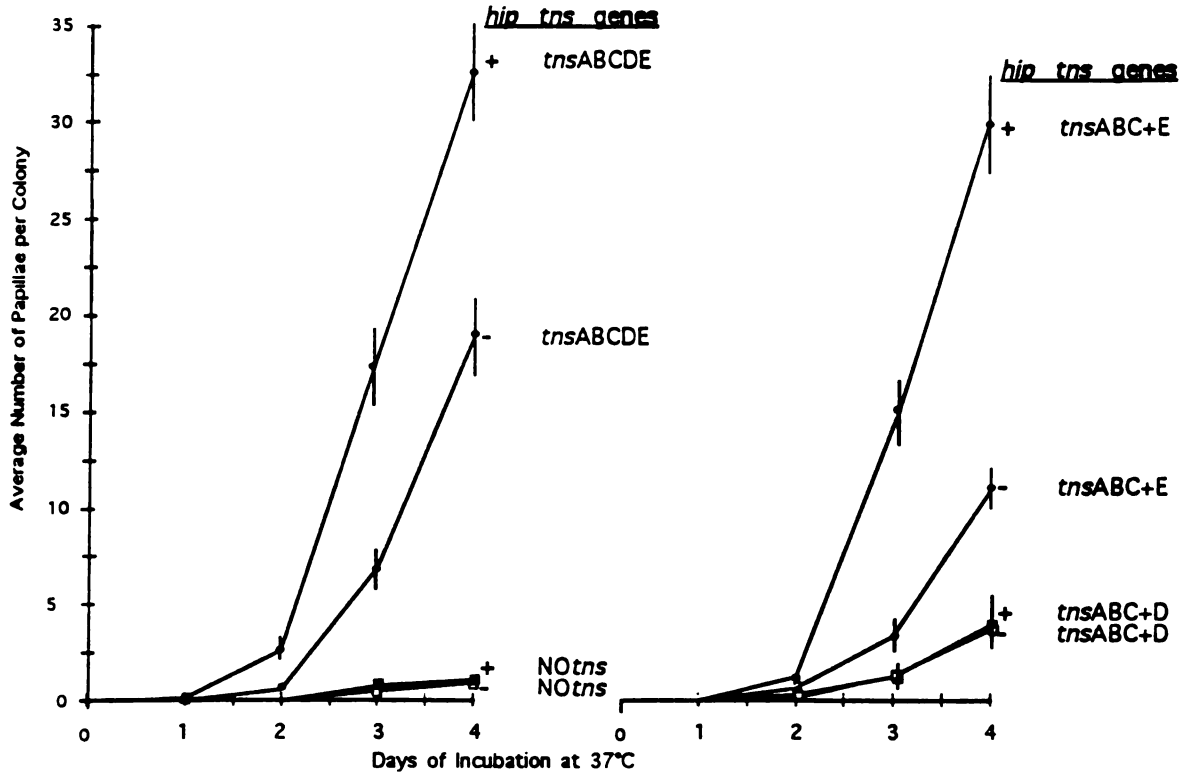
Lac$^+$ papillation of isogenic *him*A$^+$ and *him*A-825 bacterial colonies (NLC28 *att*Tn7::miniTn7-*lac* + *tns* plasmids) on MacConkey Lactose indicator plates was photographed after a 4 day incubation at 30°C. Data is from simultaneous plating of the indicated strains on the same batch of plates, and are the colonies used for Figure 4-10. Lac$^+$ papillae report miniTn7-*lac* transposition from the chromosomal *att*Tn7 site of NLC28, promoted by either *tns*ABC+E from pCW4::miniMuΩ^{107D}, or *tnsABC+D* from pCW4::miniMuΩ^{76E}. MacConkey agar was supplemented with spectinomycin at a concentration of 50 ug/ml.

Panel B:

Lac$^+$ papillation of the isogenic *him*A$^+$ and *him*A-825 strains from Panel A are patched on MacConkey Lactose plates. Strains were as indicated above, with *him*A$^+$ at the top of the plate to be compared to the isogenic *him*A-825 strain below it. *tns*ABC+D promoted papillation is presented on the left side of the plate while *tns*ABC+E promoted papillation is presented on the right side of the plate. The *him*$^-$ strains appear in patches to grow faster and have higher "background" redness on MacConkey plates, though this effect was not seen for colonies and may reflect the initial number of cells plated. Patches were photographed after a 4 day incubation at 30°C. MacConkey agar was supplemented with spectinomycin at a concentration of 50 ug/ml.

Figure 4-12

Effect of *hip*-306 on MiniTn7-*lac* Papillation From a pOX38*gen*::miniTn7-*lac* Donor Site



(NLC28*att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* p*tns*)

Figure 4-12 Legend

The Effect of *hip*-306 on MiniTn7-*lac* Papillation

From a pOX38*gen*::miniTn7-*lac* Donor Site

Time courses of Lac$^+$ papillation of isogenic *hip*$^+$ and *hip*-306 bacterial colonies (NLC28 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* + *tns* plasmids) on MacConkey Lactose indicator plates were generated by counting Lac$^+$ papillae every 24 hours during incubation at 37°C. Data is from simultaneous plating of the indicated strains on the same batch of plates. The average number of Lac$^+$ papillae on 5-10 colonies is plotted (each data point) along with the standard deviation of the average number of Lac$^+$ papillae (bar through data points). Lac$^+$ papillae report miniTn7-*lac* transposition from an F' (pOX38*gen*) donor site in NLC28, promoted as indicated by *tns*ABCDE from pCW4; *tns*ABC+E from pCW4::miniMuΩ^{107D}; *tns*ABC+D from pCW4::miniMuΩ^{76E}; and "NO *tns*" by pACYC. *att*Tn7 was occupied by miniTn7-Cm preventing miniTn7-*lac* transposition to *att*Tn7. Plasmids were maintained by tetracycline at a concentration of 20 ug/ml in the MacConkey agar. *att*Tn7 was occupied by miniTn7-KmR199. Status of *hip* and the *tns* genes present is indicated to the right of each day 4 endpoint.

Discussion

The inclusion of host components in transposition enables transposons to tie transposition to favorable host environmental conditions and take advantage of existing tools within a cell. For bacterial transposons, regulation by DNA adenine methylation and facilitation by histone-like proteins are the best characterized examples of host involvement in transposition (Kleckner, 1990). We have demonstrated here that mutations of DAM and histone-like proteins FIS, IHF, and H-NS affect Tn7 transposition.

DAM Regulation of Tn7 transposition:

DAM was shown to effect miniTn7-*lac* transposition. By both mating-out and papillation assays, *dam*-13 (a loss-of-function allele) was shown to increase miniTn7-*lac* transposition. These results suggest that a change in GATC methylation state can have an effect on Tn7 transposition, resulting in substantially increased transposition frequency under some conditions, however continuing work by Bob DeBoy in Dr. Craig's lab has not been able to reproduce the increase of Tn7 transposition as measured by mating-out, though has reproduced the effect on miniTn7-*lac* papillation (N. Craig, personal communication). Due to this inability to reproduce the mating-out results, the significance of these results is unclear.

The increase of miniTn7-*lac* transposition seen with *dam*-13 may be analogous the increased transposition of IS10 and IS5 in *dam*$^-$ strains. DAM is thought to act on both IS10 and IS5 to repress transposase transcription and block transposase binding to the end of the transposon (Dodson and Berg, 1989; Makris et al., 1988; McCommas and Syvanen, 1988; Roberts et al., 1985; Yin et al., 1988). In IS10, one DAM site lies within the inside end transposase binding site, and another lies within the -10 region of the pIN promoter.

The site of DAM action on Tn7 is unknown; however, there is only one DAM site within the *cis*-acting end sequences known to be important for Tn7 transposition (Arciszewska et al., 1989; Hauer and Shapiro, 1984; McKown et al., 1988; Ouartsi et al., 1985; Rogers et al., 1986; Smith and Jones, 1984; Waddell and Craig, 1988). A GATC sequence defining a DAM site lies 40 nucleotides in form the right end terminus of Tn7, within a TnsB binding site in the right end of the transposon (Gosti-Testu, 1982; Lichtenstein, 1982; Arciszewska et al., 1989; Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991). We hypothesize that adenine methylation at this **R**40 - GATC site alters TnsB binding to the end of Tn7 such that synaptic complex formation is inhibited and/or *tns*AB transcription is repressed.

Direct blockage of transposition by methylation at the **R**40 - GATC could be the result of blocking TnsB binding to its binding site over the **R**40 - GATC sequence; however, repressing the *tns*AB promoter (which lies nearly 50 base pairs away from **R**40 - GATC) poses a problem of action at a distance. Of the four TnsB binding sites in the right end of Tn7, **R**40 - GATC lies under the second TnsB binding site in from the end (**R**1), while the predicted *tns*AB promoter -35 region is contained in the innermost, fourth TnsB binding site in from **R**1 (Gosti-Testu, 1982; Lichtenstein, 1982; Arciszewska et al., 1989; Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991). The four TnsB binding sites within the right end of Tn7 lie head to tail in a slightly overlapping manner (Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991), raising the possibility of TnsB mediated communication between **R**40-GATC and the *tns*AB promoter through the TnsB binding sites. The -35 region of the *tns*AB promoter lies within the inner-most TnsB binding site suggest that the specific binding of TnsB to the ends of Tn7 may also regulate the expression of *tns*AB (Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991). Thus we speculate that altered TnsB binding at the **R**40 GATC affects TnsB binding over the -35 region of the *tns*AB promoter, and subsequently *tns*AB transcription. Methylation may decrease binding of

TnsB to the **R**40 site, blocking synaptic complex formation and increasing TnsB binding to the inner-most TnsB binding site, (occluding the -35 region of the *tns*AB promoter).

To further examine regulation of Tn7 transposition by DAM, and specifically to test the hypothesis that DAM's effect on Tn7 is mediated through the **R**40-GATC DNA sequence, transposition of a miniTn7 element with a mutation at the **R**40-GATC sequence should be characterized. Mutation at **R**40-GATC DNA sequence would eliminate it as a site of DAM methylation.

Mutation of the **R**40-GATC DNA sequence which lies within the TnsB binding repeat may negatively affect transposition activity, obscuring the importance of DAM methylation. However, only the G of the **R**40 - GATC sequence is conserved within all the other repeats (Gosti-Testu, 1982; Lichtenstein, 1982; Flores et al., 1990; Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991). Thus mutation at one of the other positions within the **R**40-GATC DNA sequence (possibly to **R**40-GATG) would likely generate a *cis*-acting right transposon end capable of supporting transposition.

The examination of the effect of *dam* mutation on the transposition of the **R**40-GATG mutant would test the direct involvement of the **R**40-GATC DNA sequence in the regulation of Tn7 transposition. If the **R**40-GATC DNA sequence is in fact the site of DAM regulation of Tn7, the **R**40-GATG mutant should transpose at the same frequency in the wild type strain as in the *dam*$^-$ mutant strain.

If the **R**40-GATC DNA sequence is found to be the site of DAM regulation of Tn7 transposition, the mechanism of DAM regulation could be investigated by studies of TnsB binding the right end of Tn7. The simplest investigation of DAM regulatory mechanism would look for altered binding of TnsB to methylated or unmethylated DNA fragments of the right end of Tn7 containing the **R**40-GATC DNA sequence. Because of the complex nature of the Tn7 *cis*-acting ends, the effect of DAM methylation on the

TnsB binding may be subtle and require more than the single repeat containing the **R**40-GATC DNA sequence.

In addition to altering TnsB binding to the *cis*-acting right end of Tn7, we have hypothesized that DAM methylation alters *tns*AB transcription. To test this possibility we would like to examine the effect of *tns*AB transcription from a heterologous promoter on the transposition of a miniTn7 element in both *dam*$^+$ and *dam*$^-$ strain backgrounds. This experiment would help separate the direct effects upon transposition of DAM methylation altering *cis*-acting end activity verses indirect DAM effect on transposition frequency through a direct effect on the expression of *tns*AB. Additionally, one could directly examine the expression of *tns*AB from their native promoter in both *dam*$^+$ and *dam*$^-$ strain backgrounds if the expression levels could be sensitively measured.

Histone-like Proteins and Tn7 Transposition:

Mutations in genes encoding histone-like proteins were also shown to affect Tn7 transposition. Null mutants of the genes encoding FIS, H-NS, and IHF were all shown to decrease Tn7 transposition. By both mating-out and papillation assays, a null allele of *hns* was shown to decrease Tn7 transposition. FIS and IHF were also shown to decrease miniTn7-*lac* transposition as measured by papillation. However, *fis*-null mutations show no effect on Tn7 transposition, and intact Tn7 transposition in *hip* or *him*A null mutants (encoding IHF) could not be measured because of pleiotropic effects on our assays for intact Tn7 transposition, mating-out and λ-hop.

The molecular mechanisms of histone-like protein involvement in Tn7 transposition may be more difficult to elucidate than the mechanism of DAM regulation because of the lack of a specific site-of-action to test. It is likely that histone-like proteins act in Tn7 transposition to facilitate formation of the elaborate protein-DNA complexes thought to be involved in transposition. IHF binding to the transposon ends has been show to cooperatively facilitate transposase binding for γδ (Wiater and Grindley, 1988),

and is required for IS10 outside end activity *in vivo* and *in vitro* (Morisato and Kleckner, 1987; Roberts et al., 1987; Huisman et al., 1989; Kleckner, 1989). FIS has been shown to bind to the ends of Mu (Alazard et al., 1992; Betermier et al., 1989). FIS also binds the inside end of IS50, and *fis* activity has been shown to stimulate Tn5 transposition (Weinreich and Reznikoff, 1992).

A possible clue to the mechanism of FIS involvement in Tn7 transposition lies with the observation that miniTn7-*lac* is affected to a far greater extent by a *fis*$^-$ mutation than intact Tn7. In *fis*$^-$ cells, miniTn7-*lac* transposition is reduced while intact Tn7 seems unaffected. *fis* mutation may act synthetically with the impaired miniTn7-*lac* element to reveal an effect on transposition.

MiniTn7-*lac* transposition is reduced in comparison to intact Tn7 (see Chapter 2, Table 2-3). One reasonable hypothesis is that this reduced transposition frequency is the result of deletion of a portion of the *cis*-acting right end sequence important for Tn7 transposition. The *cis*-acting sequences on the right end of miniTn7-*lac* is composed of only three of the four TnsB binding sites in the right end Tn7 (see Chapter 2 and - Gosti-Testu, 1982; Lichtenstein, 1982; Lichtenstein and Brenner, 1982; Arciszewska et al., 1989; Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991). We noted in Chapter 2 that the apparent crippling of miniTn7-*lac* can be suppressed by over-expression of the *tns* genes from a plasmid. This may suggest that increasing the concentration of TnsB can suppress the crippled transposition phenotype of miniTn7-*lac*. If FIS acts to increase the binding of TnsB to the end of Tn7, then one might see a greater effect of FIS on an element that begins with reduced TnsB binding, as we see in the case of miniTn7-*lac* vs. intact Tn7. In other words, the overexpression of TnsB would suppress the decreased transposition phenotype seen with miniTn7-*lac* in a wild-type stain background, however *fis*$^-$ mutation would again reveal a deceased transposition phenotype.

To specifically ask whether the short **R**70 end is partially responsible for the reduced papillation seen with miniTn7-*lac* in *fis*⁻ cells, we would like to examine the effect on papillation of a miniTn7-*lac* element constructed with a *cis*-acting right end containing all four of the TnsB binding repeats in intact Tn7 - i.e. **R**199 (Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991). If FIS acts at the ends of Tn7, and has a greater effect on an element composed of an **R**70 end than on one with a **R**199 end, we would expect that *fis* mutation should have less effect on the papillation of an miniTn7-*lac*(**R**199) than on the miniTn7-*lac*(**R**70) used in this work. If Lac$^+$ papillation by a miniTn7-*lac*(**R**199) element is reduced in *fis*⁻ strains exactly as miniTn7-*lac*(**R**70), then the hypothesis that FIS acts directly to aid TnsB binding and synaptic complex formation is weakened.

To directly test the hypothesis that FIS acts to stabilize *cis*-acting end complex formation one would like to examine the effect of FIS on TnsB binding to the ends *in vitro*. TnsB binding the *cis*-acting end fragments would be examined under TnsB limiting conditions, and either the presence or absence of FIS. FIS could affect either the simple binding of TnsB to *cis*-acting end fragments, or may more subtly affect the form of the binding. Simple effects if FIS on the binding of TnsB to the *cis*-acting end fragments may be detectable with mobility shift assays of TnsB binding Tn7 end fragments (McKown et al., 1987; Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991), however more subtle alterations of the form of binding may require DNA footprint studies of TnsB binding to the *cis*-acting end fragments in the presence and absence of FIS. Similar studies of TnsB transposon end binding activity could be done to investigate the involvement of IHF and HNS in Tn7 transposition.

As with DAM, the histone-like proteins may affect miniTn7-*lac* transposition by altering the transcription of the *tns* genes. A likely target of transcriptional regulation of Tn7 by FIS, IHF and HNS is the *tnsAB* promoter. Transcriptional regulation could occur through the alteration of TnsB binding over the *tnsAB* promoter. As with the case of

DAM, testing the hypothesis that *fis*, *hip*, *him*A, or *hns* mutation affects *tns*AB expression, we could examine the effect of *tns*AB transcription from a heterologous promoter on the transposition of a miniTn7 element in both wild type and mutant strain backgrounds. Again, this experiment would help separate the direct effects upon transposition of *cis*-acting end activity verses indirect effect on transposition frequency through a direct effect on the expression of *tns*AB.

The large array of *in vitro* assays for the activities of the Tns proteins, including TnsB *cis*-acting end fragment binding activity (Arciszewska and Craig, 1991; Arciszewska et al., 1991; Tang et al., 1991), TnsD and TnsC *att*Tn7 binding activity (Waddell and Craig, 1989; Bainton et al., 1993), and the reconstruction of Tn7 transposition *in vitro* (Bainton et al., 1991) makes Tn7 an attractive system for the further study of the interaction of a mobile genetic element with its host cell.

Chapter 5: New Host Mutants

Abstract:

The goal of this work was to identify E. *coli* components involved in Tn7 transposition. Through analysis of approximately 28,000 E. *coli* colonies containing independent miniTn10-*tet* insertions, 26 strains with altered Tn7 transposition frequencies were identified. The locations of the miniTn10-*tet* insertion mutations were identified by sequencing the chromosomal DNA flanking each insertion. 21 of the 26 miniTn10-*tet* insertions were grouped through sequence matching to 16 previously identified loci on the E. *coli* chromosome. Insertions in or near a wide array of E. *coli* genes were found to affect the transposition of Tn7. Insertion in *hns* decreased Tn7 transposition. *hns* encodes a histone-like protein identified in the previous chapter to be involved in Tn7 transposition. Insertion in *min*D, a gene involved in cell division control and affecting DNA super-coiling, increased miniTn7-*lac* transposition. Six of the sixteen groups are genes which affect the choice between aerobic and anaerobic growth. Two of these six groups are global regulators of transcription responding to aerobic vs. anaerobic conditions - *arc*B and possibly *sox*S (insertion located within 1.2 Kb of *sox*R). The other four groups are genes required for either aerobic or anaerobic growth: 1) *unc* (ATP synthase), 2) *sdh* (encoding the subunits of succinate dehydrogenase), 3) *suc* (encoding the subunits of α-ketogluterate dehydrogenase and succinyl-Co-A), and 4) two insertions within 2 Kb of *men*B possibly affecting the expression of the *men* operon (involved in menaquinone biosynthesis and required for anaerobic metabolism). Insertions in *pur*MN, *gua*B, and *dcd* may suggest a link between Tn7 transposition and nucleotide metabolism. MiniTn10-*tet* insertions affecting Tn7 transposition were also found in *lys*S, in the *rps* operon, and at six other chromosomal positions near previously identified genes.

Introduction

Recombination of many mobile genetic elements involves host-encoded functions as well as transposon-encoded transposases (reviewed by Kleckner, 1990; specific examples outlined below). Host factors may be involved in transposition as essential components of the transposition machinery, through the regulation of transposase expression and activity, or in the maintenance of DNA integrity as necessitated by the strand cleavage and ligation reactions of transposition.

Host components of transposition have been identified in three ways. Host components known to be involved in other recombination systems have been directly examined for effects on transposition *in vivo*. For example, IHF was originally identified through its role in phage Lamda recombination (Miller et al., 1979). IHF was subsequently found to be involved in the transposition of IS1, γδ, Tn5, IS10 and Mu (Gamas et al., 1987; Gamas et al., 1985; Morisato and Kleckner, 1987; Krause and Higgins, 1986; Surette and Chaconas, 1989; Surette et al., 1989; Wiater and Grindley, 1988; Wiater and Grindley, 1990a; Makris et al., 1990).

Host components can be identified during the *in vitro* reconstitution of transposition. An example of the biochemical identification of host components involved in transposition came with the reconstruction of Mu transposition *in vitro*. *In vitro* transposition of Mu was found to require the host-derived protein HU as well as MuA and MuB (Craigie et al., 1985). It was later demonstrated that E. *coli* *hup*A *hup*B double mutants (encoding the subunits of HU) do not support Mu's transpositional replication, supporting a direct involvement of HU in Mu transposition *in vivo* as well as *in vitro* (Huisman et al., 1989). However, biochemical reconstruction may bypass factors relevant *in vivo*.

Host components of transposition may also be identified genetically. Host mutants displaying altered transposition frequency can identify host components involved

in transposition. Perhaps the best understood host-transposon interaction is mediated by DNA adenine methylase (DAM) (reviewed by Kleckner, 1990). *dam*'s role in transposition was identified in a screen for E. *coli* mutants affecting Tn10 transposition-promoted adjacent deletions (Roberts et al., 1985). Mutations in *dam* were the major class of E. *coli* mutants which increased Tn10 transposition. Other E. *coli* mutants, either increasing or decreasing Tn10 transposition, were also isolated in the screen that identified *dam*, but remain uncharacterized.

Examples of host involvement in transposition are described in Chapter One of this thesis. In Chapter Four, the influence on Tn7 transposition of mutations of *dam*, and the histone-like proteins encoded by *fis*, *hns*, and both *hip* and *him*A (the subunits of IHF) was examined. The purpose of the work in this chapter was to extend our investigation of E. *coli* components involved in Tn7 transposition. Specifically, random insertion mutations throughout the E. *coli* chromosome were generated by miniTn10-*tet* insertion and screened for effects on Tn7 transposition.

Transposon-mediated mutagenesis is attractive in that each mutation is "tagged" by the insertion of the transposon, aiding in its manipulation and identification. However, transposon-tagged mutant screens are biased against essential host genes due to the predominance of null mutations resulting from the interruption of a gene. Thus, the mutant screen described in this chapter will underrepresented essential host functions involved in Tn7 transposition, however transposon-tagging can identify essential host functions as described below.

Mutations generated by transposon insertions have been found to generate a variety of mutation types, in addition to simple null mutation of a disrupted gene. Insertion in a gene can generate truncated forms of a protein with partial or novel activities . The physical interruption of an operon, with termination of transcription within the transposon, results in polar loss of function of downstream genes (Fiandt et al., 1972). Transposon insertions also directly generate gain of function mutations by

interrupting negative control elements or transcription from a promoter within the mutagenizing transposon into adjacent genes can result in inappropriately expressed functions (Lers et al., 1989; Dorsett et al., 1989; Weiss et al., 1989; Cookson et al., 1990; Zagorec and Steinmetz 1991; Barkan and Martienssen, 1991; Tanda and Corces, 1991). Conditional promoters within the mutagenizing transposon can be employed to generate conditional gain of function mutations. Conditional mutations have been described using the induceable tetracycline resistance gene within same miniTn10-*tet* element employed in this work (Lockman and Curtiss, 1992; Takiff et al., 1992).

I have isolated E. *coli* mutants which either increase or decrease Tn7 transposition. Some of these mutants also appear to affect Tn10 transposition. Below, I detail work aimed at the identification, characterization, and rationalization of host involvement in Tn7 transposition.

Results:

Transposon Mutagenesis:

To generate transposon-tagged E. *coli* mutations, a miniTn10 element marked with a gene conferring tetracycline resistance (miniTn10-*tet*) was employed (Way et al., 1984). MiniTn10-*tet* and the Tn10 transposase required for Tn10 transposition were carried into E. *coli* on a replication-defective phage lambda vector (λ1098 - Way et al., 1984; Maurer et al., 1980; Lichten and Fox, 1984). MiniTn10-*tet* transposed from λ1098 at a relatively high frequency, due to a high level of Tn10 transposase expression form a pTAC promoter. With the loss of the replication-defective phage vector, Tn10 transposase is also lost, so no subsequent miniTn10-*tet* transposition occurs. Figure 5-1 diagrams miniTn10-*tet* and its transposase on λ1098.

Assaying the Effect of Mutagenic miniTn10-*tet* Insertion on MiniTn7-*lac* Generated Papillation:

The effect of miniTn10-*tet* insertion mutations on Tn7 transposition was assayed by miniTn7-*lac* generated Lac^+ colony papillation. Transposition of miniTn7-*lac* from a non-transcribed donor site on an F' plasmid (pOX38*gen*) to a transcribed genetic location results in a Lac^+ phenotype in the cell in which the transposition occurred and its subsequent progeny. On MacConkey Lactose indicator plates, Lac^+ cells generated as the result of miniTn7-*lac* transposition form red papillae on a white Lac^- colony. Scoring the rate of red papillae formation in a single colony can be used to visually assess transposition frequency in that colony. The papillation assay for Tn7 transposition was characterized in Chapter 2 of this thesis.

Strain Used for the Identification of E. *coli* Mutants Affecting Tn7:

A well-characterized E. *coli* strain was chosen for miniTn10-*tet* transposon mutagenesis. NLC28 is a MC4100 derivative, in which miniTn7-*lac* transposition and papillation were characterized in the previous chapters of this thesis. Tn7 in the chromosomal *att*Tn7 site supplied *tns* transposition functions in *trans* to miniTn7-*lac*. MiniTn7-*lac* was situated on an F' plasmid, pOX38*gen*. We had intended to have a second copy of Tn7 on pOX38*gen*: (i.e. pOX38*gen*::miniTn7-*lac*, ::Tn7). However re-testing of the mutagenized strains found no Tn7 on the pOX38*gen*::miniTn7-*lac* plasmid. Thus it is likely that the bacterial strain mutagenized by miniTn10-*tet* was NLC28 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*.

The absence of Tn7 from pOX38*gen*::miniTn7-*lac* makes it is difficult to understand the relative infrequency of the four insertions into *att*Tn7::Tn7 identified (Appendix A, Table A-2), vs. 112 insertions on the pOX38*gen*::miniTn7-*lac* (Appendix A, Table A-1). The relative infrequency of scored insertion into *att*Tn7::Tn7 may be a result of a number of possibilities including target preference for miniTn10-*tet* insertion on the pOX38*gen*::miniTn7-*lac* plasmid, or recombination between *att*Tn7::Tn7 (or other share sequence near *att*Tn7::Tn7) and pOX38*gen*::miniTn7-*lac* resulting in conjugal mobilization of *att*Tn7::Tn7 resulting in *att*Tn7::Tn7 insertions actually being scored as pOX38*gen*::miniTn7-*lac* insertions.

The absence of Tn7 from pOX38*gen*::miniTn7-*lac* does not effect the results reported here in that the miniTn10-*tet* insertions were transduced into new background strains for the subsequent tests of effects on Tn7 transposition.

Screening for E. *coli* Mutants Affecting miniTn7-*lac* Transposition:

Transposition of miniTn7-*lac* from its non-transcribed location on pOX38*gen* to a transcribed location results in Lac$^+$ papillation as described in Chapter 2 of this thesis.

Colonies of E. *coli* mutants containing miniTn10-*tet* insertions were screened for altered miniTn7-*lac* educed Lac$^+$ papillation on MacConkey-Lactose indicator plates.

Seven separate infections with λ1098 carrying miniTn10-*tet* resulted in a total of approximately twenty eight thousand tetracycline-resistant colonies plated directly on MacConkey Lactose Tetracycline which were screened for effects on miniTn7-*lac* papillation. 195 tetracycline-resistant colonies were identified which displayed altered Lac$^+$ papillation phenotypes. (For details, see "Materials and Methods" section.)

A series of secondary screens were performed to identify the miniTn10-*tet* insertions in the E. *coli* chromosome which alter Tn7 and miniTn7-*lac* transposition frequency. Figure 5-2 outlines the results of secondary screens done with these miniTn10-*tet* insertion mutations. The secondary screens are described below.

Identification of Chromosomal MiniTn10-*tet* Insertions Unlinked to Tn7:

The tetracycline resistant (Tc^R) colonies selected after infection of NLC28 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* with λ1098 may contain miniTn10-*tet* insertions in either of the two replicons in the cell, the F' (pOX38*gen*::miniTn7-*lac*), or the chromosome. Evaluation of the 195 Tc^R strains with altered Lac$^+$ papillation by conjugation of the F' revealed that 83 did not contain miniTn10-*tet* on the F' and, hence, likely contained chromosomal insertions (Appendix A, Table A-1).

Of the 83 E. *coli* chromosomal miniTn10-*tet* insertions, 79 were found not to be closely linked by P1 transduction to *att*Tn7::Tn7's trimethoprim resistance marker. MiniTn10-*tet* insertions with close P1 transduction linkage to Tn7's trimethoprim resistance marker were not of interest for this work due to their likelihood of being insertions in or near Tn7's *tns* genes. Table 5-2 in Appendix A reports the P1 co-transduction linkage of chromosomal miniTn10-*tet* insertions with *att*Tn7::Tn7. Interestingly, mutant #57 was weakly linked to Tn7 (2 of 20 Tc^R transductants

cotransduced Tp^R). MiniTn10-*tet* insertion #57's weak linkage to Tn7 was found to be due to insertion in a gene near Tn7 (*unc* - see Mutant #57 in Table 5-1).

MiniTn10-*tet* Insertions Linked to the Papillation Phenotype:

60 of the 79 E. *coli* chromosomal miniTn10-*tet* insertions not linked to *att*Tn7::Tn7 were found to have altered miniTn7-*lac* papillation when P1 transduced into naive strain background. The tetracycline resistance marker of miniTn10-*tet* was P1 transduced into naive NLC28*att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* to test linkage of the altered papillation phenotype with the miniTn10-*tet* insertion. By examining 20-100 Tc^R transductants from each mutant, we found that 60 of the miniTn10-*tet* insertion mutants always co-transduce the same altered papillation phenotype with the Tc^R marker. Table A-3 in Appendix A reports the linkage of the miniTn10-*tet* Tc^R with the Lac papillation phenotype. The effects of the insertion mutants on miniTn7-*lac* Lac^+ papillation is reported qualitatively in Table 5-1 and photographically in Figure 5-4.

Mutants with Altered Intermolecular Transposition:

Twenty six of the sixty miniTn10-*tet* insertion mutants with altered miniTn7-*lac* Lac^+ papillation were also found to have altered miniTn7-*lac* and Tn7 intramolecular transposition frequency as measured by the mating-out assay. The mutants were simultaneously tested for the mating-out frequency of miniTn7-*lac* from *att*Tn7, and intact Tn7 from a second chromosomal location at $\phi 80$dIIIlac, to the F' plasmid pOX38*gen*. Tn7 supplies *tns* functions in *trans* to miniTn7-*lac* for its transposition. Transposition to pOX38*gen* requires *tns*ABC+E. The mating-out data are graphically presented in Figure 5-3A and 3B, and in detail in Table A-4, Appendix A . Twenty six mutants that showed altered mating-out phenotypes in addition to papillation were chosen to further pursue. For the mutants pursued further, the frequency of miniTn7-*lac* and

Tn7 transposition as determined by mating-out is again graphically represented in Figure 5-3C.

It is should be noted that the conjugation based ("mating-out") assay used in this thesis to examine transposition frequencies probably measures both translocation of the transposable element to the conjugal plasmid followed by conjugation of that plasmid to CW51, and a background frequency of F' donor strain mutation to nalidixic acid resistance (see Materials and Methods). Thus, numbers reported as transposition frequencies as measured by the mating-out assay in this thesis may not reflect only transposition events. This caveat is expected to be significant for transposition frequencies below 10^{-7} (Bob DeBoy and Nancy Craig, personal communication).

Mutants With Altered Tn7 Transposition Also Alter Tn10 Transposition:

The effect of the miniTn10-*tet* insertion mutants on the transposition activity of another non-replicative transposon, Tn10, was also examined. A plasmid (pSIL-14) carrying miniTn10-*lac* and the Tn10 transposase gene (Bolland and Kleckner, 1992) was transformed into each of the 26 mutants identified above. MiniTn10-*lac* Lac^+ papillation was assayed on MacConkey Lactose indicator plates, just as for miniTn7-*lac* papillation. The effects of the insertion mutants on miniTn10-*lac* Lac^+ papillation is reported qualitatively in Table 5-1 and photographically in Figure 5-5.

Identifying MiniTn10-*tet* Insertion Sites:

To identify the site of miniTn10-*tet* insertion in the E. *coli* mutants, the miniTn10-*tet* insertions and flanking chromosomal DNA was cloned and sequenced. To clone the miniTn10-*tet* flanking DNA, E. *coli* chromosomal DNA was digested with a restriction enzyme which does not recognize any site within the miniTn10-*tet* insertion. This DNA was cloned into plasmid vectors, and resulting plasmids with a miniTn10-*tet* containing insertion conferred tetracycline resistance for selection.

Three plasmid vectors were used to clone the chromosomal miniTn10-*tet* insertions. pBluescript, a high copy pUC-derived plasmid, was most frequently used. pK184 and pK194 (Jobling and Holmes, 1990), low copy pACYC, derived plasmids were used to clone insertions which may have been lethal on the high copy pBluescript vector.

The cloned chromosomal insertions were sequenced out from each end of the miniTn10-*tet* insertion, and in from the plasmid vector-chromosomal insert junctions. Sequence information was used to search for a match in the GenBank data base of bacterial DNA sequences. Searching GenBank for the identity of sequences flanking the miniTn10-*tet* insertions was performed with the blastn algorithm (Altschul et al., 1990).

The genes identified here must be thought of as tags to the actual genes directly altering Tn7 transposition. Given the polar nature of transposon insertion mutations, the genes identified by sequencing DNA flanking the insertions are not necessarily the genes directly responsible for the altered Tn7 transposition phenotypes. Possibilities for further work with these mutants addressing issues of polarity, allelism, and mechanism of action are considered in the Discussion of this chapter.

MiniTn10-*tet* Insertions In Known Genes:

Nine genetic loci which affect Tn7 transposition were identified by sequencing into the flanking E. *coli* chromosomal DNA from miniTn10-*tet* insertions. Two of these nine genetic loci - *hns* and *min*D - were represented by two separate miniTn10-*tet* insertions. The insertions at *hns* reduced miniTn7-*lac* papillation, while the *min*D insertions resulted in increased papillation.

Single miniTn10-*tet* insertions were located in seven genetic loci - *glt*A/*sdh*, *sdh*/*suc*, *pur*MN, *gua*B, *arc*B, *lys*S, and *dcd* - by sequencing into the miniTn10-*tet* flanking E. *coli* chromosomal DNA. The insertions in *sdh*/*suc* (Insertion #4), *pur*MN (#20), *arc*B (#50), *gua*B (#75), *lys*S (#132) and *dcd* (#106) decreased miniTn7-*lac* papillation, while the insertion in *glt*A/*sdh* (#182) increased miniTn7-*lac* papillation

(Figure 5-4). It should be noted that the insertion in *gltA/sdh* lies 3.5 Kb from the insertion at *sdh/suc* (#4), and the insertion in *pur*MN (#20) lies near the insertion in *gua*B (#75), though we have no indication that they affect the same genes (see Appendix A).

MiniTn10-*tet* Insertions Between Known Genes:

Two of the genetic loci affecting Tn7 transposition were identified by sequencing into known genes from both plasmid vector / chromosomal insert junctions, but the point of insertion was not identified. DNA sequence from the ends of the cloned flanking chromosomal DNA locating insertion #57 between the *gid*A and *unc*B genes, implying that insertion was in either *gid*A, *gid*B, *unc*I or *unc*B (see Appendix A). The point of insertion is unknown because no sequence was obtained reading into the flanking chromosomal DNA flanking from the miniTn10-*tet* insertion. MiniTn10-*tet* insertion between *gid* and *unc* resulted in greatly increased miniTn7-*lac* papillation (Figure 5-4).

Another miniTn10-*tet* insertion was localized to the *rps*L-*tuf*A operon, with sequence from DNA flanking miniTn10-*tet* insertion #174 locating the insertion between points in the adjoining genes *rps*G and *fus*A, implying insertion was in either of these genes (Appendix A). The point of insertion was not determined due to conflicting sequence data indicated, likely as a result of confusing plasmids, and is reported in detail in Appendix A. MiniTn10-*tet* insertion in the *rps*L-*tuf*A operon decreased miniTn7-*lac* papillation (Figure 5-4).

MiniTn10-*tet* Insertions Near Known Genes:

Five of the genetic loci affecting Tn7 transposition were found to lie near known genes by sequencing into a known gene from one of the plasmid vector-chromosomal insert junctions. The exact point of miniTn10-*tet* insertion for these mutants was not identified due to either lack of sequence identity matches in GenBank, or lack of sequence data (see Table 5-1). Insertions near *ser*S, *sox*RS, *tes*B, *men*B and *fli*C were

found by matching sequence from one end of the cloned miniTn10-*tet* insertion with sequences in GenBank. *ser*S and *men*B were each found to be near the site of two miniTn10-*tet* insertions. Two insertions within 4.5 Kb of *ser*S (Mutant #17 and #190) had opposite effects on miniTn7-*lac* papillation (Figure 5-4). The less than 4.5 Kb distance to the points of insertion from *ser*S is based on the location of the matched sequence and the plasmid insert sizes (see Appendix A). Sequence from the points of insertion within 4.5 Kb of *ser*S found no DNA sequence identity matches within the GenBank data base, and so were unlikely to be within *ser*S coding sequence, however they may alter *ser*S expression.

Two insertions within 2 Kb of *men*B (Mutant #123 and #79) had opposite effects on miniTn7-*lac* papillation (Figure 5-4). Sequence from the points of insertion found no DNA sequence identity matches within the GenBank data base, and so were unlikely to be within the *men* operon, however the less than 2 Kb distance to the point of insertion from the identified sequence in *men*B places the insertion immediately upstream of the *men* operon. The opposite effects of insertion near *ser*S and *men*B may be due to differing effects of insertion near control regions of these genes.

*sox*RS, *fli*C, and *tes*B were each identified near single insertions. Mutant #73 was found to be within 1.2 Kb of *sox*R, and very possibly within *sox*S (Appendix A). The point of insertion is unknown because no sequence was obtained reading into the flanking chromosomal DNA flanking from the miniTn10-*tet* insertion. This insertion within 1.2 Kb of *sox*R decreases miniTn7-*lac* papillation (Figure 5-4). An insertion (#53) in front of the *fli* operon also decreases miniTn7-*lac* papillation (Figure 5-4). Insertion #53 is within 2 Kb of *fli*C, however sequence from the point of insertion found no DNA sequence identity match within the GenBank data base (Appendix A), and so the insertion is unlikely to be within the *fli* operon, though the insertion may affect *fli* transcription. Insertion #6 was found within 2 Kb of *tes*B, however sequence from the point of insertion found no DNA sequence identity match within the GenBank data base (Appendix A), and

so the insertion is unlikely to be within *tes*B. Furthermore, insertion #6 is downstream of *tes*B transcription, and so seems unlikely to affect its transcription. The gene affected by insertion #6, resulting in altered Tn7 transposition is unknown. Insertion #6 increases miniTn7-*lac* papillation (Figure 5-4).

MiniTn10-*tet* Insertions in Unknown Locations:

Four miniTn10-*tet* insertions mutant could not be assigned a location based on sequence information. Sequence information from mutants #171, 61, 62, and 118 found no matches within the GenBank database. Mutants #118 and 62 dramatically decrease miniTn7-*lac* papillation, while mutants #171 and 61 increase papillation.

Genetic Confirmation of the Location of MiniTn10-*tet* Insertion by P1 Transduction:

After the identification of a GenBank match to a known gene, P1 transduction was employed to confirm the location of miniTn10-*tet* insertion. Kanamycin resistance (Km^R) markers located very near the sequence match indicated location of the miniTn10-*tet* insertion were P1 transduced into strains containing the miniTn10-*tet* insertion. These Km^R transductants were then screened for loss of the miniTn10-*tet* associated Tc^R marker. If the miniTn10-*tet* insertion is, in fact, very near the Km^R marker, then the tetracycline resistance marker should be lost at high frequency. All but one of the mutants that were tested confirmed the sequence derived localization with P1 linkage to the expected location, and this information is presented in Table 5-5, Appendix A. Mutant #6 was not confirmed to lie near a Km^R marker we expected to be near *tes*B; however, we did not confirm the location of the Km^R marker.

Discussion:

The purpose of this work was to identify host components involved in the transposition of Tn7. Screening miniTn10-*tet* insertions in the *E. coli* chromosome for altered miniTn7-*lac* Lac$^+$ papillation led to the identification of 26 mutants with altered Tn7 and miniTn7-*lac* transposition frequencies. Sequence from DNA flanking 21 of these 26 miniTn10-*tet* insertions and P1 linkage analysis enabled us to identify or localize the site of miniTn10-*tet* insertion on the E. *coli* chromosome. Though the issues of polarity and allelism have not been addressed in this work, a number of interesting possibilities for the regulation of Tn7 transposition have been raised by the identity of the E. *coli* genes interrupted.

Extent of Saturation by MiniTn10-*tet* Insertion Mutagenesis:

Approximately 28,000 miniTn10-*tet* insertions were screened for altered miniTn7-*lac* papillation. The E. *coli* chromosome is comprised of approximately 4,720 Kb of DNA. If miniTn10 insertion was random and evenly spaced throughout the chromosome, this would translate into an insertion every 169 bp, or about 6 insertions in every 1 Kb gene. It is known, however, that Tn10 insertion is not random (Lee et al., 1987).

Sequence information and P1 linkage analysis of twenty four miniTn10-*tet* insertions affecting Tn7 transposition enabled us to assign these mutants to 16 groups. Eleven of the identified groups consisted of a single member. This large number of single member groups indicates that this screen did not identify all of the E. *coli* genes affecting Tn7 transposition. Another indication that this screen did not identify all of the E. *coli* genes affecting Tn7 transposition is seen the absence of the previously identifies genes. Although two of the insertions identified in this chapter were located in or near *hns*, the other genes found to affect miniTn7-*lac* papillation in Chapter 2 (*dam*, *fis*, and

IHF's *hip* and *him*A) were not among the genes identified in this screen. Particularly surprising is the absence of *dam* among the genes implicated here by the miniTn10-*tet* insertions. *dam*-13 was shown in Chapter 4 to increase miniTn7-*lac* transposition. However none of the tetracycline-resistant colonies with altered Lac$^+$ papillation phenotypes were found to be 2-aminopurine sensitive, suggesting none of these insertions resulted in a DAM$^-$ phenotype (data not shown).

It is not known what the miniTn7-*lac* papillation phenotype of a *dam* mutant would be in the NLC28 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* strain configuration. We saw in Chapter 4 that papillation of NLC28 *att*Tn7::miniTn7-*lac* p*tns*ABC+E was increased. However papillation of NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*II::Tn7 was decreased (data not shown, though Table 4-2 in Chapter 4 shows that transposition of miniTn7-*lac* in this strain configuration as measured by mating-out is decrease for *tns*ABC+E directed transposition). Papillation of a *dam* mutant in the strain configuration used for mutagenesis was not examined.

The absence of *dam* among the identified mutants in this chapter may be a result of a screen bias. In the process of performing the papillation based screen for mutations affecting Tn7 transposition, we found that increased papillation mutants were harder to score than decreased papillation. This may have resulted in an under-representation of *dam* mutants in this screen.

hns and the Histone-Like Proteins:

hns was represented by two of the twenty one identified Tn7 transposition mutants. The involvement of *hns* in Tn7 transposition was examined in Chapter 4 of this thesis. H-NS is a histone-like protein which is thought to bind and stabilize bent DNA (Yamada et al., 1991). *hns* mutants have pleiotropic effects ranging from the deregulation of genes which respond to environmental osmotic conditions (Higgins et al.,

1988), to the deregulation of a number of recombination systems (Falconi et al., 1991; Gama et al., 1992; Higgins et al., 1988; Lejeune and Danchin, 1990; Spears et al., 1986).

A conjunction of small histone-like proteins in the control of processes requiring the formation of complex, highly ordered DNA structures has been observed in a number of systems. For example the phase variation of type 1 fimbriation in *Escherichia coli* is associated with the inversion of a short DNA element at *fim*A. Inversion of this element is dependent on IHF and dramatically affected by mutations in *hns* (Blomfield et al., 1993).

In another example, Mu repressor binding to its operators requires a particular topology of the operator DNA. IHF stimulates repressor binding to the O1 and O2 operators and enhances Mu repression. IHF, DNA supercoiling, and the H-NS protein lock the operator region into the appropriate topological conformation for high-affinity binding not only of the phage Mu transposase but also of the phage Mu repressor (Falconi et al., 1991). H-NS-dependent changes in DNA topology have also been shown to play a role in the osmo-regulation of *pro*U expression (Owen-Hughes et al., 1992).

The integration of a number of components in the construction of highly ordered DNA complexes could allow for combinatorial control by a wide variety of environmental signal. In Chapter 4 of this thesis we found that mutations of *fis*, *hip*, *him*A, and *hns* affect the frequency of Tn7 transposition. Identifying possible cellular signals FIS, IHF, and H-NS involve in Tn7 transposition will be an intriguing line of investigation. For a detailed discussion of investigation into the mechanism of action histone-like proteins in Tn7 transposition see the Discussion section of Chapter 4.

Linking Tn7 Transposition to Aerobic vs. Anaerobic Metabolism:

One of the specific environmental signals which regulate Tn7 transposition may be aerobic vs. anaerobic growth conditions. Six of the sixteen loci identified in this chapter are genes which affect the choice between aerobic and anaerobic growth - 1) one

insertion in front of *sdh* (encoding the subunits of succinate dehydrogenase (Spencer and Guest, 1974)), 2) one insertion in front of *suc* (also involved in succinate metabolism - encoding the subunits of α-ketogluterate dehydrogenase and succinyl-Co-A (Darlison et al., 1984)), 3) one insertion in *unc* (ATP synthase (Walker et al., 1984a; Walker et al., 1984b)) - and another linked by P1 transduction, 4) one insertion in *arc*B (a global regulator responding to aerobic vs. anaerobic conditions (Iuchi et al., 1990)), 5) one insertion very near *sox*R, possibly in *sox*S (both encoding global regulators responsive to O_2 (Wu and Weiss, 1991)), and 6) one insertion very near the *men* operon which is required for anaerobic growth under some conditions (Newton et al., 1971; H. Taber, 1980; Sharma et al., 1992).

*arc*B codes for the periplasmic membrane-bound sensor for a classical two-component regulatory system (Iuchi et al., 1989; Iuchi et al., 1990). ArcB phosphorylates ArcA, which then represses aerobic metabolism genes under anaerobic growth conditions. Phosphorylated ArcA also turns on a number of anaerobically expressed genes (Iuchi et al., 1992). ArcB senses anaerobic conditions by the level of an electron transport components in the reduced form (Iuchi et al., 1990). The *men* genes are required for the biosynthesis of menaquinone, a component of anaerobic electron transport to fumarate (Newton et al., 1971; H. Taber, 1980). *men* mutants block anaerobic metabolism and would feed back to global regulation of metabolically responsive genes through ArcB (Iuchi et al., 1992). Mutants in the *glt*A, *sdh*, and *suc* genes would alter succinate metabolism, possibly blocking aerobic metabolism, and thus feedback to ArcB (Andersson, 1992; Iuchi et al., 1992).

To investigate possible regulatory links of Tn7 transposition to anaerobic vs. aerobic states of metabolism, it would be important to directly assay Tn7 transposition frequency under anaerobic cell growth in comparison to Tn7 transposition under aerobic cell growth. Assaying transposition under anaerobic conditions can not be done with the papillation assay in that MacConkey indicator plates use the pH sensitive dye Neutral

Red to indicate sugar fermentation (with its contaminant drop in pH). Cells in anaerobic growth would be expected to drop their pH, and were indeed found to turn red on MacConkey plates (data not shown). To circumvent this problem, a variation on the Lac^+ papillation assay in which cells are grown under anaerobic or aerobic conditions, then plated under aerobic conditions to assay the proportion of Lac^+ colonies to Lac^- colonies, could be employed to test the effect of anaerobic metabolism on transposition. One would also like to examine the effect on Tn7 transposition of well characterized mutants with defined effects on metabolism and global gene regulation (possibly of *arc* or *sox*).

Linking Tn7 Transposition to DNA replication and the Cell Cycle:

One of the best characterized example of environmental regulation of transposition is the inhibitory effect of DNA adenine methylation on non-replicative transposition (see Chapter 4). DNA adenine methylation ties transposition to DNA replication, and thus the cell cycle (Roberts et al., 1985; and reviewed by Kleckner, 1990). In the previous chapter we examined the effect of DAM on Tn7 transposition.

Previous studies in other labs have suggested that the replicative state of the Tn7 donor replicon may modulate transposition frequency (Hauer and Shapiro, 1984). Hauer and Shapiro found a very high frequency of transposition to *att*Tn7 triggered by the Inc-P1 incompatibility reaction (possibly blocking plasmid replication) suggesting that Tn7 may sense the state of the donor replicon. It seems unlikely that DAM would be responsible for a stimulation of transposition from a replicon blocked for replication in that DAM mediated transposition stimulation is thought to be a signal to induce transposition upon replication - rather than on the cessation of replication (Roberts et al., 1985). In Chapter 3 of this thesis we also saw that there may be a significant stimulation of transposition to *att*Tn7 at a defined "ring" in a colony (Chapter 3, Figure 3-5). This ring of transposition to *att*Tn7 may occur where the pOX38*gen*::miniTn7-*lac* donor

plasmid ceases replication due to conditions within the cells - though we have no evidence to support this hypothesis. Stimulation of transposition from a non-replicating replicon would be an attractive mechanism for evolutionary success. In this chapter we found a number of mutants which may affect the state of a donor replicon.

We found that mutation of the *min*D gene (part of the *min*BCD operon) can affect Tn7 transposition. The *min*B operon is involved in cell division control (de Boer et al., 1989; Lee and Price, 1993) and it was seen that Mutants #111 and 134 displayed the minicell phenotype (data not shown). Evidence suggests MinD acts both to activate the MinC-dependent division inhibition mechanism and is also required for the sensitivity of the division inhibition system to MinE (de Boer et al., 1991; de Boer et al., 1992). There is evidence that mutants in *min*D alter DNA super coiling and display some of the characteristics of *gyr*B mutants (Mulder et al., 1990). It is noted that *gyr*B is involved in a number of transposition systems (Berg and Howe, 1989). *In vitro*, donor DNA molecule supercoiling is required or stimulatory, under some conditions, for efficient transposition of Mu, IS10, and Tn7 (Bainton, 1992; Craigie et al., 1985; Surette et al., 1987). *In vivo*, mutants of *gyr*A affect Tn5, IS10, and Mu transposition (Isberg and Syvanen, 1982; Lundblad and Kleckner, 1982; Ross et al., 1986). It is attractive to suggest that mutation of *min* affects DNA supercoiling which in turn affects Tn7 transposition by altering TnsB binding to the ends of Tn7. Altered TnsB binding at the end of Tn7 may directly affect transposition by changes in synaptic transposition complex formation, or indirectly through alteration of *tns*AB transcription.

To further examine the involvement of *min* in Tn7 transposition it would be important to first determine which of the *min* genes is responsible for the transposition phenotype. Mutant #111 is most likely a polar insertions disrupting both *min*D and *min*E expression. Complementation of mutants #111 and #134 with plasmids expressing *min*C, *min*D, or *min*E could define which gene function is affecting transposition (if mutants #111 and #134 have a recessive phenotype). Alternatively, well characterized mutations

of *min* could be examined for their effects on Tn7. These tests would define which of the *min* gene functions may be involved in Tn7 transposition, however would not address the mechanism of their involvement.

To examine the mechanism of *min* involvement in Tn7 transposition one would focus on the likely possibility of altered DNA supercoiling affecting transposition. The effect of DNA supercoiling on TnsB binding to the ends of Tn7 is unknown, however under some conditions DNA supercoiling is important for efficient transposition of Tn7 *in vitro* (Bainton, 1992). Analysis of TnsB binding has used linear DNA fragments *in vitro* (Arciszewska et al., 1990; Arciszewska and Craig, 1991; Tang et al., 1991). *In vitro* and *in vivo* examination of TnsB binding to the ends of Tn7 on circular DNA substrates would enable examination of the effects of supercoiling on TnsB binding.

min and the other host components identified in this chapter may affect Tn7 transposition by altering the transcription of the *tns* genes. To test for affects on transcription of the *tns* genes one could attempt to use *tns-lacZ* fusions to quantitate expression, however the low level of *tns* transcription may create a problem of detection sensitivity. An alternative to directly quantitating the levels of *tns* expression would be to look for loss of the mutant phenotype upon *tns* expression from a heterologous (and presumably unaffected) promoter. The ability to reconstruct Tn7 transposition *in vitro* also adds the possibility of distinguishing post-transcriptional from transcriptional effects.

Linking Tn7 Transposition to Nucleotide Metabolism:

MiniTn10-*tet* insertions in *pur*MN, *gua*B, and *dcd* may indicate a regulatory link between nucleotide metabolism and Tn7 transposition. Insertions in these genes decreased miniTn7-*lac* transposition. A link between nucleotide metabolism and transposition has not been reported for other transposons. *pur*M and N code for phosphoribosylaminoimidazole synthetase and 5'-phosphoribosyglycinamide

transformylase, both involved in purine metabolism (Smith and 3d., 1987). *gua*B codes for IMP synthetase (Tiedeman and Smith, 1985; Tiedeman et al., 1985), and *dcd* codes for 2'-deoxycytidine 5'-triphosphate deaminase (which converts dCTP to dUTP) (Wang and Weiss, 1992).

Nucleotide starvation or alterations of the nucleotide pools may indirectly regulate *tns* expression through a global regulatory response. There is some evidence for a regulatory link between *gua*B and DNA replication and the stringent response in E. coli (Tesfa-Selase and Drabble, 1992; Chiaramello and Zyskind, 1990). As with the other mutants, to continue investigation of theses mutants it would be important to first ask whether the phenotypes of the insertion mutants are a result of the loss of function of the identified gene. Complementation of the *pur*MN, *gua*B, and *dcd* insertion mutants with *pur*MN, *gua*B, and *dcd* expressed from plasmids would be helpful in defining the nature of the mutations affecting Tn7 transposition.

Tn7 Transposition and Transcription:

Two mutants identified in this chapter are involved in translation. Mutant #132 was found to be in *lys*S which codes for the E. *coli*'s major lysyl-tRNA synthetase (Emmerich and Hirshfield, 1987). Mutant #174 was in either *rps*G (encoding the 3S/S7-subunit of the small ribosomal protein complex) or *fus*A (encoding the protein elongation factor EF-G; Zengel et al., 1984) and may have affected expression of the down-stream gene *tuf*A (encoding the protein elongation factor EF-tu; Post and Nomura, 1980)(see Appendix A). How these gene functions modulate the frequency of Tn7 transposition is unknown, however it is tempting to hypothesize that a defect in E. *coli*'s major lysyl-tRNA synthetase would result in the stringent response - and thus globally affect transcription and DNA replication. Alternatively, mutants #132 and #174 may affect transposition by directly affecting *tns* transcription.

Outlook:

The goal of this work was to identify host components involved in the facilitation or regulation of Tn7 transposition. In the previous chapter of this thesis, we examined the effects of mutations in *hns*, *dam*, *fis*, and IHF's *hip* and *him*A on Tn7 transposition. The mutants identified in this chapter have allowed us to define sixteen new genetic loci affecting the frequency of Tn7 transposition. Perhaps the most interesting of these new mutants are those involved in global transcription regulation in response to the anaerobic or aerobic state of metabolism. The aerobic state of metabolism has not been connected with transposition before, yet it appears that these mutants affect Tn10 transposition as well as Tn7 transposition. Examinations of the involvement of DAM and the histone-like proteins in Tn7 transposition, however, are likely to be the most productive lines of investigation in that likely targets of their action are well defined.

Index to Detailed Information in Appendix A on Mutations Affecting Tn7 Transposition

Table 5-1
Summary of New Mutant Data

Mutant#	Transposition Phenotype: Papillation Tn7lac	Papillation Tn10lac	Mating Out Tn7	Mating Out Tn7lac	Sequence Tagging: Outside F/T3	Inside OH2	Inside OH3	Outside T7/R	Location: Seq. (min)	P1 Linkage Confirmation	Comments
wt	***	***	1.0	1.0							
Histone-Like Protein:											
43	*	*	0.02	0.4	No Match	**hns**	nd	No Match	27.25	√	
3	**	**	0.05		-	--Not Cloned --		-		√	
Cell Division Control:											
111	****	****	0.06	15	No Match	**minD**	**minD**	**minD**	26.3	√	Mini cells
134	****	****	1.1	0.6	-	--Not Cloned --		-		√	Mini cells
Anaerobic vs. Aerobic Metabolism: Insertions in genes known to affect anaerobic or aerobic metabolism.											
182	******	*****	0.6	1.5	No Match	**gltA**	**sdhC**	**sdhC**	16.5	√	Slow growth
4	**		1.4	0.2	nd	nd	**sdhB**	nd	16.5	√	Slow growth
57	******	**	0.2	0.5	**gidA**	nd	nd	**uncB**	84.2	√	Slow growth
58	**	***			-	--Not Cloned --		-		√	Slow growth
Insertion in a global transcription regulator responsive to anaerobic vs. aerobic metabolism.											
50	**	**	2.5	1.8	No Match	**arcB**	**arcB**	No Match	69.5	√	
Insertion very near (possibly in) a global transcription regulator responsive to anaerobic vs. aerobic metabolism.											
73	**	**	0.01	0.8	**soxRS**	nd	nd	No Match	92.1	√	Within 1.2 Kb
Insertion very near (possibly affecting expression of) a gene involved in anaerobic metabolism.											
123	*****	*****	0.4	0.7	**menB**	No Match	nd	No Match	49	√	Within 2 Kb
79	**	*****	0.3	0.5	**menB**	No Match	No Match	No Match	49	√	Within 2 Kb
Nucleotide Metabolism: Insertions in genes involved in nucleotide metabolism.											
20	*-	*****	2.1	0.6	**guaB**	**purN**	**purM**	No Match	53.75	√	
75	-	*-	1.2	0.2	ø	**guaB**	**guaB**	ø	53.75	√	
106	**	***	1.6	0.3	No Match	**dcd**	**dcd**	ø	46	nd	
Translation: Insertion in genes involved in translation.											
132	*-	**	0.6	0.7	No Match	**lysS**	**lysS**	nd	61.7	nd	
174	*-	***	4.2	1.0	**rpsG**	ø	ø	**fusA**	73.5	√	rpsG adjoins fusA

Table 5-1 cont.
Summary of New Mutant Data

Insertions Located Near Known Genes:

Mutant#	Transposition Phenotype				Sequence Tagging				Location		Comments
	Papillation		Mating Out		Outside	Inside	Inside	Outside	Seq.	P1 Linkage	
	Tn7lac	Tn10lac	Tn7	Tn7lac	F/T3	OH2	OH3	T7/R	(min)	Confirmation	
wt	***	***	1.0	1.0							
17	**	***	1.7	1.8	**serS**	No Match	No Match	No Match	19.9	√	Within 4.5 Kb
190	****	***	13	5.2	**serS**	No Match	No Match	No Match	19.9	√	Within 4.5 Kb
6	**	***	2.6	0.3	No Match	No Match	No Match	**tesB**	10.4	-	Within 2 Kb
53	**	***	0.5	0.2	**fliC**	No Match	nd	No Match	42.6	nd	Within 2 Kb

Insertions not Located :

	Transposition Phenotype				Sequence Tagging				Location	
	Papillation		Mating Out		Outside	Inside	Inside	Outside	Seq.	P1 Linkage
	Tn7lac	Tn10lac	Tn7	Tn7lac	F/T3	OH2	OH3	T7/R	(min)	Confirmation
wt	***	***	1.0	1.0						

Cloned Mutants With No GenBank Matches:

61	****	*****			No Match	No Match	nd	No Match		
62	*	**	0.2	0.1	No Match	No Match	No Match	nd		
118	-	-	3.6	2.6	No Match	No Match	No Match	nd		
171	*****	**	2.5	1.0	No Match	nd	nd	No Match		

Mutant Not Cloned:

49	**		0.6	0.2	-	--Not Cloned --		-		

Table 5-1 Legend

Summary of New Mutant Data

A summary of data on the twenty six E. *coli* mutants affecting Tn7 transposition is presented in Table 5-1. The table presents both papillation and mating out phenotypes, of each mutant under "Transposition Phenotype", the identity of DNA sequence flanking the miniTn10-*tet* insertions under "Sequence Tagging", and the location of the GenBank matched sequences as well as P1 transduction confirmation of the sequence assigned location under "Location" . Comments on growth rate and the distance of the miniTn10-*tet* insertion to GenBank matched sequence are noted. Each of these categories is explained below in detail.

Transposition Phenotype:

Papillation:

From left to right across the table, the mutant identification number is followed by representation of miniTn7-*lac* and miniTn10-*lac* papillation activity in the mutant background. The greater the papillation activity, the more "*"s, with *** equal to the papillation activity within the non-mutant (top "wt" line). Papillation was evaluated in NLC28*att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* for miniTn7-*lac* papillation, and NLC28 pSIL(miniTn10-*lac*) for miniTn10-*lac* papillation. Photographs of miniTn7-*lac* papillation in the mutant backgrounds is reported in Figure 5-4, and photographs of miniTn10-*lac* papillation in Figure 5-5. Note: Mutant # 174 was originally scored as increasing miniTn7-*lac* papillation in colonies, however upon retesting transductants of mutant #174 papillation in patches was found to be decreased (see Figure 5-4.174).

Mating-Out:

Translocation of Tn7 and miniTn7-*lac* in NLC28*att*Tn7::miniTn7-*lac* ϕ80dlacII::Tn7 pOX38*gen* to pOX38*gen* was measured by the mating-out assay as described and reported in Figures 3 and Appendix A, Table A-4. Transposition frequency is reported as the ratio of transposition frequency for Tn7 or miniTn7-*lac* in the mutant strain background to the non-mutant strain background. Transposition to pOX38*gen* is *tns*ABC+E dependent. The data for this section is graphically reported in Figure 5-3, and tabulated in Appendix A, Table A-4.

Identification by Sequence Tagging:

Sequence from four different locations on the cloned miniTn10-*tet* insertion mutations was used to probe GenBank using the Blastn sequence matching algorithm (Altschul et al., 1990). In order to sequence from either end of the transposable element into flanking chromosomal DNA, oligo-nucleotide primers unique to *tet*R (OH3) and *tet*A (OH2) were employed. To sequence in from both of the vector / chromosomal insert junctions, either the T7 and T3 primers for pBluescript clones, or the F(orward) and R(everse) primers for pK184 and pK194 clones were employed.

Sequence identity matches from the plasmid vector / chromosomal insert junctions are reported under "Outside" - F or T3, and R or T7 sequence primer headings. Sequence identity matches from the miniTn10-*tet* insert / chromosomal junctions are reported under "Inside" - OH2, and OH3 sequence primer headings. Where no sequence information was obtained "nd" is noted in the table. Where identity of obtained sequence could not be assigned by matches with sequences in GenBank, "No Match" is indicated. Conflicting sequence data indicated as "ø" was obtained for mutants #75, 106, and 174, likely as a result of confusing plasmids, and is reported in detail in Appendix A. The information in these "Identification by Sequence Tagging" section is drawn from the detailed information presented for each mutant in Appendix A.

Location:

The location on the E.*coli* chromosome of the GenBank matched sequence is indicated in minutes. The miniTn10-*tet* insertions were tested to confirm genetic linkage by P1 transduction to a marker near the GenBank sequence match assigned location (see Table 5-5). Mutations confirmed to be near the sequence indicated position are designated by "√", while mutants not tested are indicated by "nd".

Comments:

A few of the miniTn10-*tet* insertion generated mutations are associated with notable growth phenotypes in addition to the transposition phenotypes they were selected for. As noted, mutants #182, 4, 57 and 58 grow slowly, while mutants #111 and #134 produce "mini-cells" as expected for the mutations in the *min*B locus (de Boer et al., 1989). Distance to the GenBank matched sequence is indicated for mutants where the point of miniTn10-*tet* insertion was not located. Distance to insertion is based on insert size of cloned mutant minus the 3 Kb size of miniTn10-*tet*.

Figure 5-1

MiniTn10-*tet* On λ1098 Used For Transposon Mutagenesis

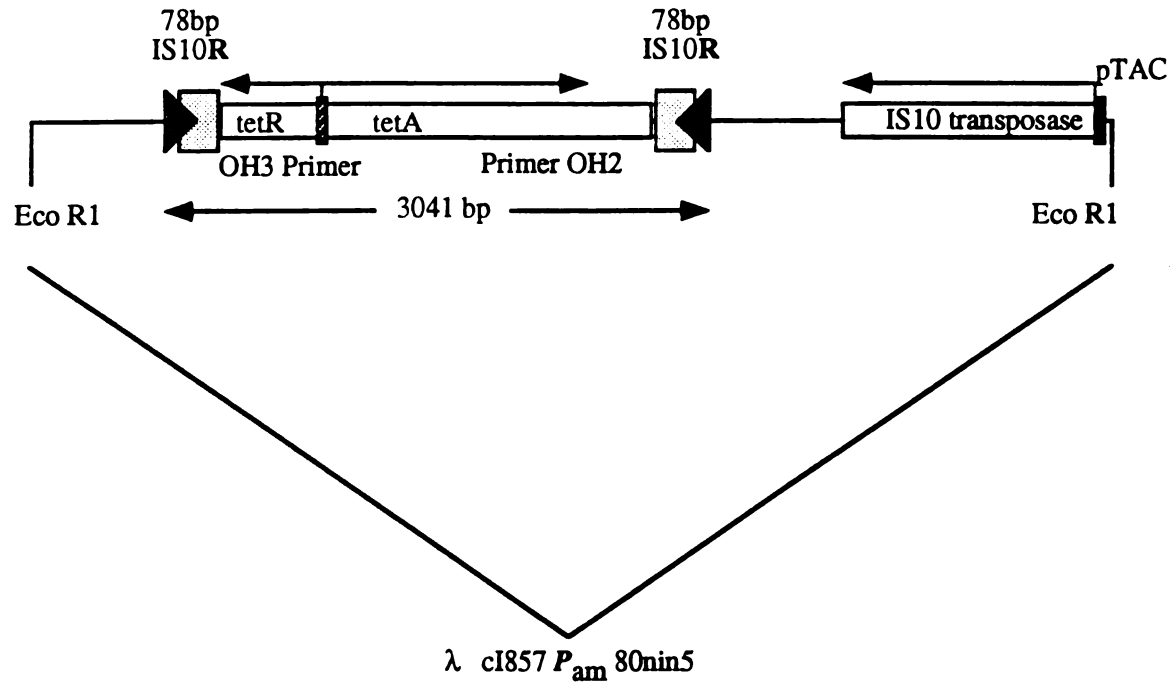


Figure 5-1 Legend

MiniTn10-*tet* On λ1098 Used For Transposon Mutagenesis

The miniTn10-*tet* transposon donor used to mutagenize E. *coli* in this chapter is depicted (not to scale). MiniTn10-*tet* is composed of a 2885 bp *tet*AR fragment from Tn10, flanked on each end by the 78 terminal bases of the right end of IS10 (IS10**R**) (Way et al., 1984). Transposase for miniTn10-*tet* is expressed from a pTAC promoter, resulting in a transposition frequency of 10^{-5}. The DNA fragment containing miniTn10-*tet* and transposase are carried on λ1098. λ1098 is a replication/recombination defective phage, with cI857 P_{am} 80*nin*5.

Figure 5-2

Screening For E. *coli* Mutants Affecting Tn7 Transposition

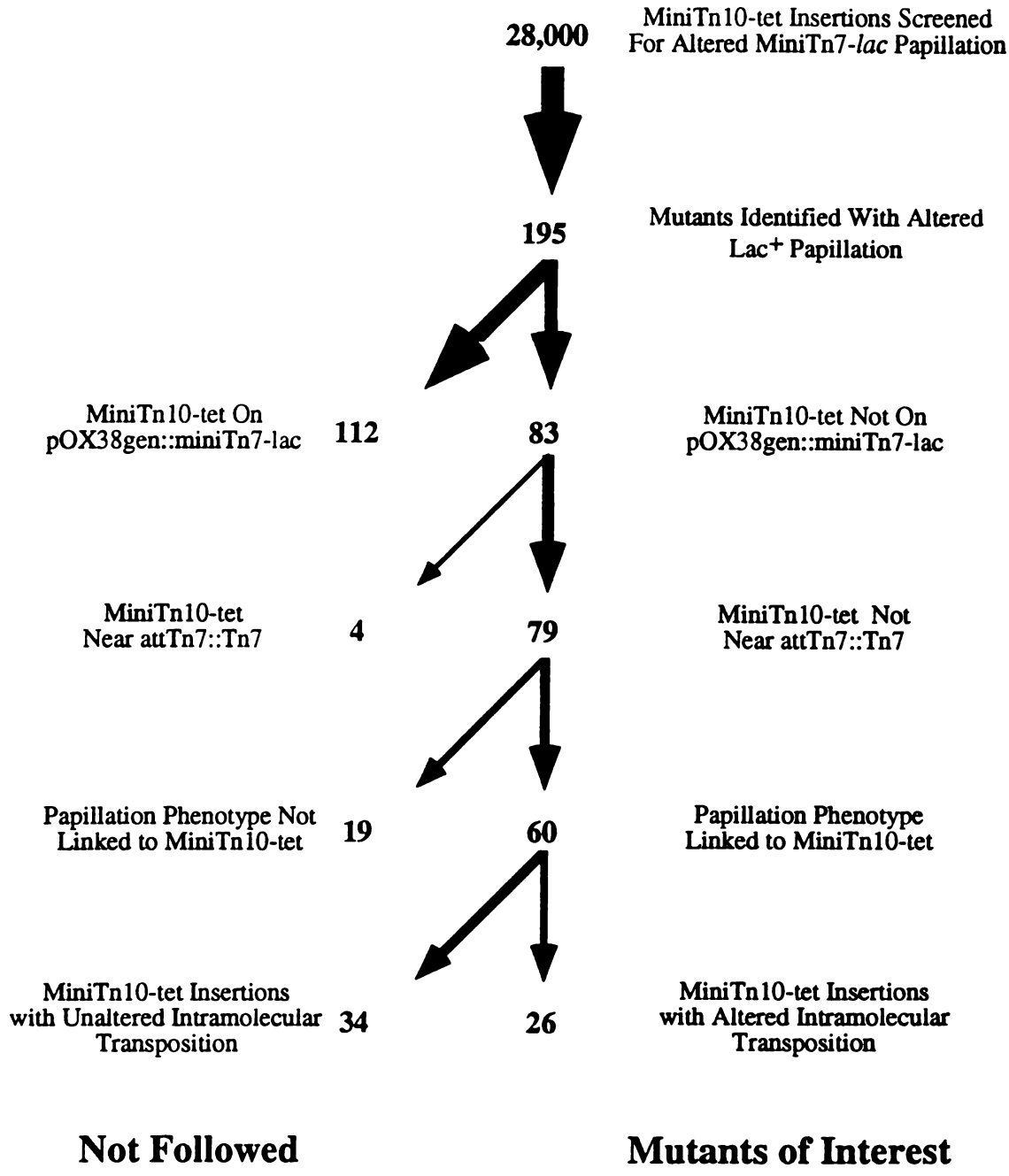


Figure 5-2 Legend

Screening For E. *coli* Mutants Affecting Tn7 Transposition

A summary of the genetic screens used to identify E. *coli* mutants affecting Tn7 transposition is depicted. Infection of NLC28*att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* with λ1098 was used to generate 28,000 tetracycline-resistant colonies that were screened for altered miniTn7-*lac* papillation on MacConkey Lactose indicator plates (as described in Materials and Methods). 195 colonies with altered Lac^+ papillation where chosen and tested for chromosomal or F' insertion location of miniTn10-*tet* by conjugation of F' to CW51 where tetracycline resistance was scored (see Appendix A, Table A-1). The 83 chromosomal miniTn10-*tet* insertions were tested for miniTn10-*tet* insertion near *att*Tn7::Tn7 by P1 transduction (see text and Appendix A, Table A-2). The 79 miniTn10-*tet* insertions not linked by P1 transduction to *att*Tn7::Tn7 were retested for altered miniTn7-*lac* papillation upon P1 transduction of miniTn10-*tet* into naive NLC28*att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* (see Appendix A, Table A-3). 60 miniTn10-*tet* insertions with altered miniTn7-*lac* papillation were tested for altered Tn7 and miniTn7-*lac* intramolecular transposition frequency as measured by the mating-out assay (see Figure 5-3 and Appendix A, Table A-4). 26 miniTn10-*tet* insertions with altered Tn7 and miniTn7-*lac* intramolecular transposition frequency as measured by the mating-out assay were chosen for further characterization (see Table 5-1, and sequence information in Appendix A).

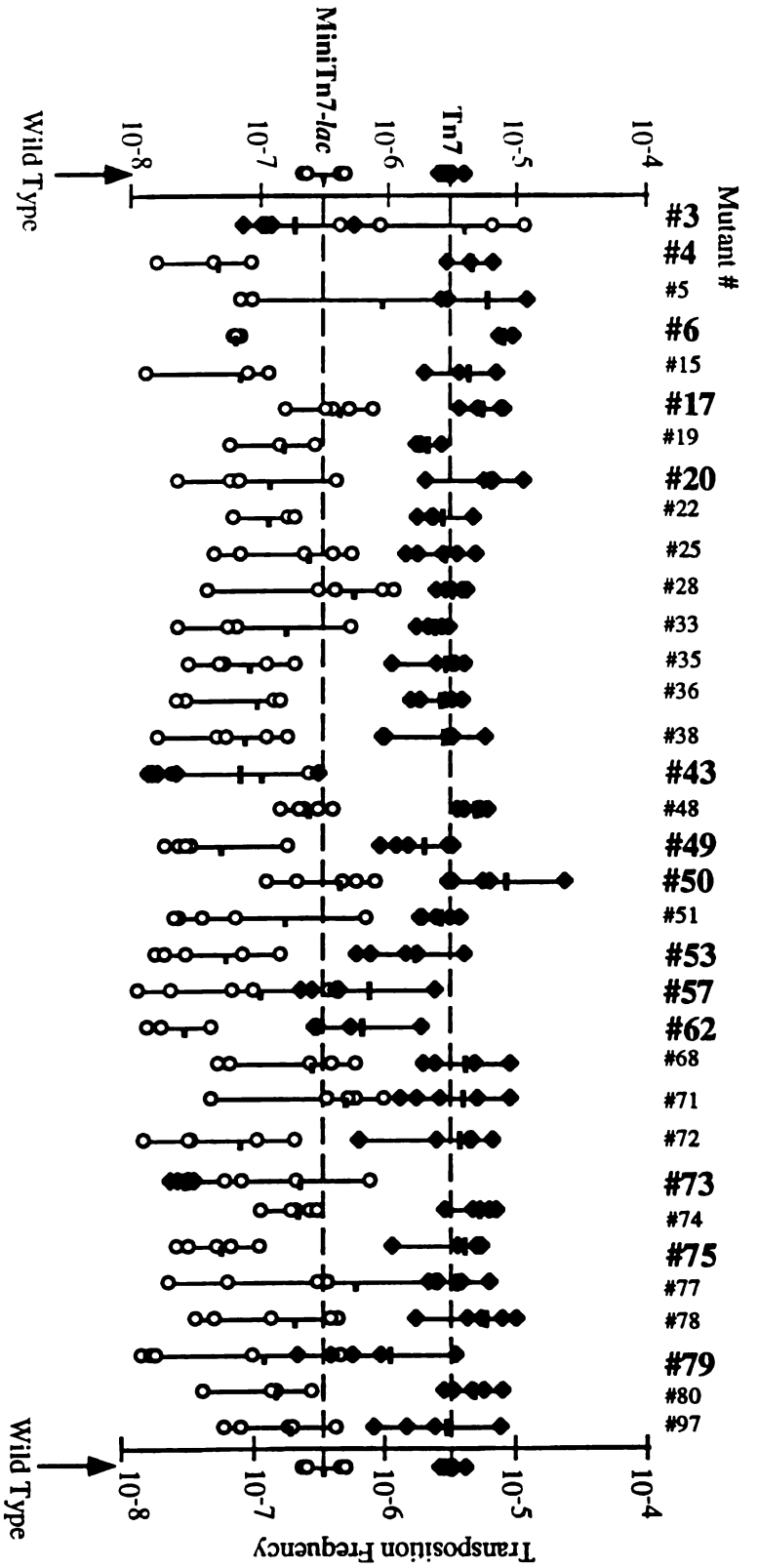


Figure 5-3A

Transposition Frequency of Tn7 and MiniTn7-*lac* in Mutants 3-97

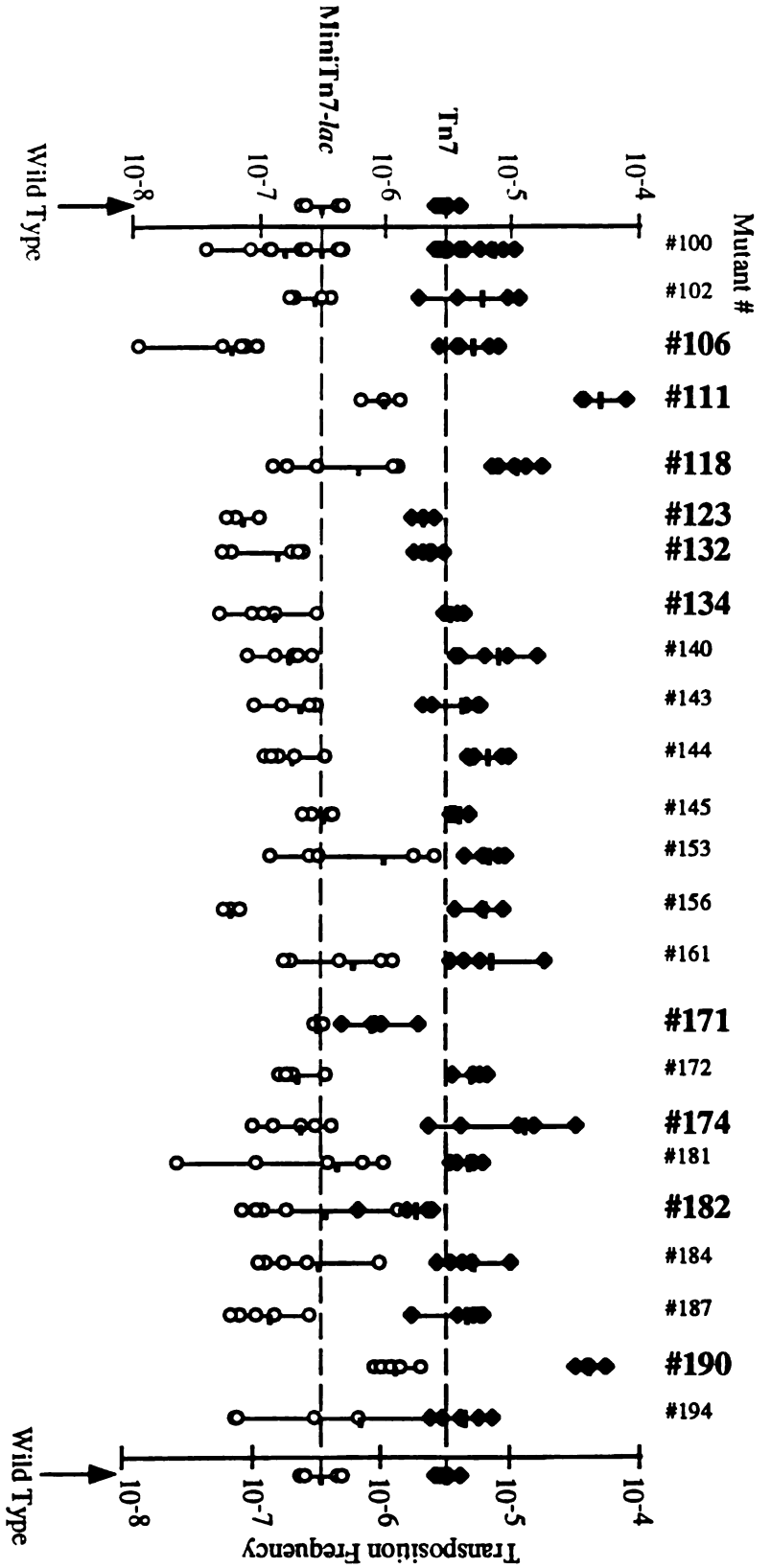


Figure 5-3B

Transposition Frequency of Tn7 and MiniTn7-*lac* in Mutants 100-194

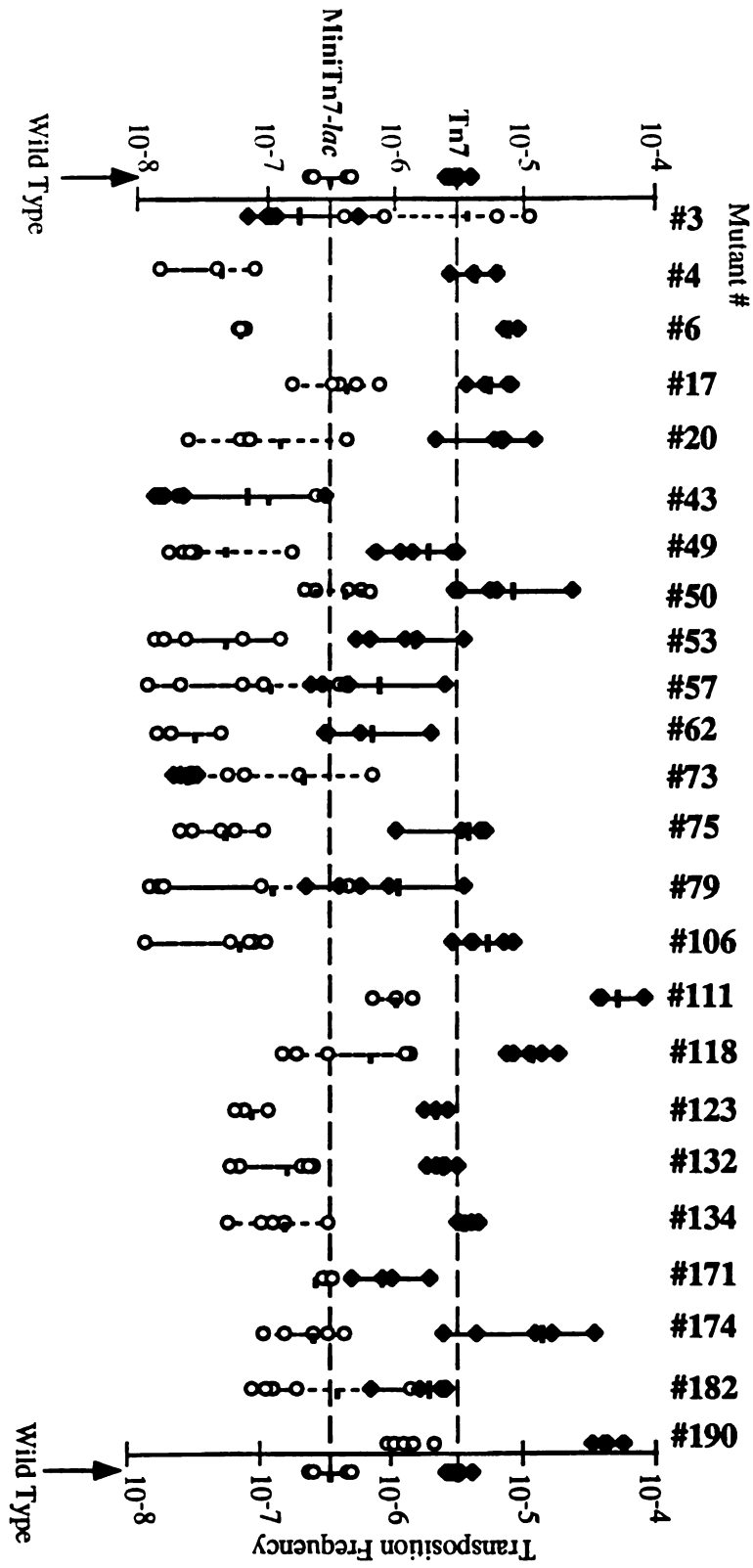


Figure 5-3C

Transposition Frequency of Tn7 and MiniTn7-*lac* in Mutants Chosen For Further Study

Figure 5-3 Legend

Transposition Frequency of Tn7 and MiniTn7-*lac*

Results of mating-out assays to test the transposition frequency of Tn7 and miniTn7-*lac* to the F' pOX38*gen* in miniTn10-*tet* insertion mutants are depicted. Figure 5-3A reports mating-out frequencies from mutants #3 through #97. Figure 5-3B reports data from mutants #100 through #194, and Figure 5-3C summarizes information from mutants that displayed altered transposition and were chosen for further characterization. The F' donor strain for these mating-out assays was NLC28*att*Tn7::miniTn7-*lac* ϕ80dlacII::Tn7 pOX38*gen*, in which miniTn7-*lac* transposes from the chromosomal *att*Tn7 site, and Tn7 transposes from a second chromosomal donor site, ϕ80dlacII. The mathing-out assay was performed as described in Materials and Methods. The result of 5 independent trials for each mutant is plotted (open circles for miniTn7-*lac*, and filed diamonds for Tn7), along with their arithmetic averages (bar). Tn7 and miniTn7-*lac* transposition frequencies in wild type NLC28 (no miniTn10-*tet*) are indicated on each side of the data set, and their average values cross (dashed lines) the data set. Transposition frequencies represent the proportion of F's onto which Tn7 or miniTn7-*lac*. have transposed, and are plotted on a logarithmic scale between 10^{-8} and 10^{-4}. Mutants selected for further characterization are indicated by the large, bold font labels. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

Figure 5-4.3
MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #3
(NLC28::miniTn10-*tet*$^{\#3}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

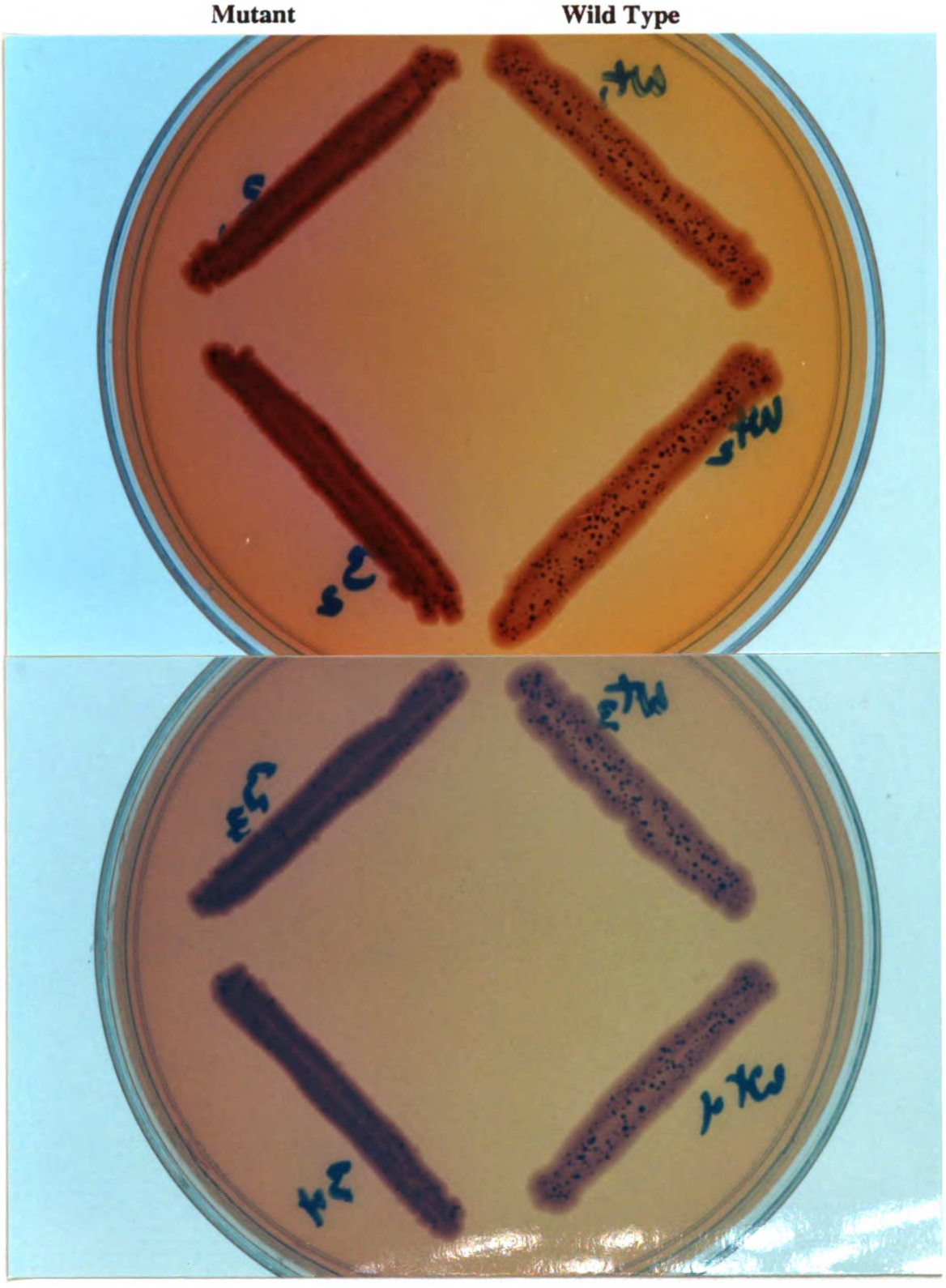


Figure 5-4.4
MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #4
(NLC28::miniTn10-*tet*$^{\#4}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

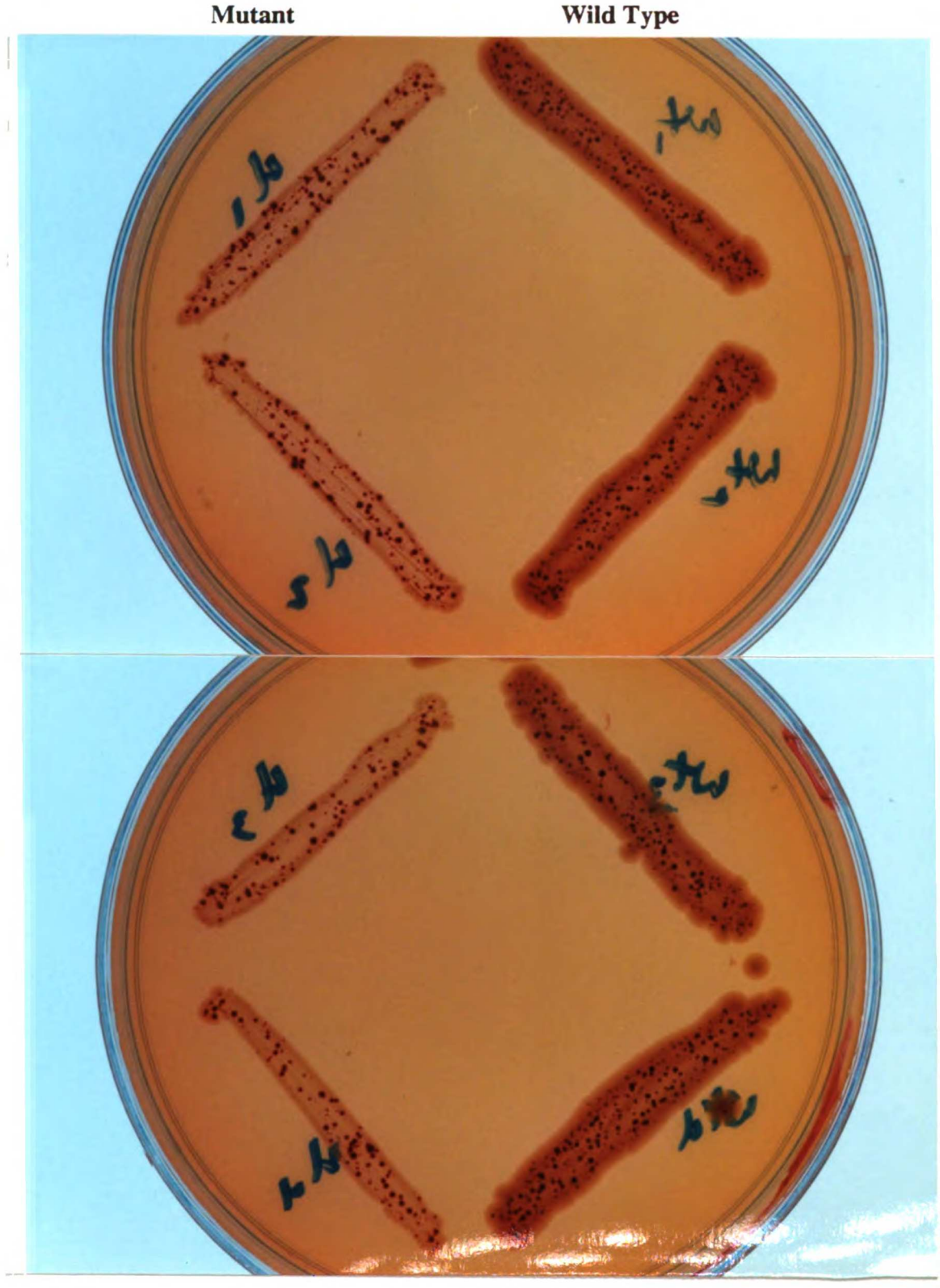


Figure 5-4.6
MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #6
(NLC28::miniTn10-*tet*$^{\#6}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

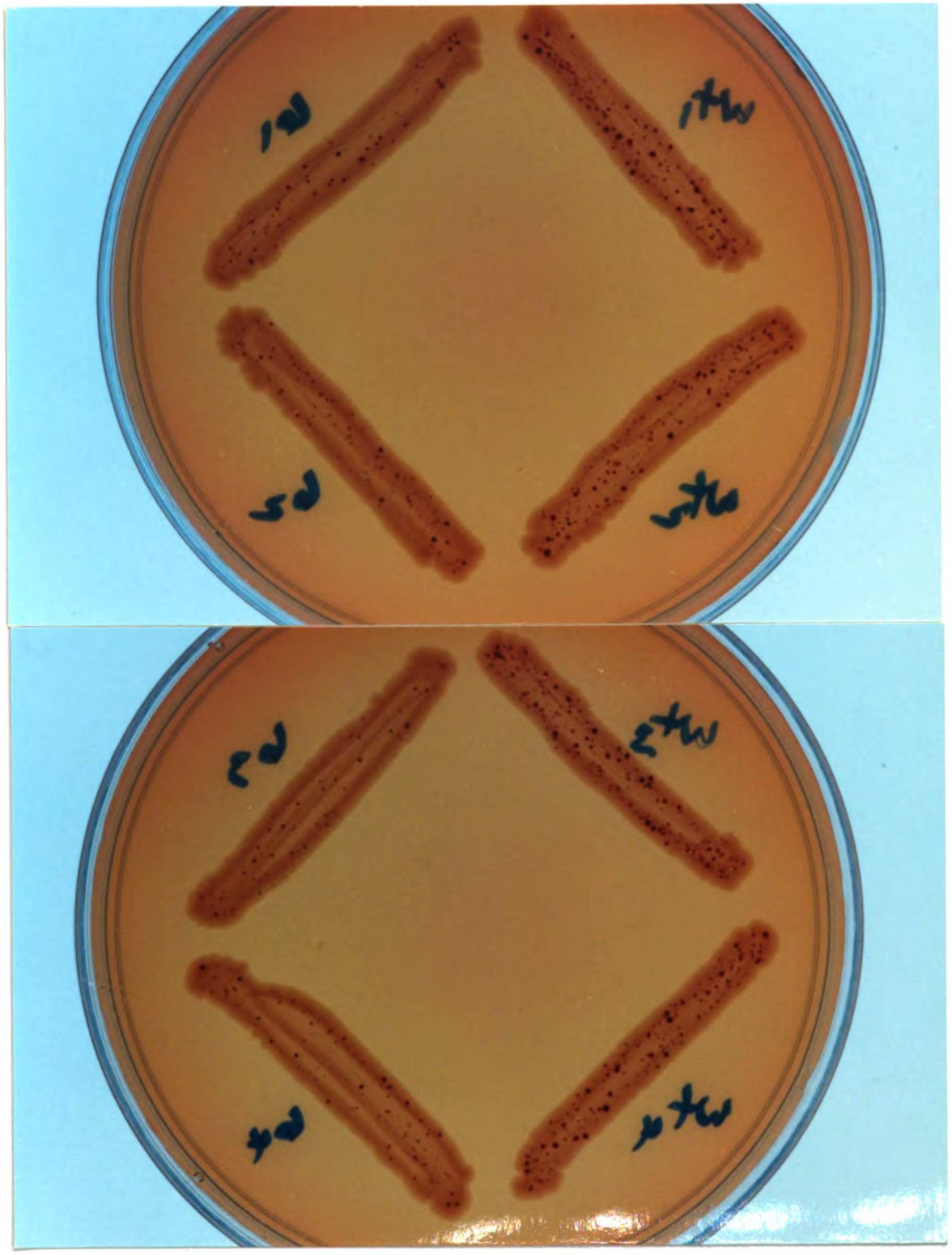


Figure 5-4.17
MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #17
(NLC28::miniTn10-*tet*$^{\#17}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

Mutant **Wild Type**

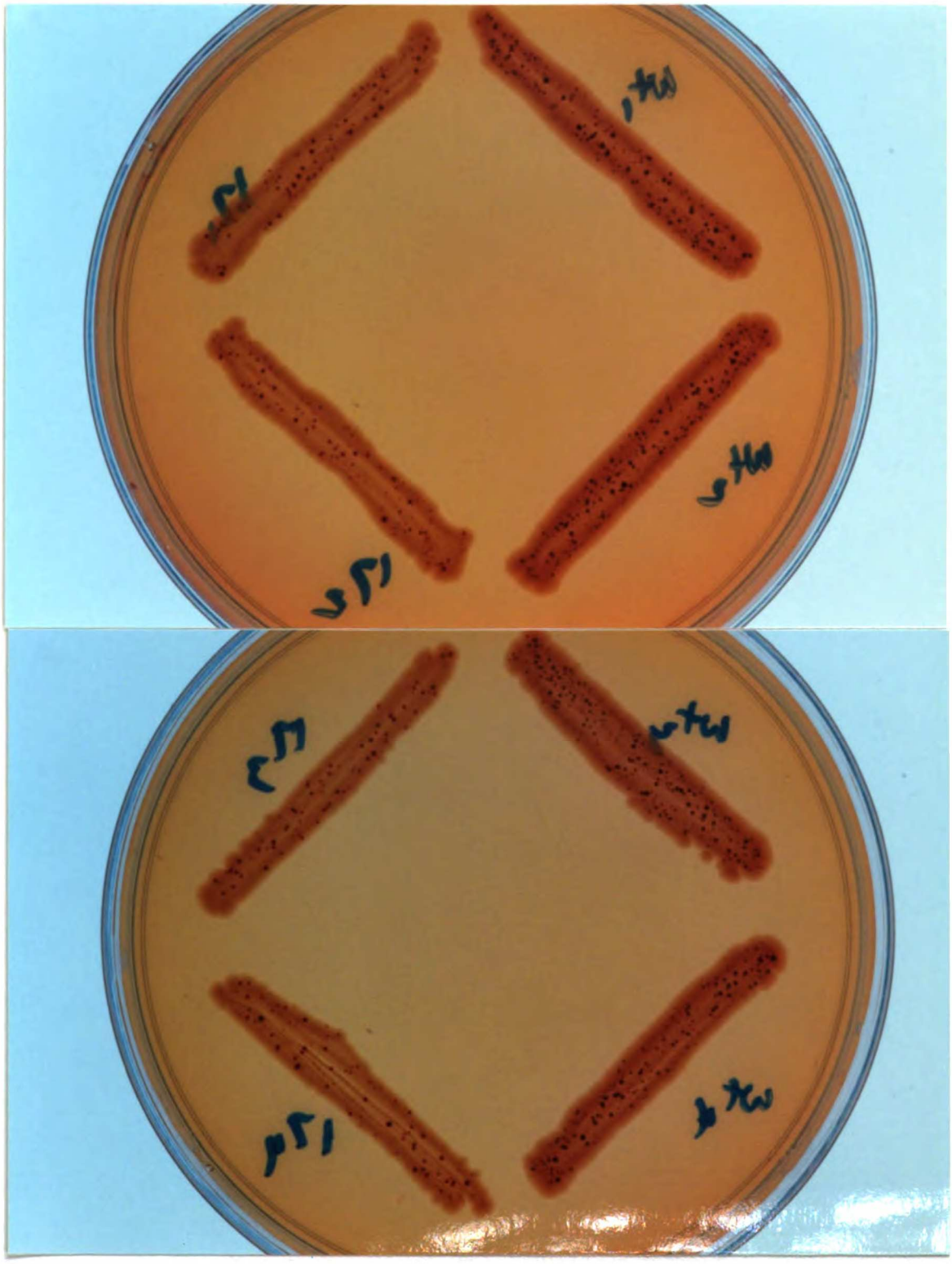


Figure 5-4.20

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #20
(NLC28::miniTn10-*tet*$^{\#20}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

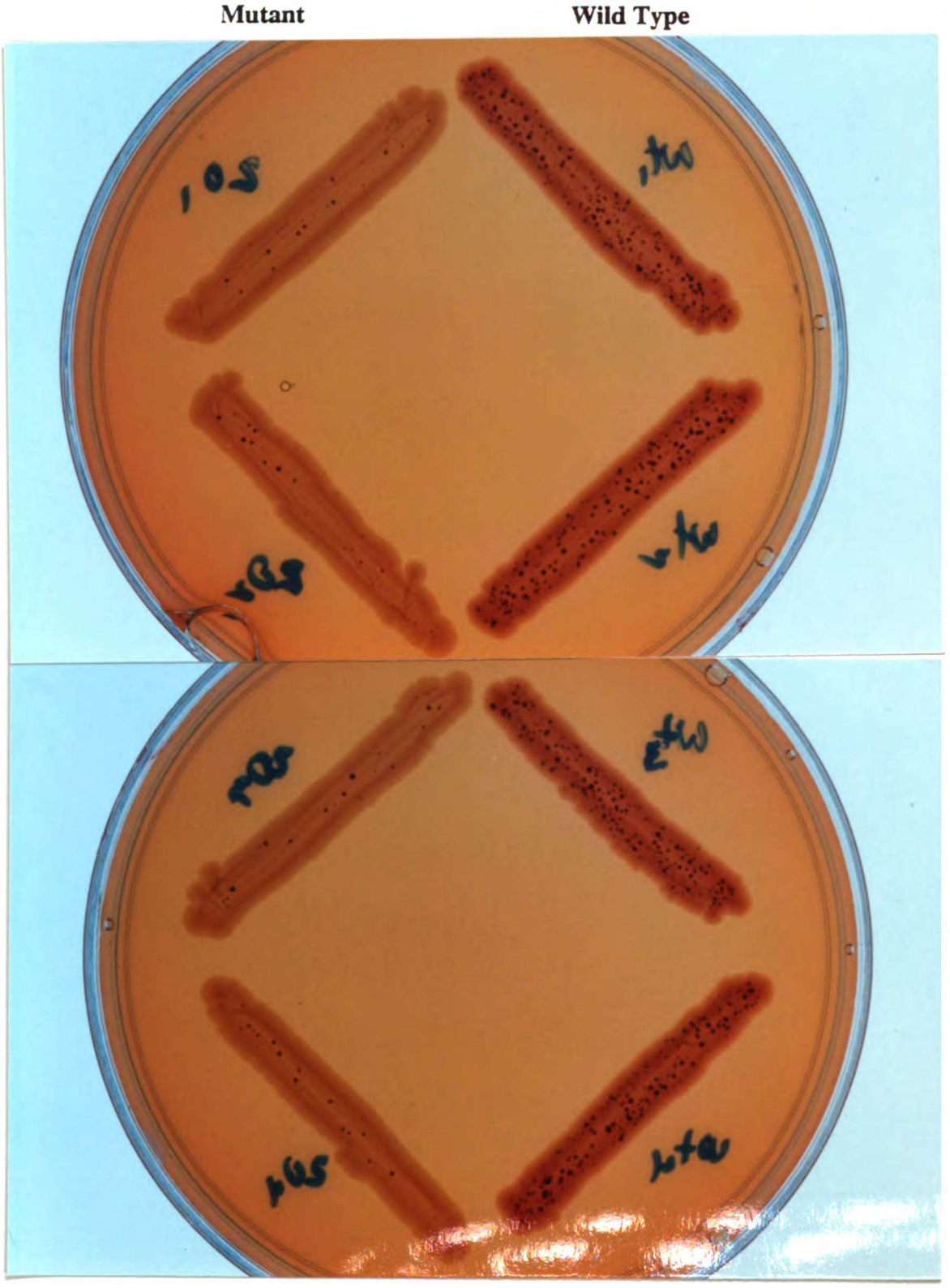


Figure 5-4.43

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #43
(NLC28::miniTn10-*tet*$^{\#43}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

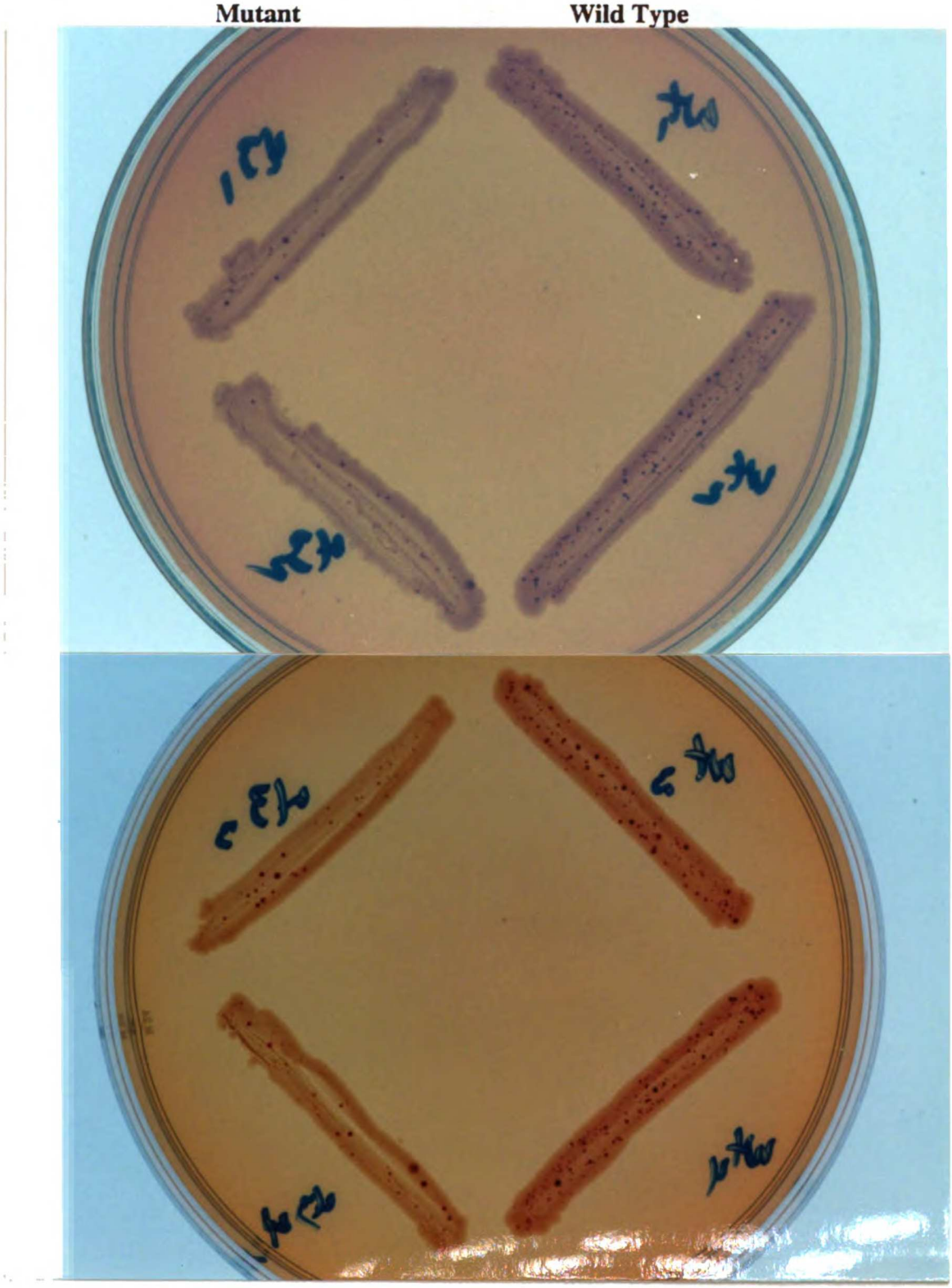


Figure 5-4.49
MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #49
(NLC28::miniTn10-*tet*$^{\#49}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

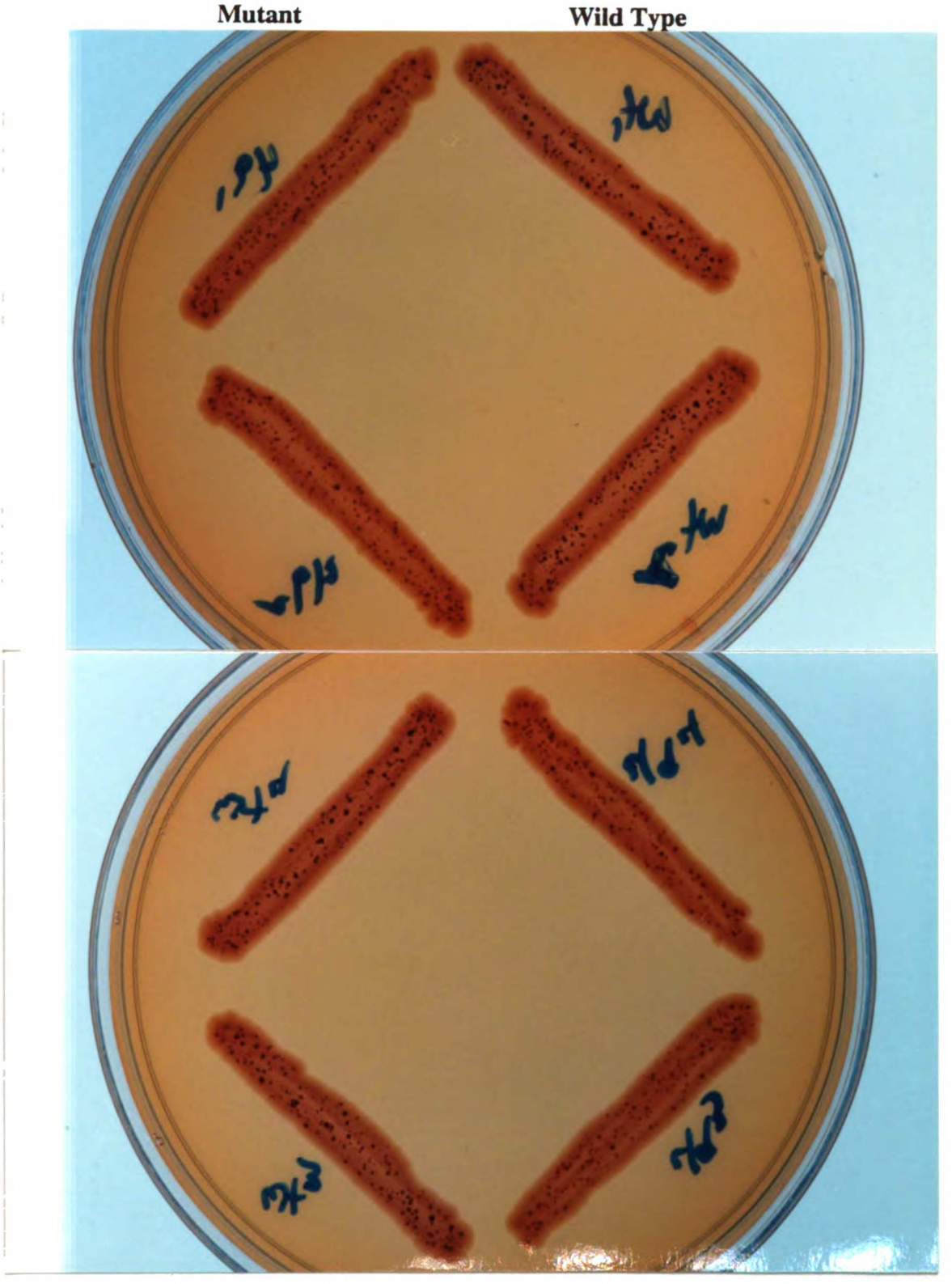


Figure 5-4.50

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #50
(NLC28::miniTn10-*tet*$^{\#50}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

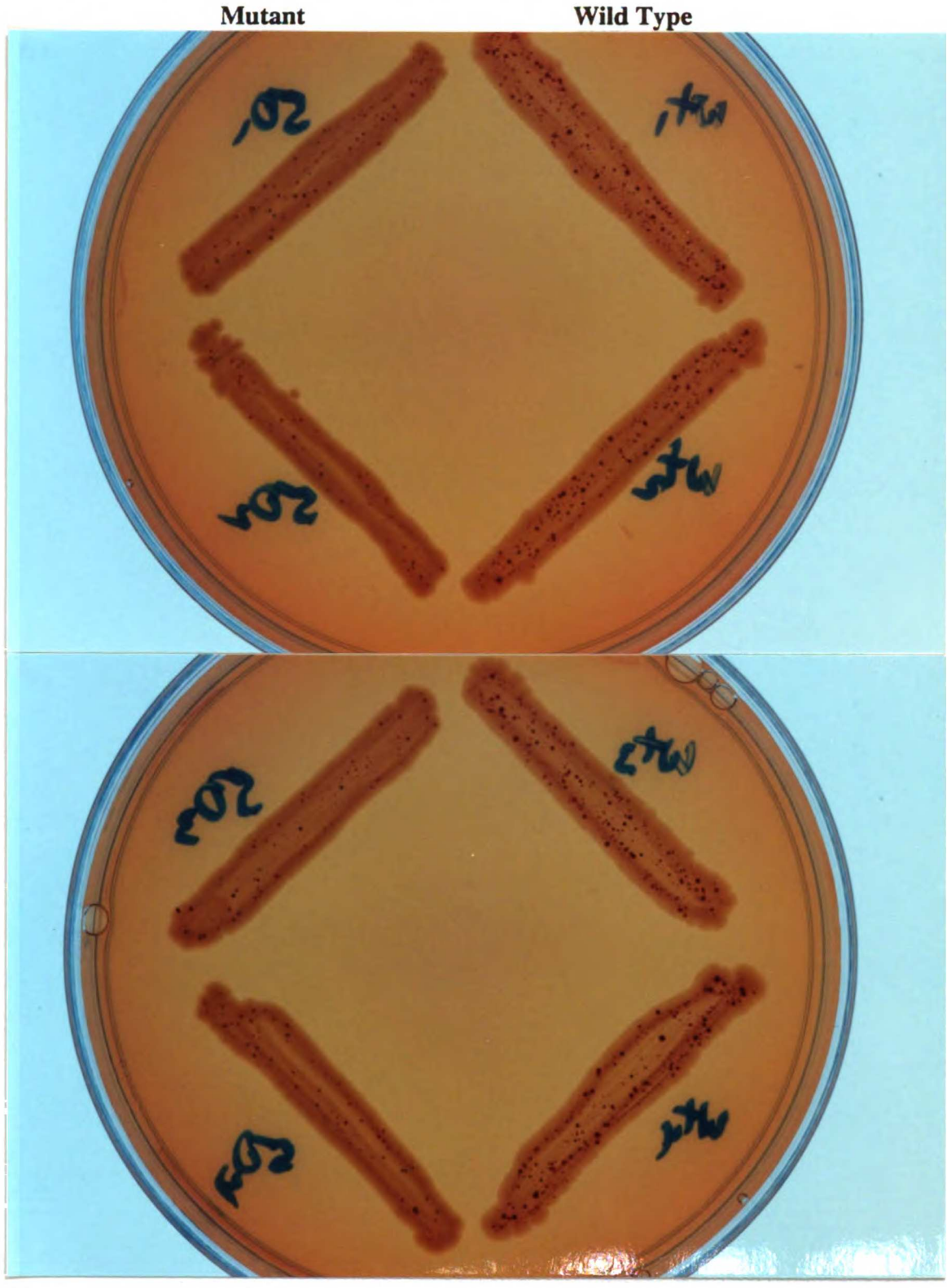


Figure 5-4.53
MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #53
(NLC28::miniTn10-*tet*$^{\#53}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

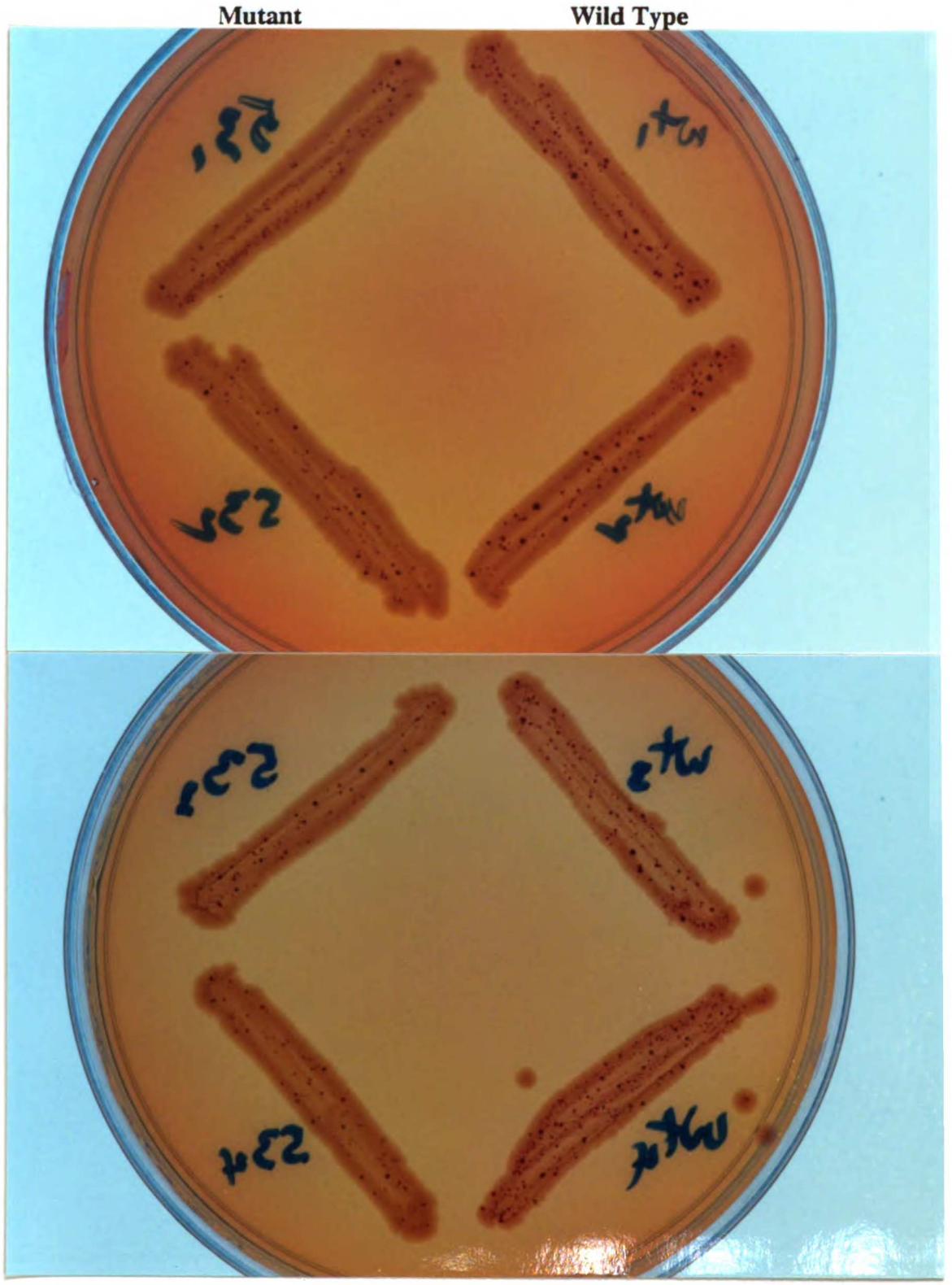


Figure 5-4.57

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #57
(NLC28::miniTn10-*tet*$^{\#57}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

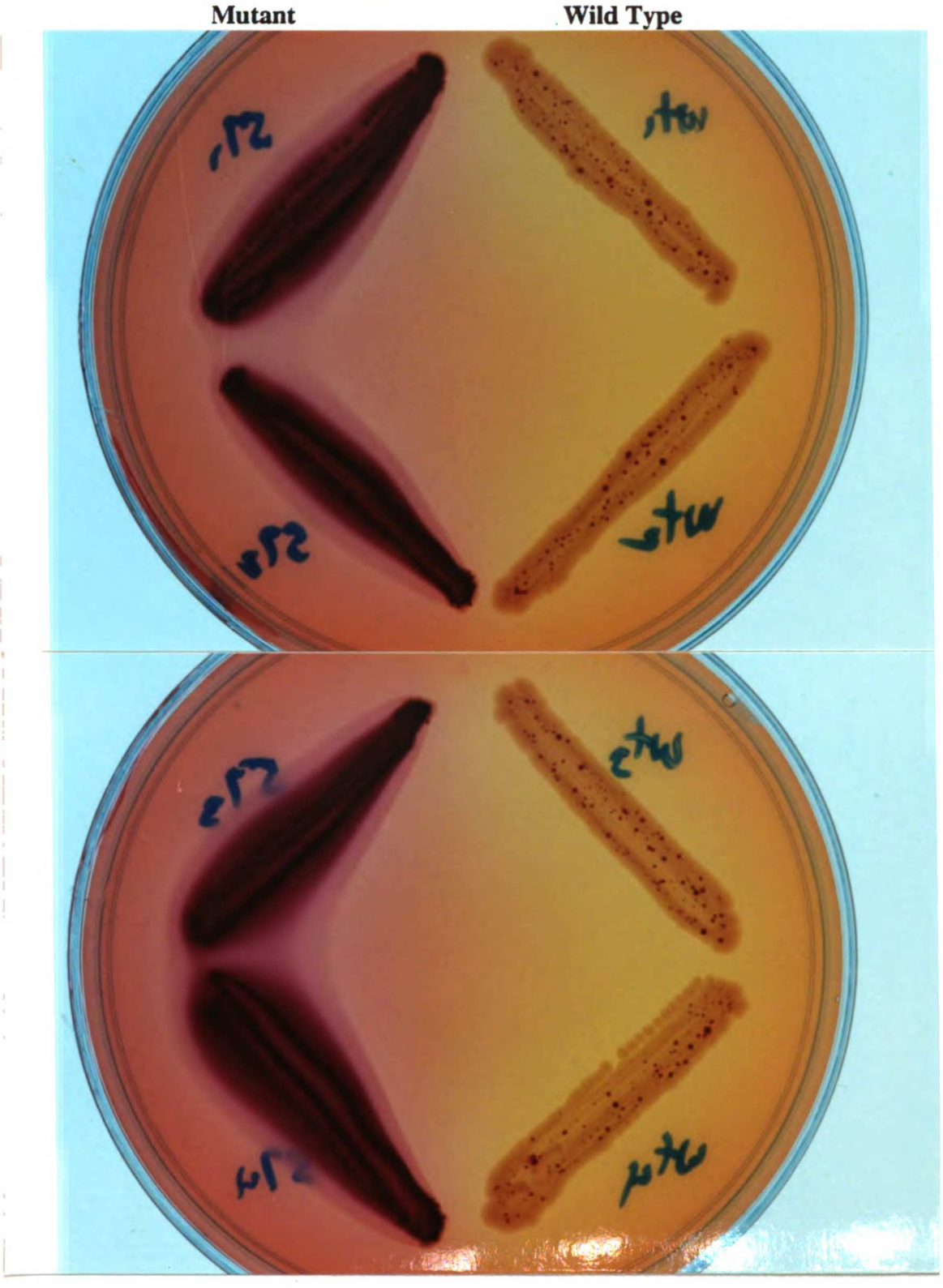


Figure 5-4.58

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #58
(NLC28::miniTn10-*tet*$^{\#58}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

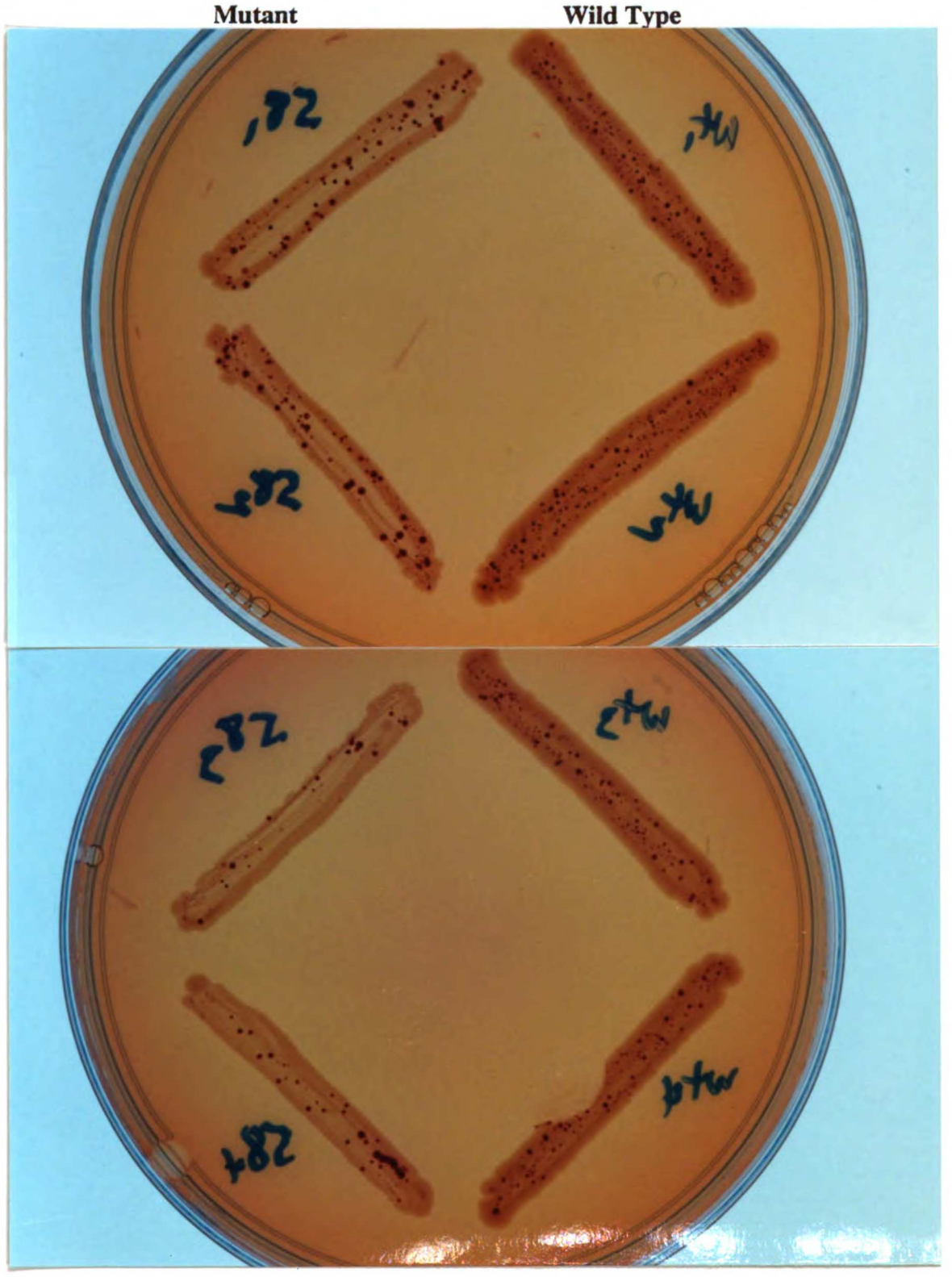


Figure 5-4.61

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #61

(NLC28::miniTn10-*tet*$^{\#61}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

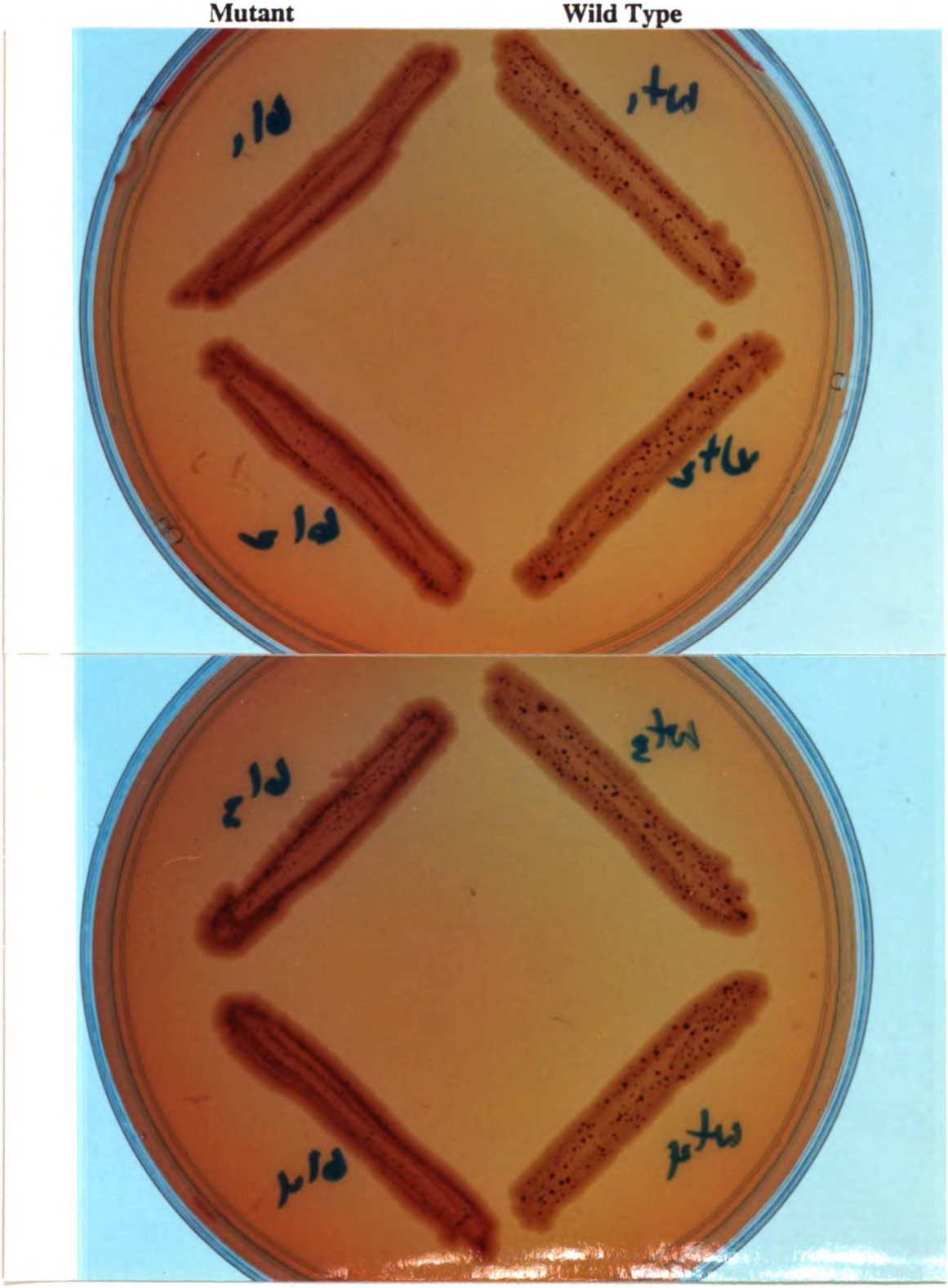


Figure 5-4.62

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #62

(NLC28::miniTn10-*tet*$^{\#62}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

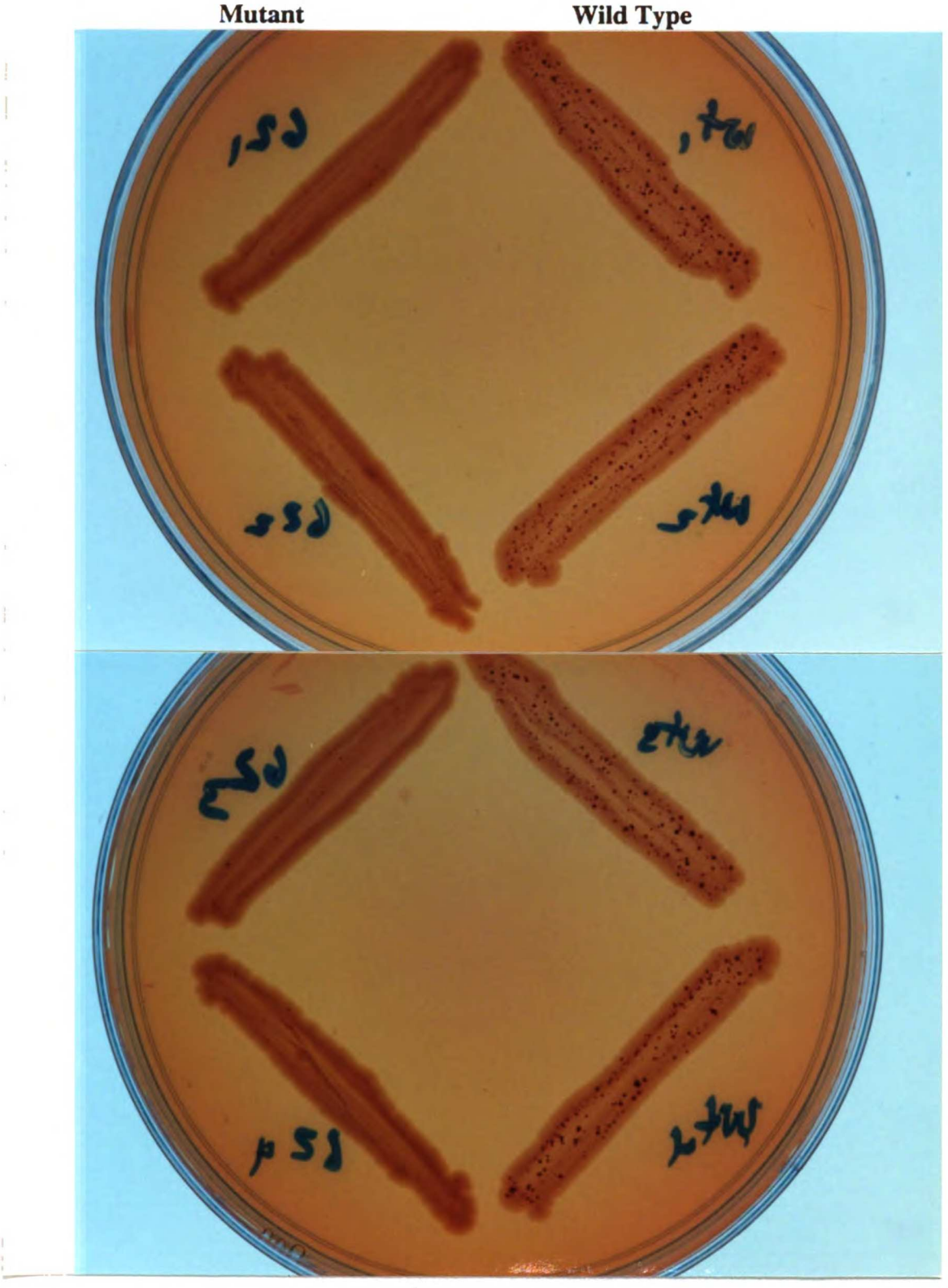


Figure 5-4.73

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #73

(NLC28::miniTn10-*tet*$^{\#73}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

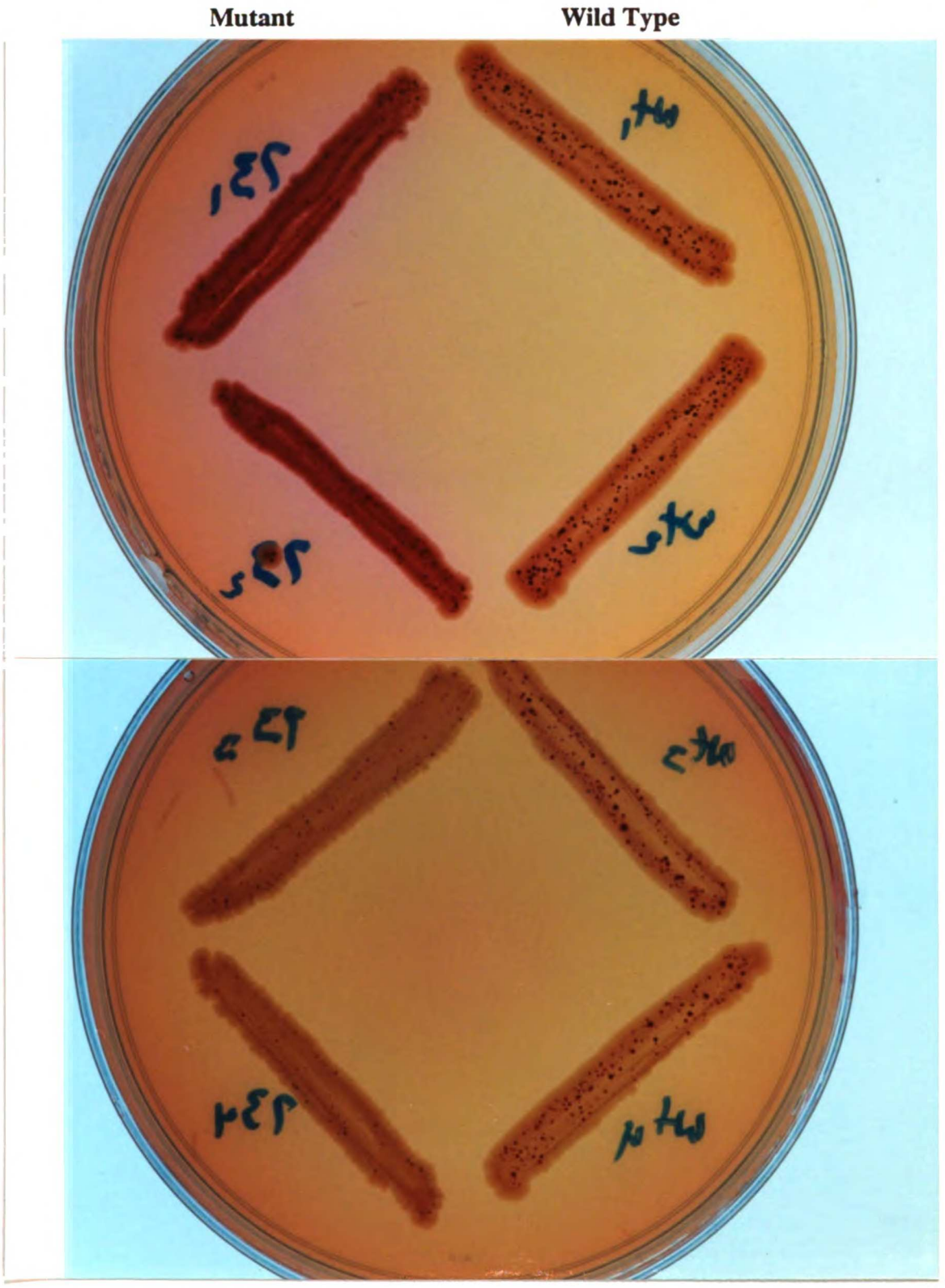


Figure 5-4.75

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #75
(NLC28::miniTn10-*tet*$^{\#75}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

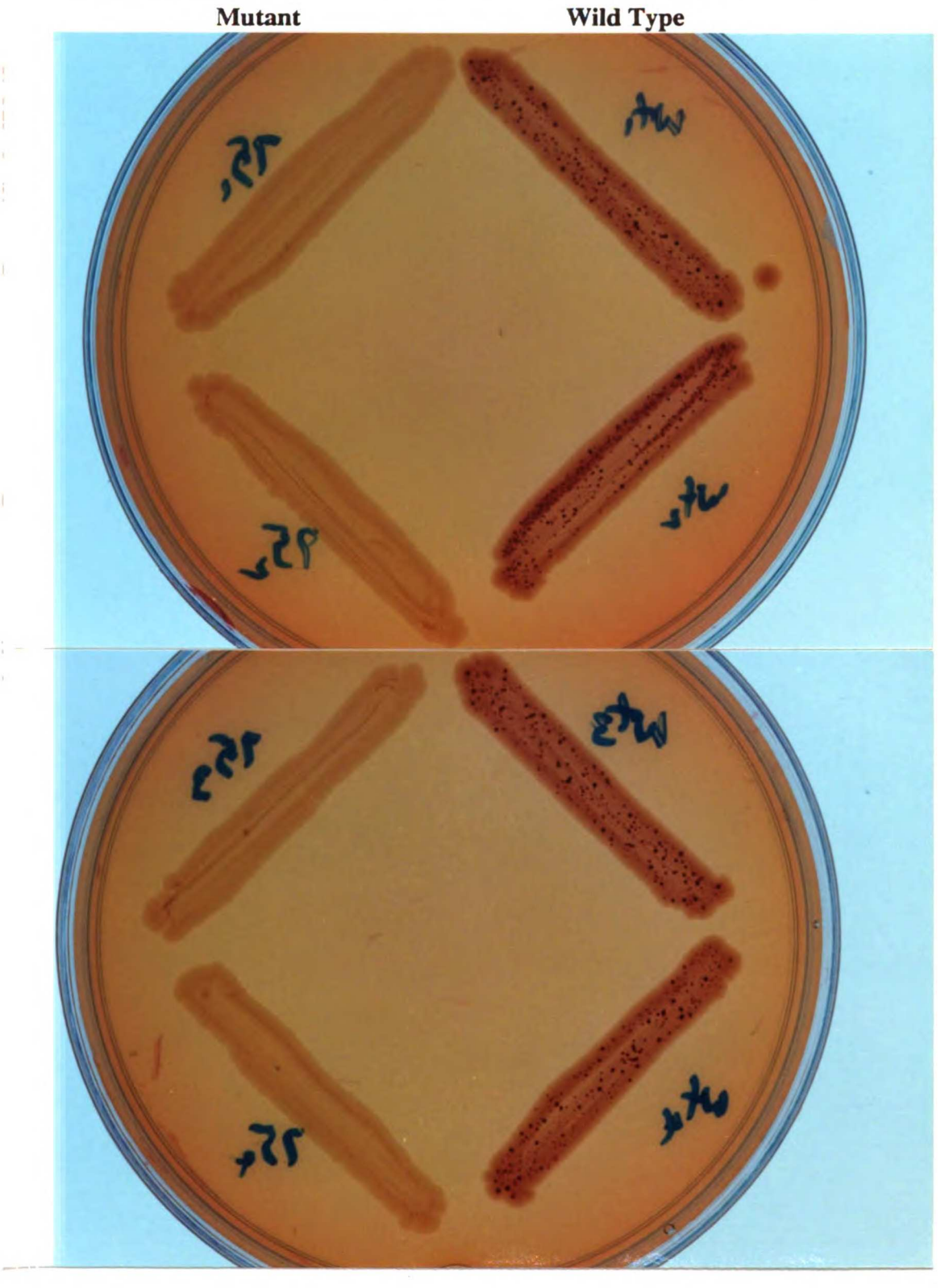


Figure 5-4.79

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #79
(NLC28::miniTn10-*tet*$^{\#79}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

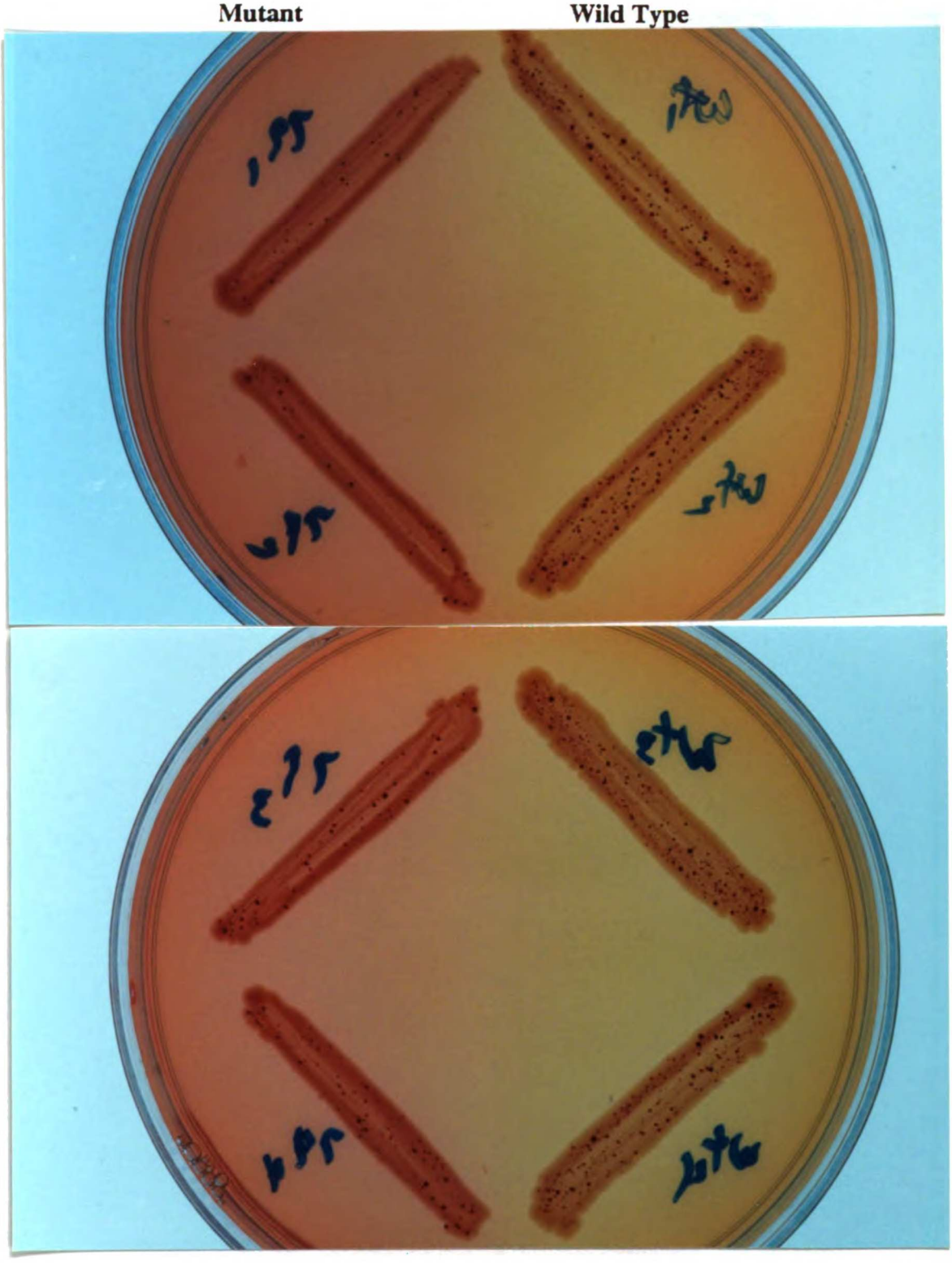


Figure 5-4.106

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #106
(NLC28::miniTn10-*tet*$^{\#106}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

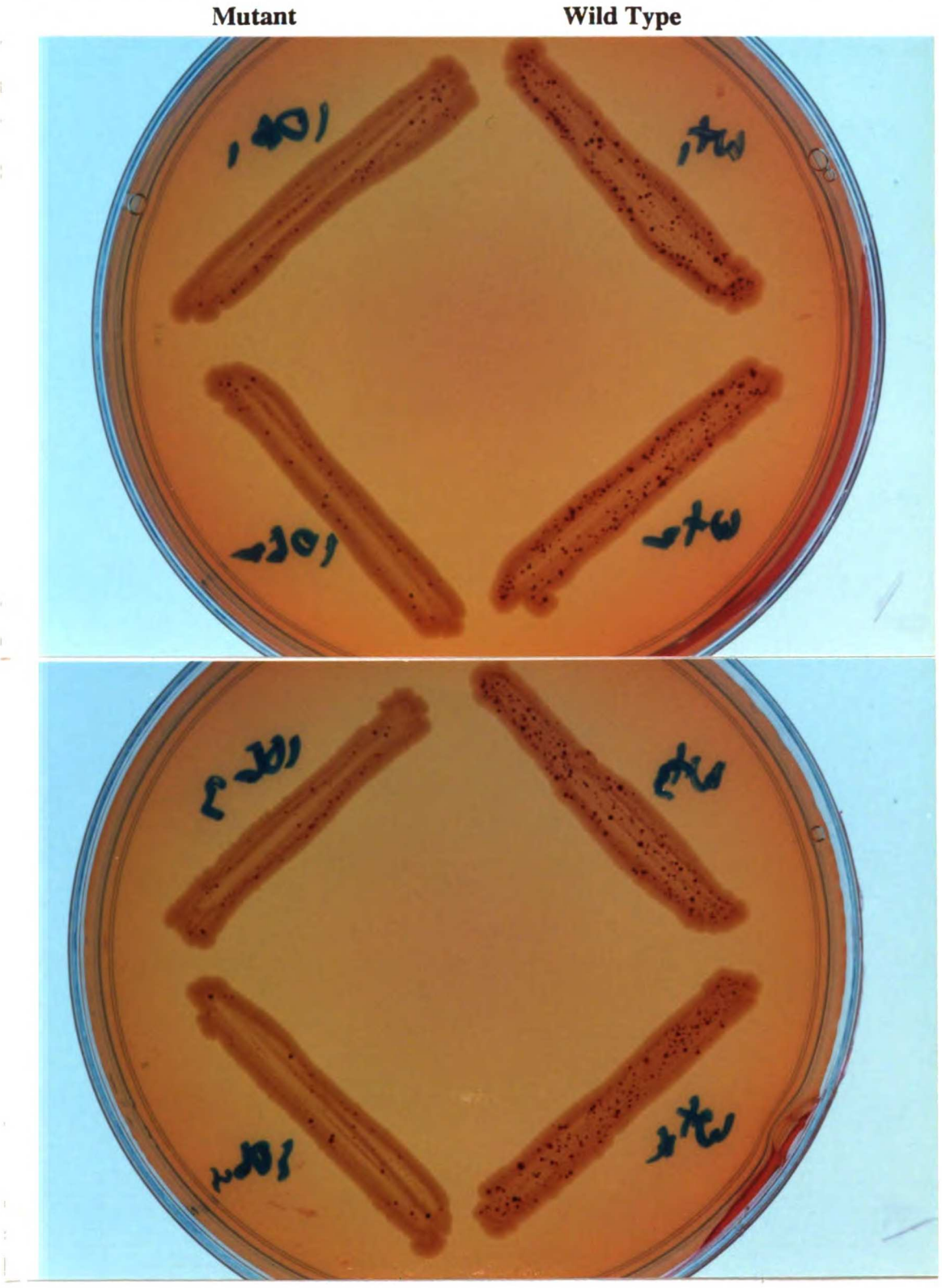


Figure 5-4.111

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #111
(NLC28::miniTn10-*tet*$^{\#111}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

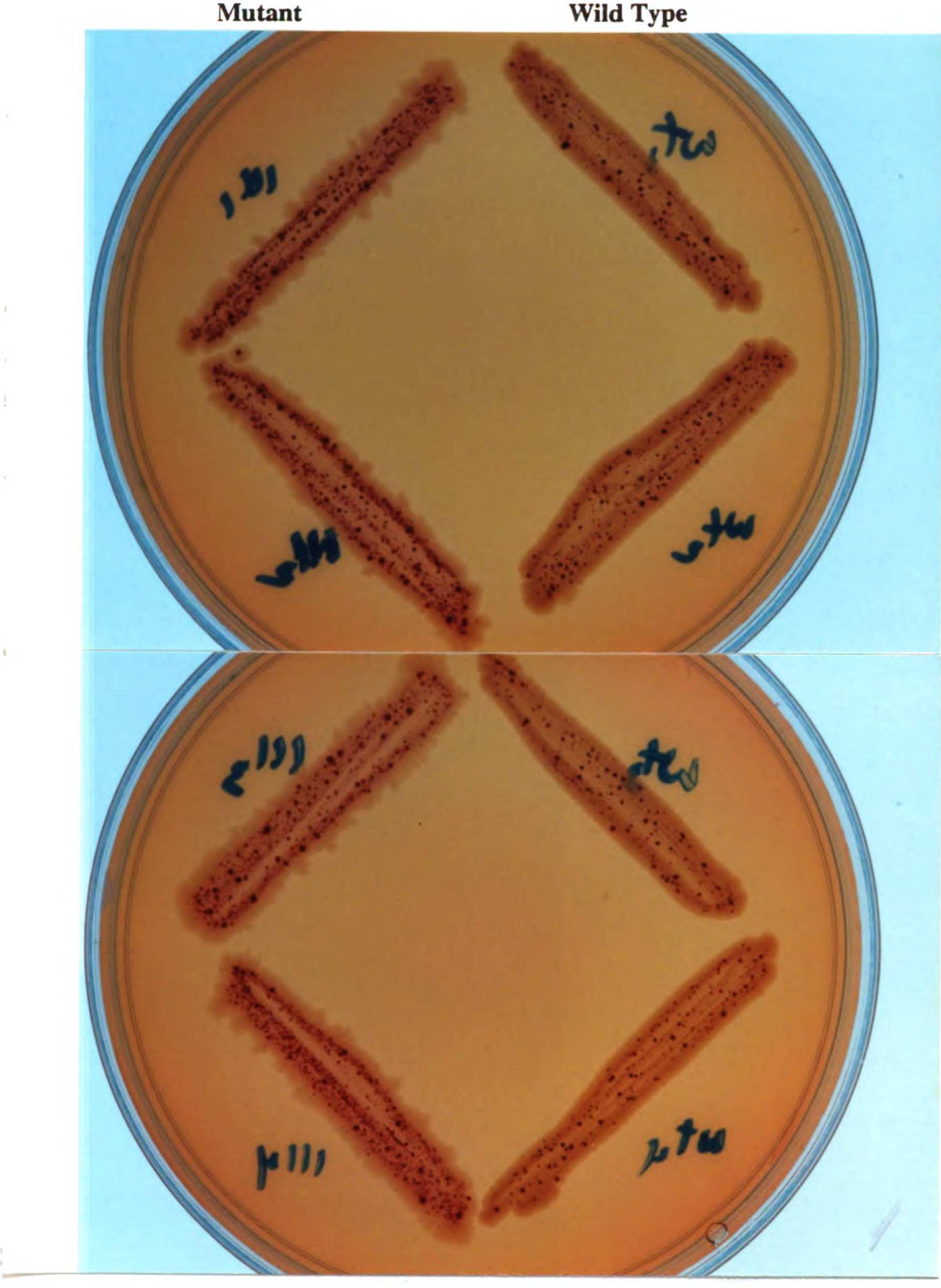


Figure 5-4.118

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #118
(NLC28::miniTn10-*tet*$^{\#118}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

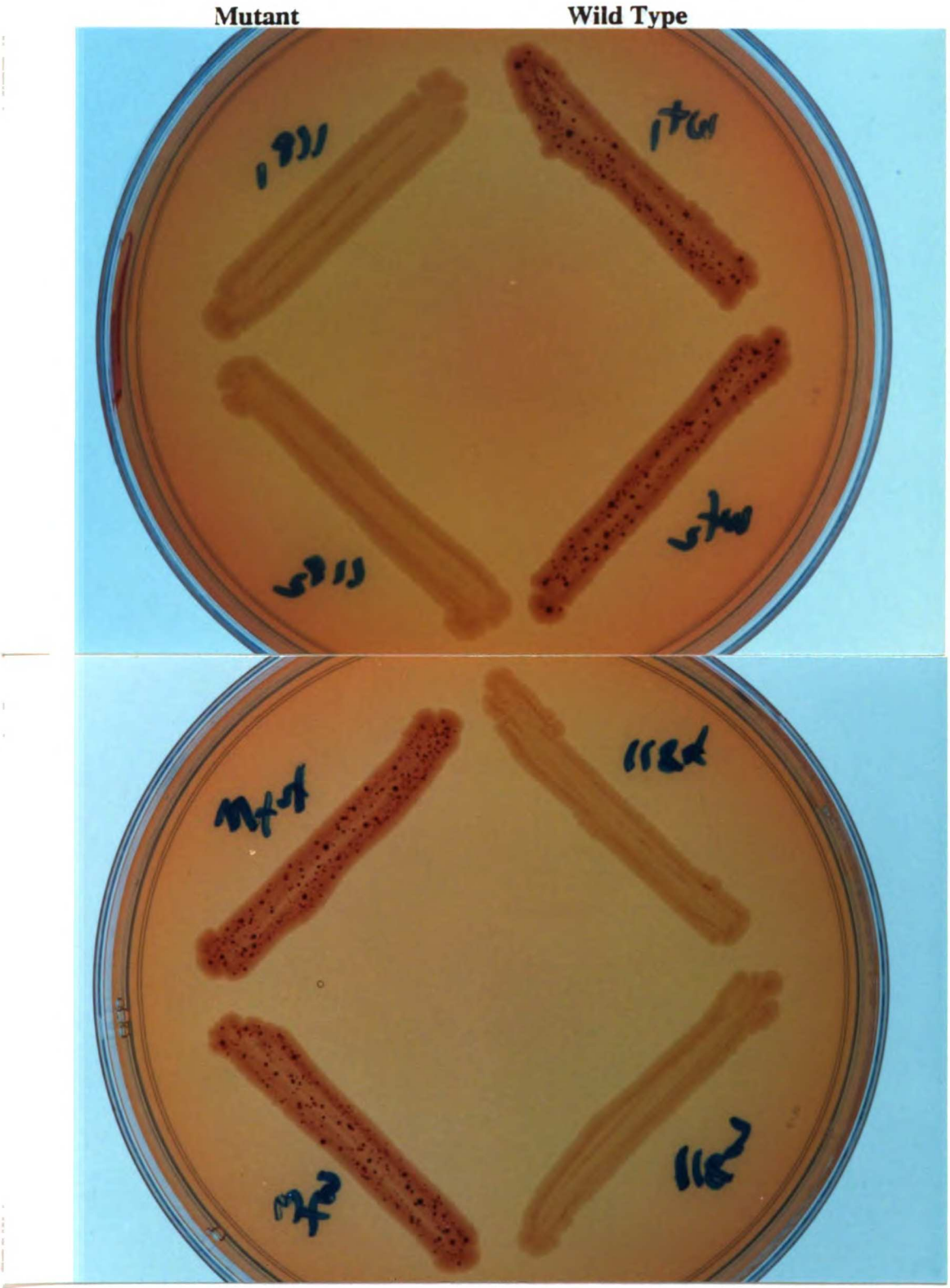


Figure 5-4.123

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #123
(NLC28::miniTn10-*tet*$^{\#123}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

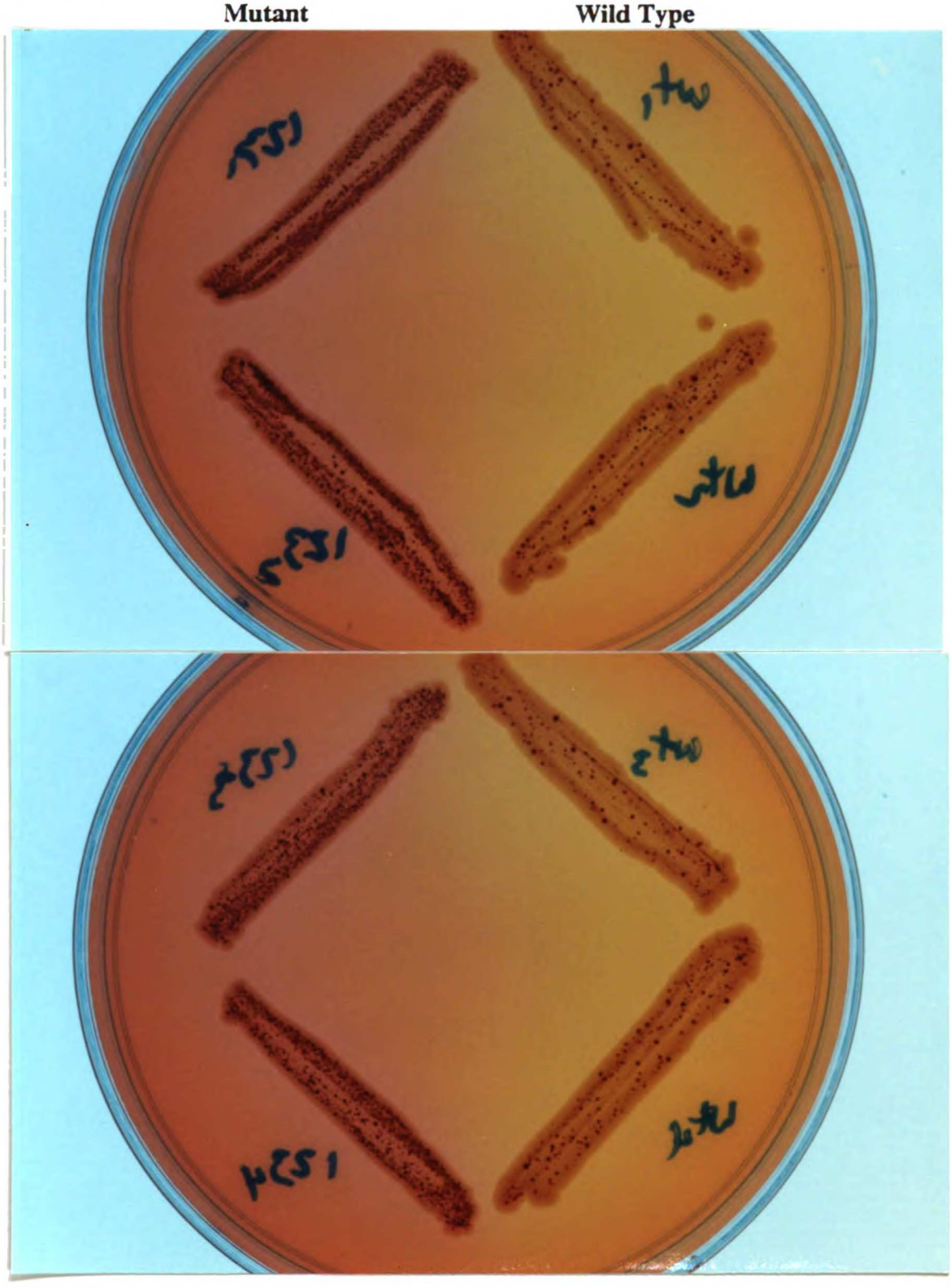


Figure 5-4.132
MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #132
(NLC28::miniTn10-*tet*$^{\#132}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

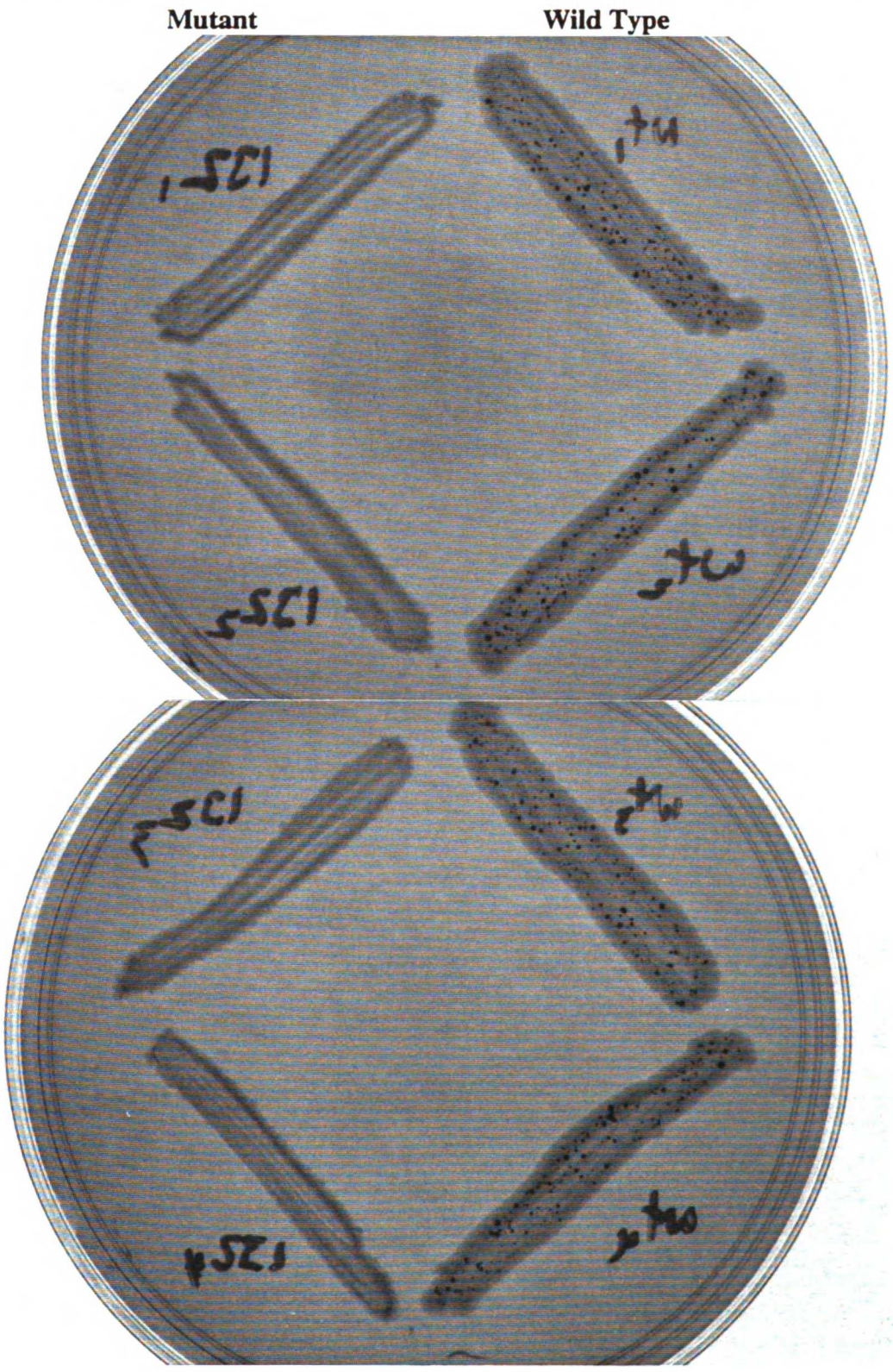


Figure 5-4.134

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #134
(NLC28::miniTn10-*tet*$^{\#134}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

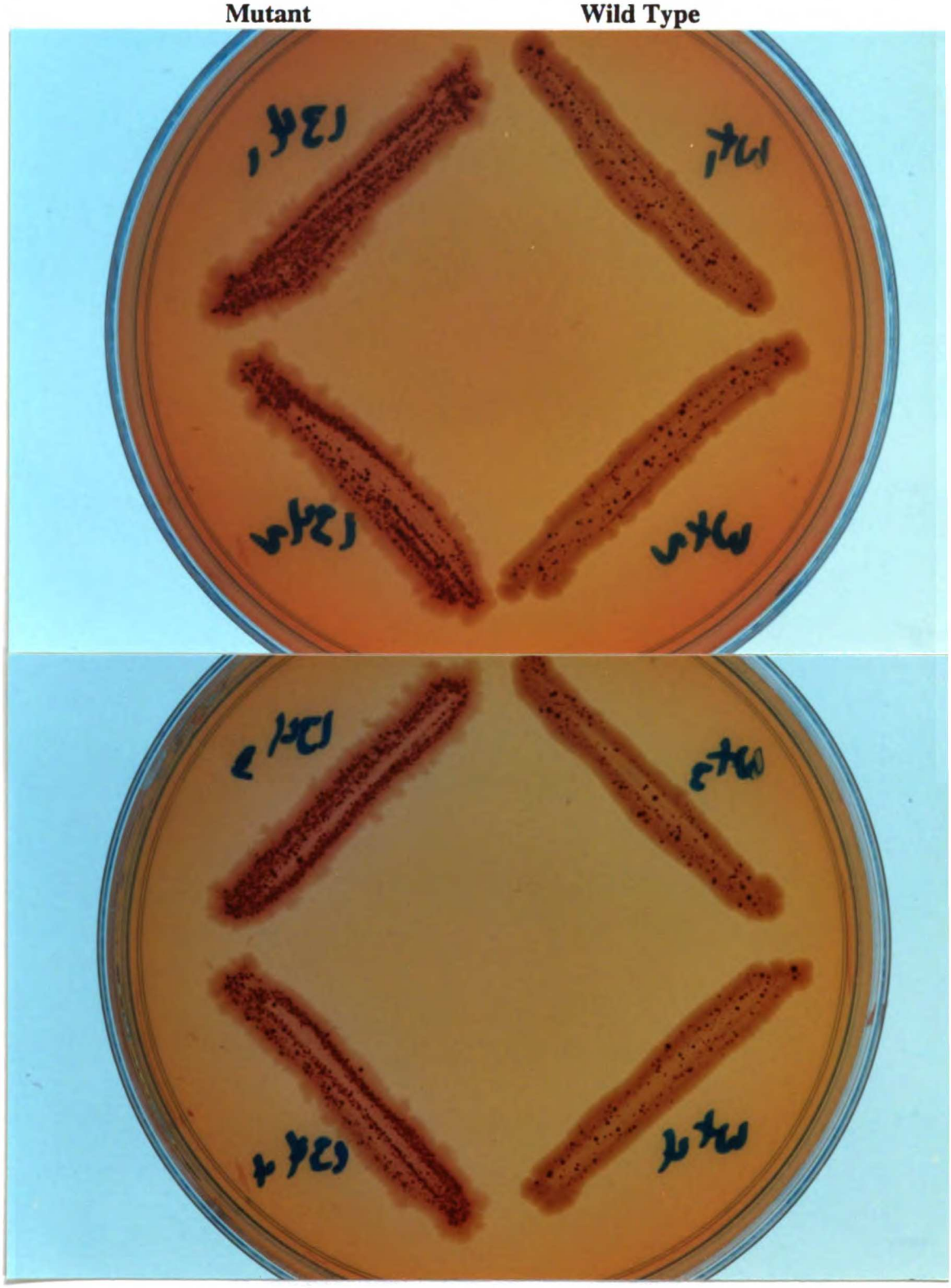


Figure 5-4.171

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #171
(NLC28::miniTn10-*tet*$^{\#171}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

Figure 5-4.174

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #174
(NLC28::miniTn10-*tet*$^{\#174}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

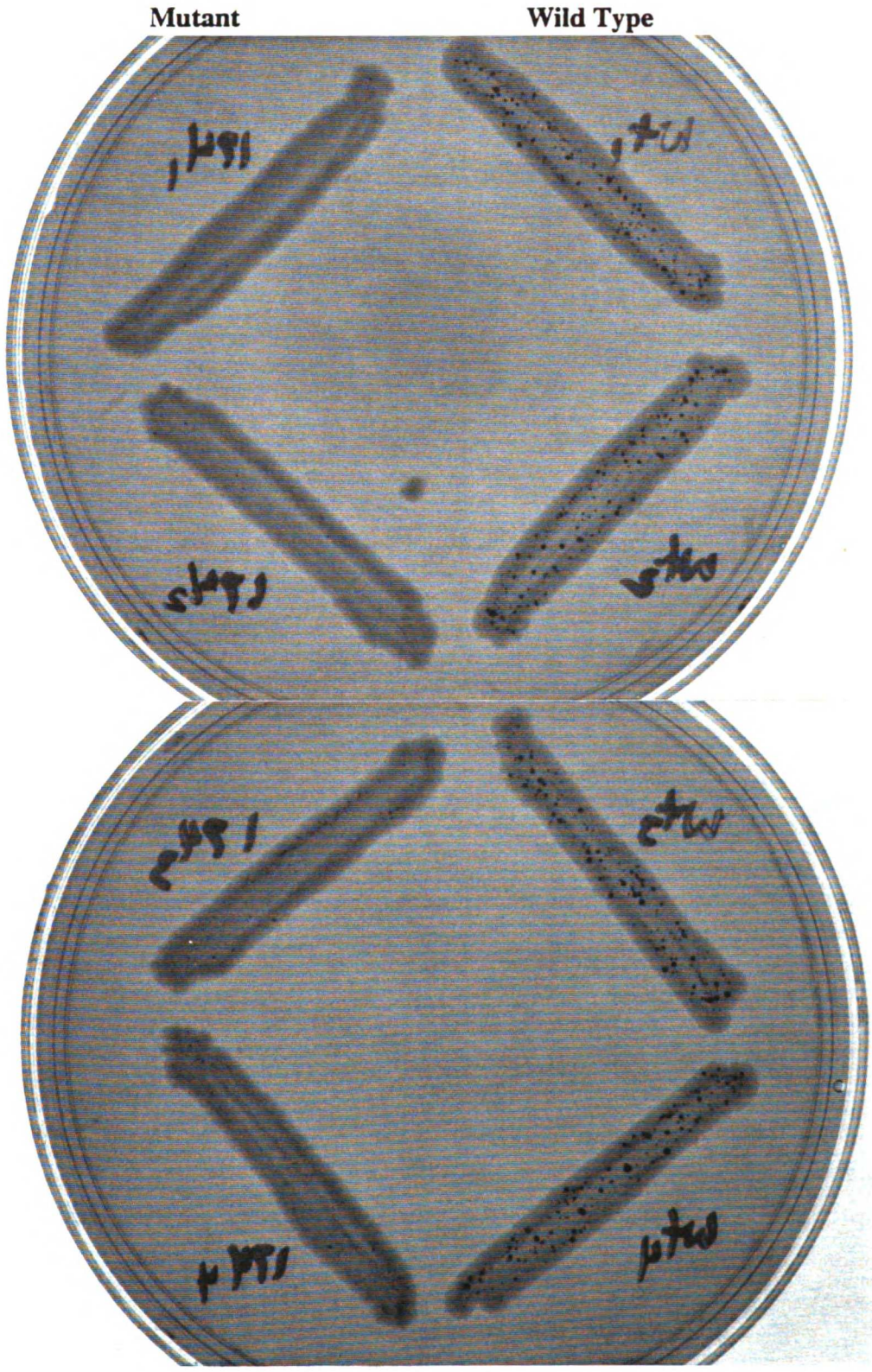


Figure 5-4.182

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #182
(NLC28::miniTn10-*tet*$^{\#182}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

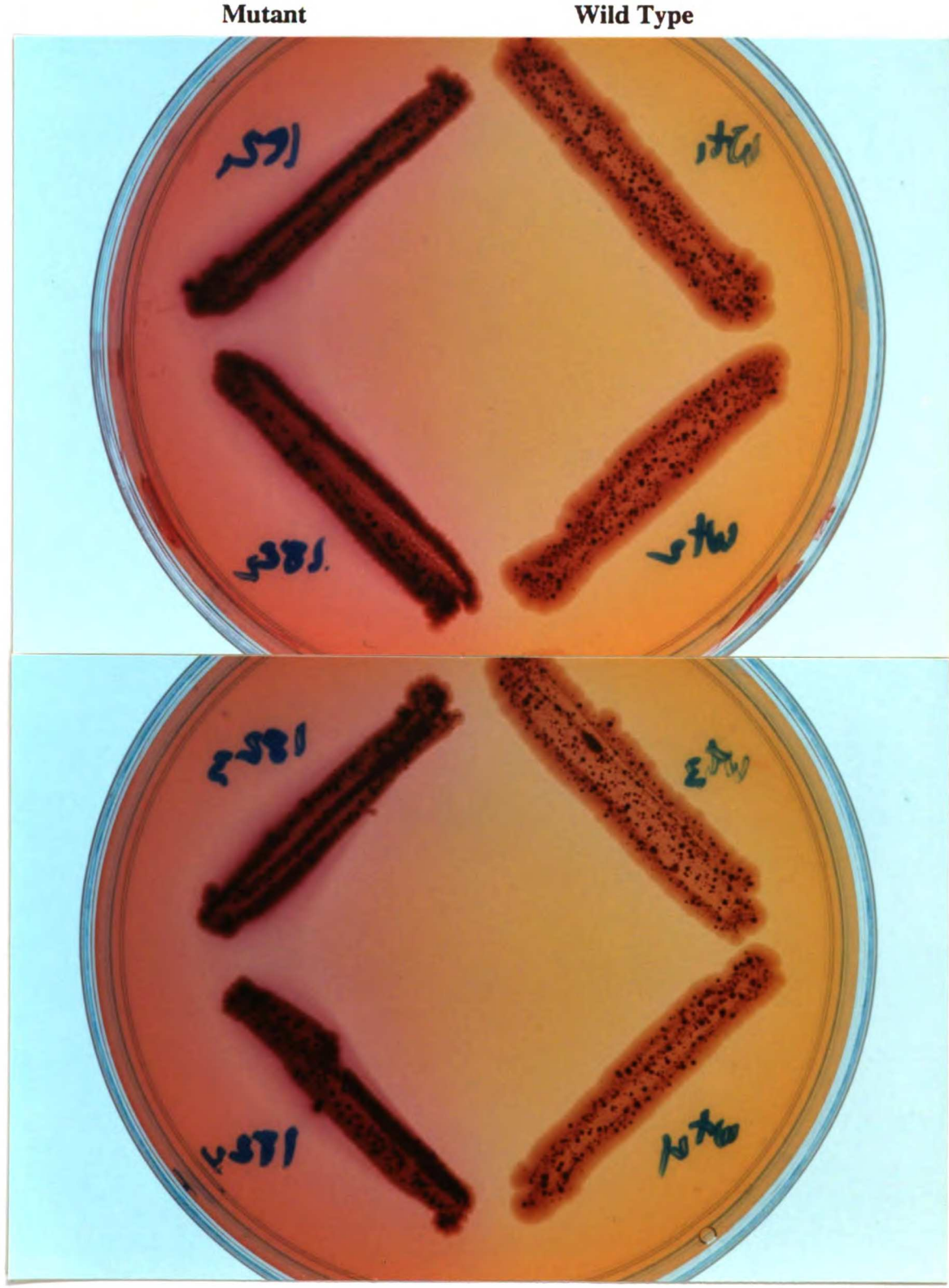


Figure 5-4.190

MiniTn7-*lac* Papillation in Tn7 Transposition Mutant #190
(NLC28::miniTn10-*tet*$^{\#190}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*)

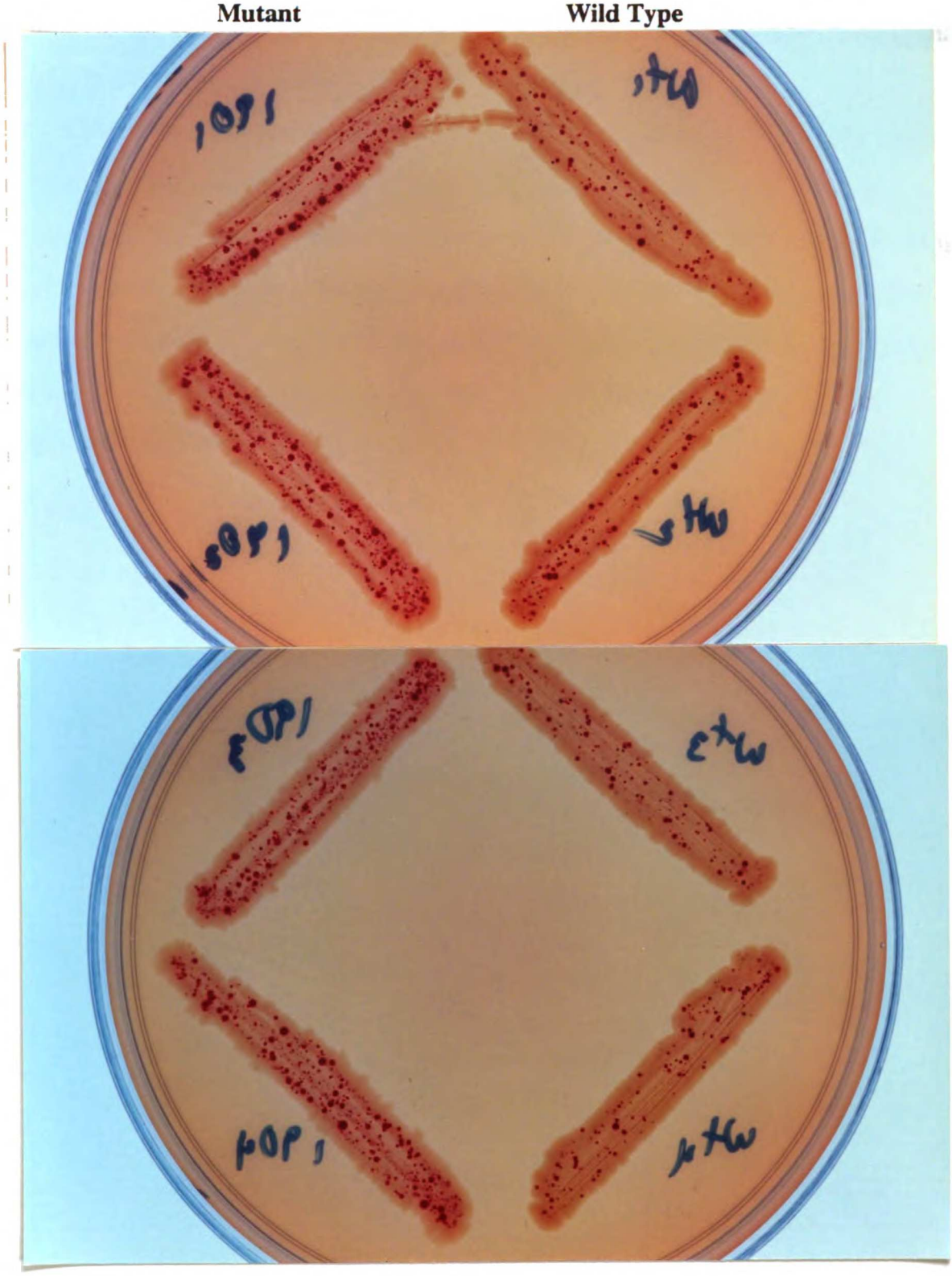


Figure 5-4 Legend

MiniTn7-*lac* Papillation in Tn7 Transposition Mutants

MiniTn7-*lac* generated Lac$^+$ papillation of 26 E. *coli* mutants generated by chromosomal miniTn10-*tet* insertion is depicted. Papillation is of NLC28*att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* carrying the indicated miniTn10-*tet* insertion P1 transduced from the original mutant strain. Four P1 transductants from each mutant (left side of photograph) are compared to four isolates of the isogenic wild type strain (not containing a miniTn10-*tet* insertion - right side of photographs). The same four wild type isolates were used for each mutant. Cells were patched on MacConkey Lactose indicator plates supplemented with 50 ug/ml of spectinomyocin and photographed after a four day incubation at 30°C.

Figure 5-5

MiniTn10-*lac* Papillation in Tn7 Transposition Mutants
(Papilllation of NLC28 pSIL(miniTn10-*lac* +Transposase))

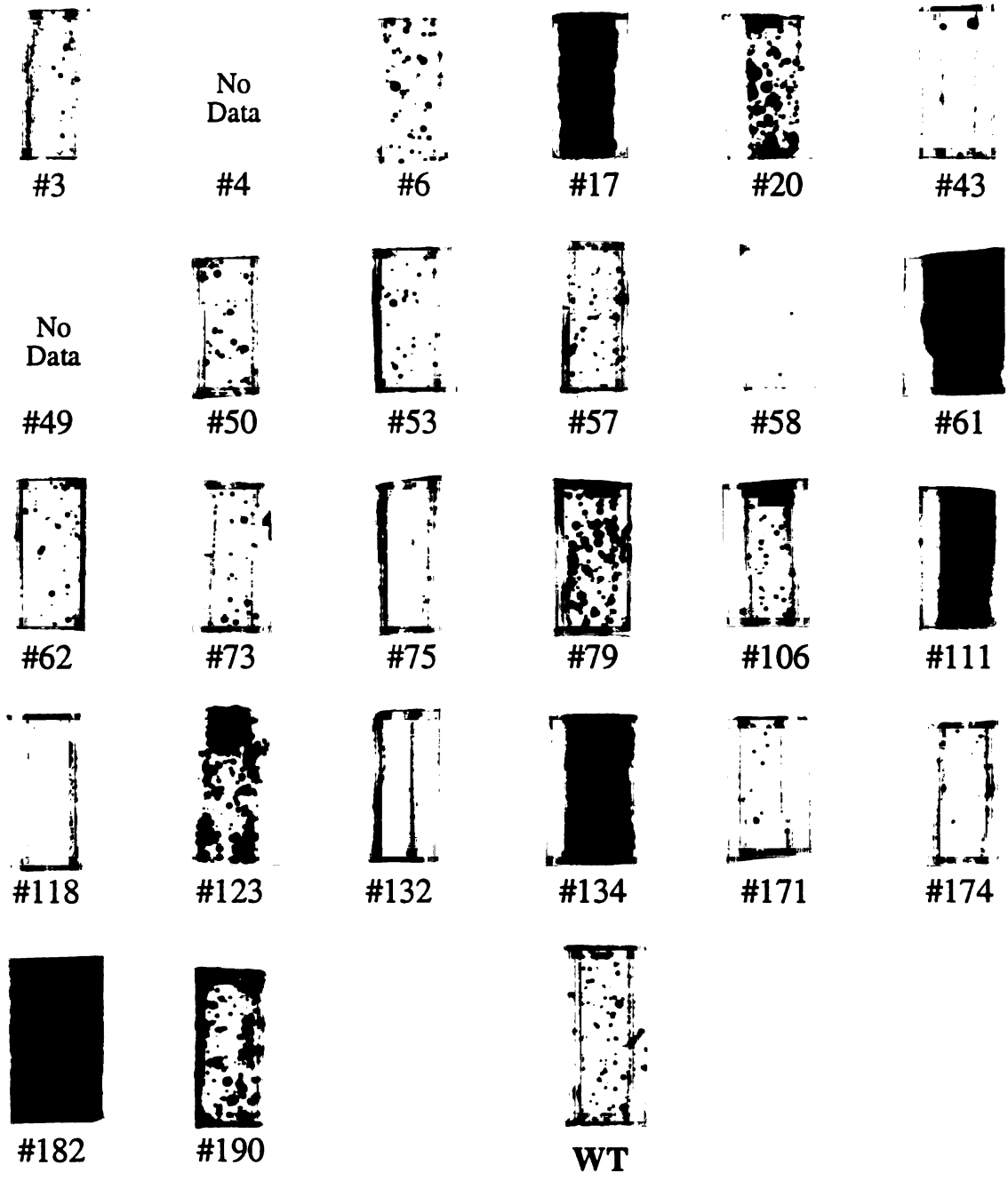


Figure 5-5 Legend

MiniTn10-*lac* Papillation in Tn7 Transposition Mutants

MiniTn10-*lac* generated Lac$^+$ papillation generated by the transposition of miniTn10-*lac* is depicted for the same E. *coli* mutants presented in Figure 5-4. Papillation is of NLC28 pSIL. The pSIL-14 plasmid carries miniTn10-*lac*, and the Tn10 transposase (Bolland and Kleckner, 1992). Cells were patched simultaneously on MacConkey Lactose indicator plates, supplemented with chloramphenicol to hold the pSIL-14 plasmid, and photographed after a 48 hour incubation at 37°C.

Appendix A

The following section reports the sequence data, sequence matches, and genetic loci information from each miniTn10-tet insertion examined.

Table A-1

Identification of E. *coli* Chromosomal MiniTn10-*tet* Insertions

MiniTn10-*tet* Insertion Not on pOX38*gen*::miniTn7-*lac*:

2	20	38	58	77	112	144	170	187
3	22	43	61	78	117	145	171	190
4	23	44	62	79	118	153	172	194
5	25	46	67	80	123	155	174	
6	26	48	68	97	127	156	175	
8	27	49	71	100	132	157	176	
13	28	50	72	101	134	158	180	
15	33	51	73	102	140	160	181	
17	35	53	74	106	142	161	182	
19	36	57	75	111	143	167	184	

MiniTn10-*tet* Insertion on pOX38*gen*::miniTn7-*lac*:

1	24	42	64	85	95	110	125	138	154	177	193
7	29	45	65	86	96	113	126	139	159	178	195
9	30	47	66	87	98	114	128	141	162	179	
10	31	52	69	88	99	115	129	146	163	183	
11	32	54	70	89	103	116	130	147	164	185	
12	34	55	76	90	104	119	131	148	165	186	
14	37	56	81	91	105	120	133	149	166	188	
16	39	59	82	92	107	121	135	150	168	189	
18	40	60	83	93	108	122	136	151	169	191	
21	41	63	84	94	109	124	137	152	173	192	

Table A-1 Legend

Identification of E. *coli* Chromosomal MiniTn10-*tet* Insertions

The initial 195 candidates with altered miniTn7-*lac* papillation were examined for chromosomal or F' location of the miniTn10-*tet* insertion. F's were transconjugated from the Tc^R colonies with altered miniTn7-*lac* papillation (NLC28*att*Tn7::Tn7 + miniTn10-*tet*#^{X} pOX38*gen*::miniTn7-*lac*) to CW51 and scored for tetracycline resistance phenotype. Insertion of miniTn10-*tet* on pOX38*gen*::miniTn7-*lac* should give the CW51 transconjugate a Tc^R phenotype. Because the initial candidates carry only the chromosomal and F' replicons, it was assumed that if miniTn10-*tet* insertion was not on the F' (pOX38*gen*::miniTn7-*lac*), then the insertion was somewhere in the E. *coli* chromosome. Under "MiniTn10-*tet* Insertions Not On pOX38*gen*::miniTn7-*lac*" are listed the mutants which gave tetracycline sensitive transconjugates, and thus are expected to be chromosomal insertions further characterized in Table A-2. Under "MiniTn10-*tet* Insertions On pOX38*gen*::miniTn7-*lac*" are listed the mutants which gave tetracycline-resistant transconjugates, expected to be F' insertions which were not further characterized.

Table A-2

Identification of E. *coli* Chromosomal MiniTn10-*tet* Not Linked To Tn7

Chromosomal MiniTn10-*tet* Insertions Not Closely Linked to *att*Tn7::Tn7

2	22	44	62	79	123	156	175
3	25	46	67	80	127	157	176
4	26	48	68	97	132	158	180
5	27	49	71	100	134	160	181
6	28	50	72	101	140	161	182
8	33	51	73	102	143	167	184
15	35	53	74	106	144	170	187
17	36	57	75	111	145	171	190
19	38	58	77	117	153	172	194
20	43	61	78	118	155	174	

MiniTn10-*tet* Insertions Closely Linked to *att*Tn7::Tn7

13
23
112
142

MiniTn10-*tet* Insertion Weakly Linked (10% Co-transduction) to *att*Tn7::Tn7

57

Table A-2 Legend

Identification of E. *coli* Chromosomal MiniTn10-*tet* Insertions Not Linked to Tn7

The 83 chromosomal miniTn10-*tet* insertions identified in Table A-1 were tested for P1 linkage of the miniTn10-*tet* insertion to the chromosomally located *att*Tn7::Tn7 which is the source of *tns* genes for miniTn7-*lac* papillation. Five to twenty P1 transductants of miniTn10-*tet*'s Tc^R marker from each of the initially identified Tc^R strains with altered miniTn7-*lac* papillation (NLC28*att*Tn7::Tn7 + miniTn10-*tet*#^{X} pOX38*gen*::miniTn7-*lac*), into NLC28 were scored for cotransduction of Tn7's trimethoprim resistance (Tp^R) marker. Under "Chromosomal MiniTn10-*tet* Insertions Not Closely Linked to *att*Tn7::Tn7" is listed the 79 mutants for which Tn7's Tp^R did not cotransduce with miniTn10-*tet*'s Tc^R marker. Under "Chromosomal MiniTn10-*tet* Insertions Linked to *att*Tn7::Tn7" is listed the four mutants for which Tn7's Tp^R always cotransduced with miniTn10-*tet*'s Tc^R marker. One of the mutants (#57) was found to be weakly linked (in 2 of 20 transductants) to *att*Tn7::Tn7.

Table A-3

MiniTn10-*tet* Linked To Altered MiniTn7-*lac* Papillation

Chromosomal MiniTn10-*tet* Insertions Affecting Papillation After Transduction of MiniTn10-*tet.*

3	28	53	75	118	161
4	33	57	77	123	171
5	35	58	78	132	172
6	36	61	79	134	174
15	38	62	80	140	181
17	43	68	97	143	182
19	48	71	100	144	184
20	49	72	102	145	187
22	50	73	106	153	190
25	51	74	111	156	194

MiniTn10-*tet* Insertions Not Affecting Papillation

2	155
8	157
26	158
27	160
44	167
46	170
67	175
101	176
117	180
127	

Table A-3 Legend

MiniTn10-*tet* Linked to Altered MiniTn7-*lac* Papillation

The 79 chromosomal miniTn10-*tet* insertions not linked to *att*Tn7::Tn7 listed in Appendix A, Table A-2 were tested for linkage of the miniTn10-*tet* insertion to the altered miniTn7-*lac* papillation phenotype. P1 transductants of the miniTn10-*tet* insertions into naive NLC28*att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* (the same strain configuration used for the initial mutagenesis) were scored for cotransduction of miniTn7-*lac* papillation phenotype. Approximately 20-100 Tc^R transductants from each mutant were examined. Under "Chromosomal MiniTn10-*tet* Insertions Affecting Papillation" is listed the 60 mutants for which an altered papillation phenotype always cotransduced with miniTn10-*tet*'s Tc^R marker. Under "Chromosomal MiniTn10-*tet* Insertions Not Affecting Papillation" is listed the 19 mutants for which an altered papillation phenotype did not cotransduce with miniTn10-*tet*'s Tc^R marker, and were not further characterized.

Table A-4

Intermolecular miniTn7-*lac* and Tn7 Transposition Frequency in miniTn10-*tet* Insertion Mutants

Mutant #	miniTn7-*lac* x10^{-7}	Ratio mutant/wt	Tn7 x10^{-6}	Ratio mutant/wt
3	38 ± 48	15	0.2±0.2	0.06
4	0.5±0.4	0.2	4.6±1.9	1.4
5	9.3± 15	3.7	6.0±5.5	1.8
6	0.8±0.05	0.3	8.5±1.3	2.6
15	0.7±0.06	0.3	4.2±2.6	1.3
17	4.5±2.3	1.8	5.7±2.1	1.7
19	1.6±1.0	0.6	2.1±0.5	0.6
20	1.4±1.7	0.6	6.9±3.7	2.1
22	1.3±0.7	0.5	2.8±1.4	0.8
25	2.6±2.1	1.0	2.9±1.4	0.9
28	5.7±4.7	2.3	3.3±0.7	1.0
33	1.7±2.4	0.7	2.4±0.6	0.7
35	0.9±0.7	0.4	2.8±1.1	0.8
36	1.0±0.7	0.4	2.6±0.9	0.8
38	0.9±0.7	0.4	2.9±2.1	0.9
43	1.1±1.3	0.4	0.1±0.1	0.02
48	2.5±0.8	1.0	4.6±1.0	1.4
49	0.5±0.6	0.2	1.9±1.0	0.6
50	4.4±2.8	1.8	8.4±8.8	2.5

Table A-4 cont.

Intermolecular miniTn7-*lac* and Tn7 Transposition Frequency in miniTn10-*tet* Insertion Mutants

Mutant #	miniTn7-*lac* $x10^{-7}$	Ratio mutant/wt	Tn7 $x10^{-6}$	Ratio mutant/wt
51	1.7±3.0	0.7	2.6±0.8	0.8
53	0.6±0.6	0.2	1.7±1.3	0.5
57	1.2±1.5	0.5	0.8±1.0	0.2
62	0.3±0.2	0.1	0.7±0.7	0.2
68	2.6±2.1	1.0	3.8±2.7	1.2
71	4.8±3.3	1.9	3.8±3.1	1.2
72	0.7±0.7	0.3	3.6±2.2	1.1
73	2.1±2.8	0.8	0.03±0.004	0.01
74	2.1±0.7	0.8	5.1±1.6	1.5
75	0.6±0.3	0.2	3.9±1.7	1.2
77	5.7±8.7	2.3	3.3±1.7	1.0
78	2.1±1.9	0.8	5.8±3.2	1.8
79	1.2±1.9	0.5	1.1±1.3	0.3
80	1.5±0.9	0.6	5.0±2.0	1.5
97	1.8±1.4	0.7	2.9±2.6	0.9
100	1.8±1.7	0.7	7.6±2.5	2.3
102	3.0±0.9	1.2	6.4±4.4	1.9
106	0.7±0.04	0.3	5.2±2.3	1.6
111	11±3.5	4.4	53±25	16

Table A-4 cont.

Intermolecular miniTn7-*lac* and Tn7 Transposition Frequency in miniTn10-*tet* Insertion Mutants

Mutant #	miniTn7-*lac* x10^{-7}	Ratio mutant/wt	Tn7 x10^{-6}	Ratio mutant/wt
118	6.6±6.1	2.6	12±4.6	3.6
123	0.9±0.3	0.4	2.2±0.4	0.7
132	1.6±0.9	0.6	2.3±0.5	0.7
134	1.5±1.0	0.6	3.5±0.6	1.1
140	2.0±0.8	0.8	8.4±5.6	2.5
143	2.4±1.0	1.0	4.3±1.9	1.3
144	1.8±0.9	0.7	6.0±2.1	1.8
145	3.5±0.8	1.4	4.1±0.7	1.2
153	11±11	4.4	7.1±1.9	2.2
156	0.7±1.0	0.3	6.5±2.8	2.0
161	6.3±4.9	2.5	7.3±6.7	2.2
171	2.6±1.5	1.0	8.3±7.0	2.5
172	2.2±0.8	0.9	4.9±1.4	1.5
174	2.5±1.3	1.0	14±13	4.2
181	4.5±4.2	1.8	4.7±1.0	1.4
182	3.7±5.5	1.5	1.9±0.8	0.6
184	3.2±3.6	1.3	5.1±3.0	1.5
187	1.4±0.9	0.6	4.7±1.9	1.4
190	13±4.7	5.2	42±10	13
194	7.2±10	2.9	4.6±2.1	1.4

Table A-4 Legend

Intermolecular miniTn7-*lac* and Tn7 Transposition Frequency in miniTn10-*tet* Insertion Mutants

The 60 chromosomal miniTn10-*tet* insertions affecting miniTn7-*lac* papillation listed in Appendix A, Table A-3 were evaluated for their effect on the frequency of intermolecular translocation as measured by the mating-out assay. Mating-out assays determined the transposition frequency of Tn7 and miniTn7-*lac* to the F' pOX38*gen* in miniTn10-*tet* insertion mutants, with the results tabulated here, and graphically depicted in Chapter 5, Figure 5-3. The F^+ strain was NLC28*att*Tn7::miniTn7-*lac* ϕ80dlacII::Tn7 pOX38*gen*, in which miniTn7-*lac* transposes from the chromosomal *att*Tn7 site, and Tn7 transposes from a second chromosomal donor site, ϕ80dlacII. The F' recipient was CW51. The average transposition frequency and standard deviation of 5 independent trials for each mutant is reported along with the ratio of the frequency of transposition in the mutant strain background to the non-mutant (wt) strain background. Transposition frequencies represent the proportion of F's onto which Tn7 or miniTn7-*lac*. have transposed, and are reported, along with their standard deviations. Note: reported transposition frequencies may not reflect only transposition events (see Materials and Methods).

Table A-5

P1 Linkage of MiniTn10-*tet* Insertions to Known Locations

Mutant #	Matched Gene	Location of Matched Gene	Location of Km^R Marker	Degree of miniTn10-tet Linkage to Km^R by P1 Transduction
43	hns	27.25	27.25	100/100
3			27.25	98/100
111	minD	26.3	25.75	5/100
134			25.75	5/100
20	purMN	53.75	54	62/100
75	guaB	53.75	54	71/100
50	arcB	69.5	70	66/100
57	(unc)	84.2	84.5	94/100
58			84.5	48/50
174	rpsG/fusA	73.5	74	37/100
17	(serS)	19.9	20	55/60
190	(serS)	19.9	20	94/100
73	(soxRS)	92.1	92.5	65/100
6	(tesB)	10.4	10.5	0/200
123	(menB)	49	48.5	8/100
79	(menB)	49	49.5	14/100

Table A-5 Legend

P1 Linkage of MiniTn10-*tet* Insertions to Known Locations

Kanamycin resistance (Km^R) markers (from a collection of strains designed for genetic mapping) located very near the GenBank sequence match indicated location of the miniTn10-*tet* insertion, were P1 transduced into strains containing the miniTn10-*tet* insertion. For each of the indicated mutants, P1 transductants of Km^R into NLC28 ::miniTn10-tet^X was scored for the loss of the miniTn10-*tet* associated Tc^R. The degree of P1 linkage is indicated as the fraction of transductants that had lost Tc^R over the total number of Km^R transductants tested (fraction on far right). If the miniTn10-*tet* insertion is, in fact, very near the Km^R marker, then the tetracycline resistance marker should be lost at high frequency. To the right of the mutant number are is the gene implicated by sequence from the cloned miniTn10-*tet* insertion. The genes in parentheses are genes near the site of insertion rather than the actual site of insertion. To the right of the gene is its location on the E. *coli* chromosome. The location of the Km^R marker used to test linkage is indicated to the right of to location of the implicated gene. The Km^R markers were transduced from the following Km^R strains kindly provided by Carol Gross (Singer et al., 1989): 27.25 min. is CG18551, 25.27 min. is CG18544, 54 min. is CG18631, 70 min. is CG18605, 84.5 min. is CG18599, 74 min. is CG18556, 20 min. is CG18528, 92.5 min. is CG18630, 10.5 min. is CG12107, 48.5 min. is CG12183, 49.5 min. is CG18552 (see Materials and Methods).

Mutant #6 was not confirmed to lie near the Km^R marker we expected to be near *tes*B (0 of 200 Km^R transductants lost Tc^R); however, we did not confirm the location of the Km^R marker.

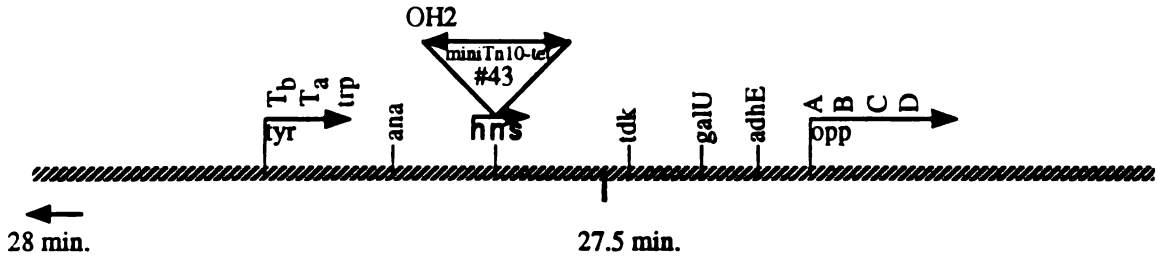
Legend to Detailed Information on Mutations Affecting Tn7 Transposition

In the following pages, four types of information is presented for each mutant, or group of mutants affecting Tn7 transposition. First, a genetic map of the region near the site of miniTn10-*tet* insertion is diagrammed, under which are listed the genes indicated on the map and their function. Next, a description of the mutation's phenotypes, and how it was assigned to a location from the sequence information. Next, the sequence that was obtained from each of the cloned miniTn10-*tet* insertions is reported. Each sequence is proceeded by an identification number and followed by the result of blastn sequence match search of the GenBank sequence data base. The multiple sequence entries under one primer reflects sequence from multiple runs when indicated by permuted letters, and sequence from separate clonings when indicated by permuted numbers. Last, the obtained sequences are displayed on their GenBank matches, with the identification number of the obtained sequence indicating the matched sequence to the GenBank entry.

Index to Detailed Information on Mutations

Affecting Tn7 Transposition

hns
Mutants #43 and #3



Mutant #3 was not cloned.

Insertion #43 in *hns*D coding sequence.

Known Genes Near *hns*

Gene		Function
tyr	T_a T_b	Tandemly duplicated tryosine tRNA genes.
ana		A protamine like protein.
hns		Histone-like protein
galU		Glucose-1-phoshate uridylytransferase
adhE		Co-A linked acetaldehyde + alcohol deydrogenase
opp	A B C D	Oligo-peptide transport

***hns* - Mutants #43 and #3:**

One of the miniTn10-*tet* generated Tn7 transposition mutants of E. *coli* characterized here, mutant #43, was found to interrupt *hns*. *hns* encodes is a histone-like protein which is thought to bind and stabilize bent DNA (Yamada et al., 1991). *hns* mutants have pleiotropic effects ranging from the deregulation of genes which respond to environmental osmotic conditions (Higgins et al., 1988), to the deregulation of a number of recombination systems (Falconi et al., 1991; Gama et al., 1992; Higgins et al., 1988; Lejeune and Danchin, 1990; Spears et al., 1986).

MiniTn10-*tet* insertion number 43 is within the coding sequence of *hns*, with sequence from the OH2 primer reading out from the miniTn10-*tet* insertion matching *hns* sequence. MiniTn10-*tet* insertion mutant #3 displays the same transposition phenotypes as mutant #43. Both miniTn10-*tet* insertions #43 and #3 were genetically linked by P1 transduction to a marker very near *hns*.

MiniTn10-*tet* insertions #43, and #3 were found to decrease both miniTn7-*lac* and miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). MiniTn10-*tet* insertions #43 and #3 both show a dramatic reduction of Tn7 transposition to pOX38*gen* as measured in the mating out assay. Tn7 transposition is decreased 50 fold by mutant #43, while miniTn10-*tet* insertions #3 decreases Tn7 transposition 20 fold. MiniTn7-*lac* transposition to pOX38*gen* is also reduced in mutant #43, though with only a 2.1 effect.

Mutant 43

Sequences Obtained:

T3 Primer
From pOH50 - 43H2T3:
CTGATGAATCCTCAATCGATCTCGTCAACATCTAGTACGTCGATACACATTTGCATACGATCCGATTGACA
GenBank Match - NONE

OH2 Primer
From pOH50 - 43H2OH2:
CAGCAAGGCTATTCAGCAGTTCGTTCGGGTCAATACGTCAGGGATCAGCATTTCGCGATATTGCAGGACTG
CAAGCTTGTCCTTAG
GenBank Match - >**ECOHNS** (GenBank) E. coli hns gene for DNA-binding protein H-NS.

OH3 Primer
No Sequence

T7 Primer
From pOH50 - 43H2T7:
CGGATGCTGCATGATGCGGATGCG
GenBank Match - NONE

***hns* Sequence**

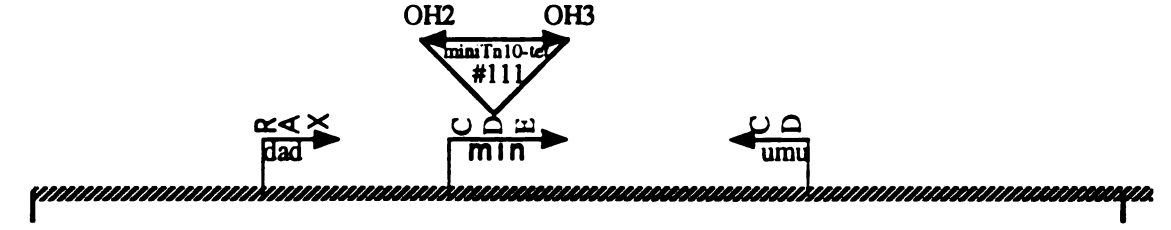
HNS Coding Sequence = 850-1317
Located at min. 27.75

4.5kb PstI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion in Coding Sequence
One PstI cloning site located.

***hns*:**

```
      1311        1301        1291        1281        1271        1261
AATCCGCCGC TGGCGGGATT TTAAGCAAGT GCAATCTACA AAAGATTATT GCTTGATCAG

      1251        1241        1231        1221        1211        1201
GAAATCGTCG AGGGATTTAC CTTGCTCATC CATTGCTTTT TTGATTACAG CTGGAGTACG

      1191        1181        1171        1161        1151        1141
GCCTTGGCCA GTCCAGGTTT TAGTTTCGCC GTTTTCGTCA ACGTAGCTAT ATTTTGCCGG

      1131        1121        1111        1101        1091        1081
ACGCTGAGCA CGTTTAGCTT TGGTGCCAGA TTTAACGGCA GCAAGGCTAT TCAGCAGTTC
                                          :: :::::::::: :::::::::: 
                    miniTn10-tet-43H2OH2>CA GCAAGGCTAT TCAGCAGTTC

                                        PstI 1037
      1071        1061        1051        1041        1031        1021
GTTCGGGTCA ATACCGTCAG CGATCAGCAT TTCGCGATAT TGCTGCAGCT TACGAGTGCG
:::::::::: :::: :::::  ::::::::: :::::::::: ::: :   pBluescript
GTTCGGGTCA ATAC  GTCAG GGATCAGCAT TTCGCGATAT TGCAGGACTGCAAGCTTGTCCTTAG


      1011        1001         991         981         971         961
CTCTTCAACT TCAGCAGCAG CCGCGCTTTC TTCTTCGCGA CGTTCGTTAA CGACAACTTC

       951         941         931         921         911         901
TAATTTTTCC AGCATTTCTT CCAGCGTTTC AAGTGTACAT TCTCTTGCCT GCGCACGAAG

                                          Ribosome Binding
       891         881         871         861         851         841
AGTACGGATG TTGTTCAGAA TTTTAAGTGC TTCGCTCATT GTAGTAATCT CAAACTTATA
```

*min*D
Mutants #111 and #134



Mutant #134 was not cloned.

Insertion #111 in *min*D coding sequence.

Known Genes Near *min*D

Gene		Function
dad	R	Regulatory gene.
	A	D-amino acid dehydrogenase.
	X	Alanine racemase.
min	C	Non-specific inhibitor of septation
	D	Activator of minC
	E	topological identifier of septation site
umu	C	U.V. inducible mutagenisis; error prone repair.
	D	

***min*D - Mutants #111 and #134:**

Mutant #111 was found to interrupt *min*D. *min*D, as part of the *min*B operon, is involved in cell division control. The *min* mutants generate "mini" cells as a result of aberrant fission from each end of the bacterial rod (de Boer et al., 1989). Evidence suggest that MinC is the proximate cause of the septation block and that MinD plays two roles in the MinCDE system - it activates the MinC-dependent division inhibition mechanism and is also required for the sensitivity of the division inhibition system to the MinE topological specificity factor (de Boer et al.,1991; de Boer et al.,1992). MiniTn10-*tet* insertion #111 is within the coding sequence of *min*D, with sequence reading out from each end of miniTn10-*tet* insertion #111 (OH2 and OH3 primers) matching *min*D sequence. MiniTn10-*tet* insertion mutant #111 was cloned into a pBluescript plasmid after digestion of the chromosomal DNA with PstI. Sequencing into the cloned chromosomal DNA flanking miniTn10-*tet* insertion #111 from the pBluescript vector also matched *min*D sequence.

MiniTn10-*tet* insertion mutant #134 displays the same increased papillation phenotype as mutant #111, and both #111 and #134 display a "mini-cell" phenotype, though no gross growth rate defect (previously characterized *min*B mutants also have no growth rate defect). Both miniTn10-*tet* insertions #111, and #134 were genetically linked by P1 transduction to a marker at minute 25.75, near *min*D at min. 26.3.

MiniTn10-*tet* insertions #111 and #134 were found to increase both miniTn7-*lac* and miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). As measured by the mating-out assay, miniTn10-*tet* insertion #111 produces a 17 fold reduction of Tn7 transposition to pOX38*gen*, while miniTn7-*lac* transposition is conversely increased 15 fold. Surprisingly, miniTn10-*tet* insertion #134 has little effect on Tn7 transposition to pOX38*gen*, while increasing miniTn7-*lac* transposition 1.7 fold.

Mutant 111

Sequences Obtained:

T3 Primer
From pOH51 - 111H-T3:
GGAAGAACTGTCGTGATGAGATTGATGATCGCTGCGTACTCGACTGATGATGGCC
GenBank Match - NONE

OH2 Primer
From pOH51 - 111HOH2.2:
CGCGGCGTGATCGACGCAGATGCTAAATACGTCAGAGTCGCGTACTGAGGAGACTCGGTGTGTATATGCTCG
CTG
GenBank Match - >**ECOMINB** (GenBank) E.coli minicell (minB) locus encoding minC, minD, and minE (required for the proper placement of the division septum)

OH3 Primer
From pOH51 - 111H2OH3:
CTACGACTGTACCTCGCTTAGTGGCCTGGGTTATAGGGCCTAAGCAGCGGT
GenBank Match - >**ECOMINB** (GenBank) E.coli minicell (minB) locus encoding minC, minD, and minE (required for the proper placement of the division septum)

T7 Primer
From pOH51 - 111HT7.2:
GTTAAATTGATCCCTTTTTAACAAGGAATTTCTATGGCACGCATTATTGTTGTTAC
GenBank Match - >**ECOMINB** (GenBank) E.coli minicell (minB) locus encoding minC, minD, and minE (required for the proper placement of the division septum)

***minB* Sequence**

minD Coding Sequence = 915-1727
Located at min. 26.3

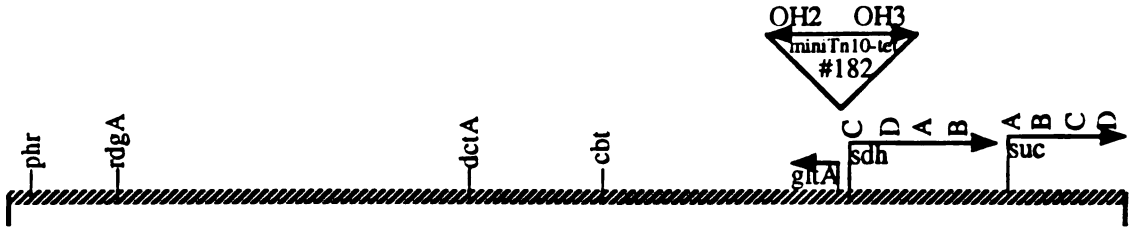
3.9kb PstI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion in *min*D coding sequence
Both PstI cloning sites located and agree with 3.9kb insert size in pBluescript plasmid.

ECOMINB:

```
      1791        1781        1771        1761        1751        1741
TCTGCAGCCG TTCTTTTGCA ATGTTGGCTG TGTTTTTCTT CCGCGAGAGA AAGAAATCGA
  PstI
      1731        1721        1711        1701        1691        1681
GTAATGCCAT AACTTATCCT CCGAACAAGC GTTTGAGGAA GCCTTTCTTC TCTTCTTCAA

      1671        1661        1651        1641        1631        1621
TGAAGCGGAA AGGACGTTCT TCTCCCAACA GACGTTCTAC GGTATCTGCG TAGGCTTTAC

      1611        1601        1591        1581        1571        1561
CCGCATCGGC GTTAATGTCG AGAATGACCG GTTCACCCTG GTTAGAGGCG CGCAATACTG

      1551        1541        1531        1521        1511        1501
ATTGATCCTC TGGGATCACG CCGACGAGTT TGATGCGCAG GATCTCCAGC ACATCTTCCA

      1491        1481        1471        1461        1451        1441
TGCTCAGCAT GTCACCTCTG CTTACGCGGC CTGGGTTATA GCGCGTTAAC AGCAGGTGCT
  ::/\:/\: :: :::: : :::: : ::: :::::::::: : :: : ::: ::: :::
  CTACGACT GT ACCT CG CTTA GTGGC CTGGGTTATA GGGCCT AAGCAGC GGT<111H2OH3

      1431        1421        1411        1401        1391        1381
CTTTAATAGG CTCTTCGCCA TTTTCTGCGC GGCGTGATTT CGACGCCAGA ATGCCTAAAA
                       ::: ::::::::   :::::::: :  ::: :::::
                  111H2OH2.2>CGC GGCGTGAT   CGACGCAG A  TGC TAAA

      1371        1361        1351        1341        1331        1321
TACGGTCAGA GTCGCGTACT GAGGAGACTT CCGGGTTGGT GGTAATAATG GCTTCGTCTG
::: :::::: :::::::::: :::::::::   :::  : ::  ::: :: ::  :: :: :::
TAC GTCAGA GTCGCGTACT GAGGAGACT   CGG  T  GT  GTA TA TG  CT CG CTG


     1311        1301        1291        1281        1271        1261
CAAAATAGAG TGCCATTAAC GCACCGGTTT CAATCCCTGC CGGGGAGTCA CAAACGATAA

      1251        1241        1231        1221        1211        1201
ATTCAAAATC CATCGCTTTC AGATCATCAA GAACTTTGGC GACCCCTTCA CGGGTGAGGG

      1191        1181        1171        1161        1151        1141
CATCTTTATC GCGTGTTTGC GATGCCGGCA GAATATAGAG ATTTTCAGTA CGCTTATCTT

      1131        1121        1111        1101        1091        1081
TAATTAACGC CTGATTTAGC GTTGCATCGC CCTGAATGAC GTTGACGAAA TCGTAAACGA

      1071        1061        1051        1041        1031        1021
CCCGGCGTTC ACAACCCATA ATCAGGTCGA GATTACGCAG GCCGATATCA AAATCTATCA
```

```
      1011        1001         991         981         971         961
CGACAGTTTT CTTTCCCTTC TGGGCCAAAC CAGTGGCGAT GGCCGCGCTG GAGGTTGTCT

       951         941         931         921         911         901
TACCAACACC CCCTTTGCCC GAAGTAACAA CAATAATGCG TGCCATAGAA ATTCCTTGTT
                        ::::::: :::::::::: :::::::::: ::::::::::
                        GTAACAA CAATAATGCG TGCCATAGAA ATTCCTTGTT
       891         881         871         861         851         841
AAAAAGGGAT CAATTTAACG GTTGAACGGT CAAAGCGTTT TCGACTAACT GCAGTCGCGC
:::::::::: :::::::::
AAAAAGGGAT CAATTTAAC<111HT7.2                       PstI
```

glt/sdh Mutant #182



Insertion #182 between *glt*A and *sdh*C.

Known Genes Near *glt/sdh*

Gene		Function
phr		Deoxyribodipyrimidine photolyase
rdgA		Dependence of growth upon recA gene product
dctA		uptake of C4 dicarboxylic acids
cbt		Dicarboxylate-binding proteinprotein production
gltA		citrate synthase
sdh	C	succinate dehydrogenase, cytochrome b_{556}
	D	, hydrophobic subuinit
	A	, flavoprotein subunit
	B	, iron/sulfur protein
suc	A	α-ketoglutarate dehydrogenase, decarboxylase subunit
	B	, dihydrolipotranssuccinase component
	C	Succinyl-Co-A synthase, β subunit
	D	, α subunit

***gltA/sdh* - Mutants #182:**

MiniTn10-*tet* insertion #182 was found to lay between *glt*A and *sdh*C. *gltt*A codes for citrate synthase (Hull et al., 1983), while the *sdh* genes are involved in succinate metabolism (succinate dehydrogenase) (Spencer and Guest, 1974). The *sdh*, and *glt*A genes are thus involved in aerobic metabolism. Mutant #182 grows slowly, both in liquid media (LB), and on plates (LB and MacConkey).

Sequencing into flanking chromosomal DNA from the OH2 and OH3 primer ends of miniTn10-*tet* insertion #182 indicated that the insertion lies between nucleotides #2905 and #2995 of the "ecoglta" locus. With *glt*A coding sequence between nucleotides #1091 and #2374, and *sdh*C coding sequence between nucleotides #3081 and #3470, miniTn10-*tet* insertion #182 lies between *glt*A and *sdh*C. MiniTn10-*tet* insertion #182 was genetically linked by P1 transduction to a marker at minute 16.25 - near *glt*A at min. 16.5.

MiniTn10-*tet* insertion #182 was found to dramatically increase both miniTn7-*lac* and miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). As measured by the mating-out assay, mutant #182 increases miniTn7-*lac* transposition to pOX38*gen*. 1.5 fold; however, it decreases Tn7 transposition 1.7 fold.

Mutant 182

Sequences Obtained:

T3 Primer
From pOH53 - 7.181.182i.37.t3:
AGGGGGGAGCCAGTTTTCTGAGTGCCACGCCAGCCAGGCTTGCGGAATACTAATATTCAGTTGCTGATTGGC
TTGATCGAAATTGAAGAGCATTTCAGGTCGGGAACTGAAATCAATACACCGATCTATCTGTGGCAAGGACTG
ACGGATATCTGGTTTTAAACCAAATTTATCGACCAGTGAATCATTGATGCATGGAATGG
GenBank Match - NONE

OH2 Primer
From pOH53 - 7.181.182i.37.oh2:
ACGTTACAACGCTGGGTGGCTCGGGATTGCAGGGTGTTCCGGAGACCTGGCGGCAGTATAGGCTGTTCACAA
AATCATTACAATTAACCTACATATAGTTTGTCGGG
GenBank Match - >**ECOGLTA** (GenBank) E.coli gltA gene, sdhCDAB operon and sucABCD operon encoding complete proteins.

OH3 Primer
From pOH53 - 7.181.182i.37.oh3:
TCTCCAGGTAACAGAAAGTTAACCTCTGTGCCCGTAGTCCCCAGGGAATAATAAGAACAGCATGTGGGCGTT
ATTCATGATAAGAAATGTGAAAAA
GenBank Match - >**ECOGLTA** (GenBank) E.coli gltA gene, sdhCDAB operon and sucABCD operon encoding complete proteins.

T7 Primer
From pOH53 - 7.181.182i.37.t7:
AATGGACGCTATCGCCGTGATGGGGAACCGGATGGTCTGTAGGTCCAGATTAACAGGTCTTTGTTTTTTCAC
ATTTCTTATCATGAATAACGCCCACATGCTGTTCTTATTATTCCCTGGGGACTACGGGCACAGAGGTTAACT
TTCTGTTACCTGGAGACGTCGG
GenBank Match - >**ECOGLTA** (GenBank) E.coli gltA gene, sdhCDAB operon and sucABCD operon encoding complete proteins.

***gltA/sdh* Sequences**

gltA Coding Sequence = 1091-2374
sdhC Coding Sequence = 3081-3470
sdhD Coding Sequence = 3464-3811
sdhA Coding Sequence = 3811-5577
sdhB Coding Sequence = 5593-6309
sucA Coding Sequence = 6609-9410
Located at min. 16.5

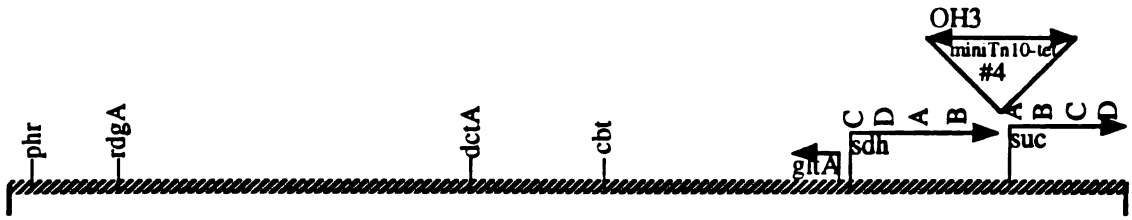
Mutant #182:
6.5kb KpnI insertion in pBluescript plasmid.
"Forward" end of cloned chromosomal DNA ends in guaB.
MiniTn10-*tet* insertion between gltA and sdhC coding sequence (between #2910 and #2995).
One KpnI cloning site located.

***gltA, sdhC*:**

```
C1 ( 1f): |>u 1>+++++ ecoglta (13063 bases)+++++>u 13063>|
      2710        2720        2730        2740        2750        2760
GGTAATGTTT GTAACAACTT TGTTGAATGA TTGTCAAATT AGATGATTAA AAATTAAATA

      2770        2780        2790        2800        2810        2820
AATGTTGTTA TCGTGACCTG GATCACTGTT CAGGATAAAA CCCGACAAAC TATATGTAGG
                                             :::::::::: ::::::::::
                                             CCCGACAAAC TATATGTAGG

      2830        2840        2850        2860        2870        2880
TTAATTGTAA TGATTTTGTG AACAGCCTAT ACTGCCGCCA GTCTCCGGAA CACCCTGCAA
:::::::::: :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
TTAATTGTAA TGATTTTGTG AACAGCCTAT ACTGCCGCCAGGTCTCCGGAA CACCCTGCAA

      2890        2900        2910        2920        2930        2940
TCCCGAGCCA CCCAGCGTTG TAACGTGTCG TTTTCGCATC TGGAAGCAGT GTTTTGCATG
:::::::::: :::::::::: :::::
TCCCGAGCCA CCCAGCGTTG TAACG<7.181.182i.37.oh2

      2950        2960        2970        2980        2990        3000
ACGCGCAGTT ATAGAAAGGA CGCTGTCTGA CCCGCAAGCA GACCGGAGGA AGGAAATCCC
                                                                 ::
                                                                 CC
7.181.182i.37.oh3>
    TCTCCA GGTAACAGAA AGTTAACCTC TGTGCCCGTA GTCCCCAGGG AATAATAAGA
    ::3010 ::::::3020 ::::::3030 ::::::3040 ::::::3050 ::::::3060
GACGTCTCCA GGTAACAGAA AGTTAACCTC TGTGCCCGTA GTCCCCAGGG AATAATAAGA
:::::::::: :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
GACGTCTCCA GGTAACAGAA AGTTAACCTC TGTGCCCGTA GTCCCCAGGG AATAATAAGA
                                             7.181.182i.37.t7

ACAGCATGTG GGCGTTATTC ATGATAAGAA ATGTGAAAAA
::::::3070 ::::::3080 ::::::3090 ::::::3100        3110        3120
ACAGCATGTG GGCGTTATTC ATGATAAGAA ATGTGAAAAA ACAAAGACCT GTTAATCTGG
:::::::::: :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
ACAGCATGTG GGCGTTATTC ATGATAAGAA ATGTGAAAAA ACAAAGACCT GTTAATCTGG
                                             7.181.182i.37.t7
```

```
      3130        3140        3150        3160        3170        3180
ACCTACAGAC CATCCGGTTC CCCATCACGG CGATAGCGTC CATTCTCCAT CGCGTTTCCG
:::::::::: :::::::::: :::::::::: :::::::::: ::::
ACCTACAGAC CATCCGGTTC CCCATCACGG CGATAGCGTC CATT
                                             <7.181.182i.37.t7

      3190        3200        3210        3220        3230        3240
GTGTGATCAC CTTTGTTGCA GTGGGCATCC TGCTGTGGCT TCTGGGTACC AGCCTCTCTT
                                               KpnI 3229

      3250        3260        3270        3280        3290        3300
CCCCTGAAGG TTTCGAGCAA GCTTCCGCGA TTATGGGCAG CTTCTTCGTC AAATTTATCA

      3310        3320        3330        3340        3350        3360
TGTGGGGCAT CCTTACCGCT CTGGCGTATC ACGTCGTCGT AGGTATTCGC CACATGATGA

      3370        3380        3390        3400        3410        3420
TGGATTTTGG CTATCTGGAA GAAACATTCG AAGCGGGTAA ACGCTCCGCC AAAATCTCCT

      3430        3440        3450        3460        3470        3480
TTGTTATTAC TGTCGTGCTT TCACTTCTCG CAGGAGTCCT CGTATGGTAA GCAACGCCTC

      3490        3500        3510        3520        3530        3540
CGCATTAGGA CGCAATGGCG TACATGATTT CATCCTCGTT CGCGCTACCG CTATCGTCCT

      3550        3560        3570        3580        3590        3600
GACGCTCTAC ATCATTTATA TGGTCGGTTT TTTCGCTACC AGTGGCGAGC TGACATATGA

      3610        3620        3630        3640        3650        3660
AGTCTGGATC GGTTTCTTCG CCTCTGCGTT CACCAAAGTG TTCACCCTGC TGGCGCTGTT

      3670        3680        3690        3700        3710        3720
TTCTATCTTG ATCCATGCCT GGATCGGCAT GTGGCAGGTG TTGACCGACT ACGTTAAACC

      3730        3740        3750        3760        3770        3780
GCTGGCTTTG CGCCTGATGC TGCAACTGGT GATTGTCGTT GCACTGGTGG TTTACGTGAT

      3790        3800        3810        3820        3830        3840
TTATGGATTC GTTGTGGTGT GGGGTGTGTG ATGAAATTGC CAGTCAGAGA ATTTGATGCA

      3850        3860        3870        3880        3890        3900
GTTGTGATTG GTGCCGGTGG CGCAGGTATC GCGCGCGCGC TGCAAATTTC CCAGAGCGGC
```

sdh/suc
Mutant #4



Insertion #4 between *sdh*B and *suc*A.

Known Genes Near *sdh/suc*

Gene		Function
phr		Deoxyribodipyrimidine photolyase
rdgA		Dependence of growth upon recA gene product
dctA		uptake of C4 dicarboxylic acids
cbt		Dicarboxylate-binding proteinprotein production
gltA		citrate synthase
sdh	C	succinate dehydrogenase, cytochrome b_{556}
	D	, hydrophobic subuinit
	A	, flavoprotein subunit
	B	, iron/sulfur protein
suc	A	α-ketoglutarate dehydrogenase, decarboxylase subunit
	B	, dihydrolipotranssuccinase component
	C	Succinyl-Co-A synthase, β subunit
	D	, α subunit

***sdh/suc* - Mutant #4:**

MiniTn10-*tet* insertion #4 lies between *sdh*B and *suc*A. The *sdh* and *suc* genes are involved in succinate metabolism, with *sdh*B encoding the iron/sulfur binding subunit of succinate dehydrogenase (Spencer and Guest, 1974), and *suc*A encodes α-ketoglutarate dehydrogenase (Darlison et al., 1984). The *suc* and *sdh* genes are thus involved in aerobic metabolism. Mutant #4 grows slowly, both in liquid media (LB), and on plates (LB and MacConkey).

Sequence from the OH3 primer end of miniTn10-*tet* insertion #4 indicated that insertion lies just above nucleotide #6430 of the "ecoglta" locus. With *sdh*B coding sequence between nucleotides #5593 and #6309, and *suc*A coding sequence between nucleotides #6609 and #9410, miniTn10-*tet* insertion #182 lies between *sdh*B and *suc*A. MiniTn10-*tet* insertions #4 was genetically linked to a marker at minute 16.75 - both near *glt*A at min. 16.5.

MiniTn10-*tet* insertion #4 decreases both miniTn7-*lac* papillation and miniTn7-*lac* transposition to pOX38*gen* 5 fold, while slightly increasing Tn7 transposition (see Chapter 5, Figure 5-4 and Appendix A, Table A-4).

Mutant 4

Sequences Obtained:

T3 Primer
No Sequence

OH2 Primer
No Sequence

OH3 Primer
From pOH55 - p4d1oh3:
TGATGCGCTGCGTTATCAGGCCTACGGGTAATGACTTGTAGGCCCGGATAAGGGTTCACCCATCcACTGGTT
GCTGATGCGACGCTTGCCGTTATCAGGCTCAGGTTTACGCATTACGTTGCAACAACATCGACTTGATATGGC
CGATGCAGCCTCGACAACCTGACGCGTCATGATGCTGTGACAGCGATACGCTGATGCATACTCAACGTCGAG
CGCTGTCAG
GenBank Match - >**ECOGLTA** (GenBank) E.coli gltA gene, sdhCDAB operon and sucABCD operon encoding complete proteins.

T7 Primer
No Sequence

***sdh/suc* Sequences**

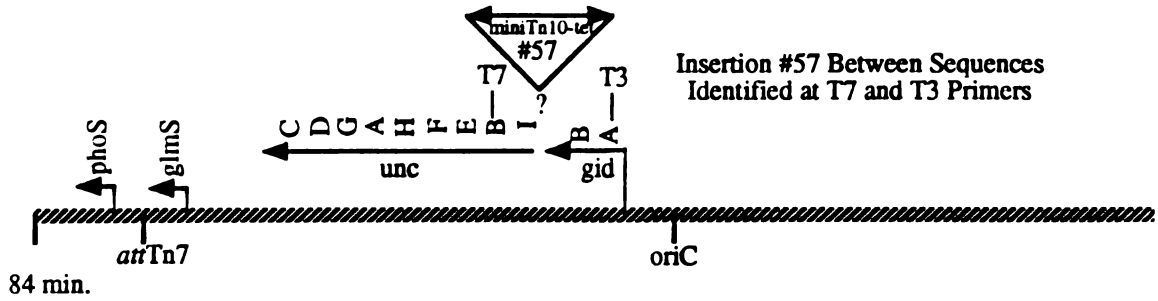
gltA Coding Sequence = 1091-2374
sdhC Coding Sequence = 3081-3470
sdhD Coding Sequence = 3464-3811
sdhA Coding Sequence = 3811-5577
sdhB Coding Sequence = 5593-6309
sucA Coding Sequence = 6609-9410
Located at min. 16.5

Mutant #4:
5-6kb PstI/SacI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion between sdhB and sucA coding sequence (just above #6430).

sdhB, sucA:

```
C1 ( 1f): |>u 1>+++++ ecoglta (13063 bases)+++++>u 13063>|
      6190        6200        6210        6220        6230        6240
TTGAGTGATG CATTCAGCGT ATTCCGCTGT CACAGCATCA TGAACTGCGT CAGTGTATGT

                GCATCAGGCGTAACAAAGAAGTATGCATCAGCGTATCGCTGTCACAGCA
                                                          p4d1oh3

      6250        6260        6270        6280        6290        6300
CCGAAGGGGC TGAACCCGAC GCGCGCCATC GGCCATATCA AGTCGATGTT GTTGCAACGT
                          ::  :::: :::::::::: :::::::::: ::::::::::
TCATGACGCGTCAGGTTGTC GAGGCTGCATC GGCCATATCA AGTCGATGTT GTTGCAACGT
                                                          p4d1oh3

      6310        6320        6330        6340        6350        6360
AATGCGTAAA CCGTAGGCCT GATAAGACGC GCAAGCGTCG CATCAGGCAA CCAGTGCCGG
:::::::::: :: ::::::: :::::  ::  :::::::::: :::::: ::: :::::   ::
AATGCGTAAA CC TGAGCCT GATAA  CG  GCAAGCGTCG CATCAG CAA CCAGT   GG
                                                          p4d1oh3

      6370        6380        6390        6400        6410        6420
ATGCGCGTGA ACGCCTTATC CGGCCTACAA GTCATTACCC GTAGGCCTGA TAAGCGCAGC
::: : :::: :: ::::::: :::::::::: :::::::::: :::::::::: ::::    ::
ATG G GTGA AC CCTTATC CGGCCTACAA GTCATTACCC GTAGGCCTGATAACGCAGCGC
                                                          p4d1oh3

      6430        6440        6450        6460        6470        6480
ATGCAGGAAA TCTTTAAAAA CTGCCCCTGA CACTAAGACA CTGACAGCGC TCGACGTTGA
:: ::
AT CA<p4d1oh3

      6490        6500        6510        6520        6530        6540
GTTTTTAAAG GTTCCTTCGC GAGCCACTAC GTAGACAAGA GCTCGCAAGT GAACCCCGGC
                                          SacI 65523
```

unc/gid
Mutants #57 and #58



Insertion #57 Between Sequences Identified at T7 and T3 Primers

Mutant #58 was not cloned.

Insertion #57 between *unc*B and *gid*A.

Known Genes Near *unc/gid*

Gene		Function
phoS		Phosphate-specific trans periplasmic transport
glmS		D-fructose-6-phosphate aminotransferase
unc	C	Membrane-bound ATP synthase, F_1 ε-subunit
	D	, F_1 β-subunit
	G	, F_1 γ-subunit
	A	, F_1 α-subunit
	H	, F_1 δ-subunit
	F	, F_0 subunit b
	E	, F_0 subunit c
	B	, F_0 subunit a
	I	, Unknown
gid	B	Glucose-inhibited division; chromosome replication
	A	

***gid/unc* - Mutants #57 and #58:**

Mutant #57 lies between the *gid*A and *unc*B genes. The *gid* and *unc* genes are located immediately counter-clockwise of OriC (Bachmann, 1990). The *unc* genes, isolated as succinate non-utilizing mutants, code for the major membrane-bound ATP synthase complex (Walker et al., 1984a; Walker et al., 1984b). *gid* is thought to be involved in the control of *ori*C activity in that *gid* transcription increases *ori* activity (possibly by affecting local supercoiling) and stringent response repression of *gid* transcription decreases *ori* activity (Ogawa and Okazaki, 1991). Mutants #57 and #58 grow slowly both in liquid media (LB) and on plates (LB and MacConkey).

MiniTn10-*tet* insertion #57 is located between *gid*A, and *unc*B - in a 2.4 Kb stretch of DNA encoding *gid*A, *gid*B, the *unc* operon promoter, *unc*I, and *unc*B. The location of miniTn10-*tet* insertion #57 was determined by sequencing into each end of the cloned miniTn10-*tet* containing chromosomal DNA from the cloning vector plasmid (pBluescript). Sequence from the T3 pBluescript primer located the PstI cloning site at nucleotide #1882 of the "ecouncc" *gid/unc* operons, and continued through *gid*A. Sequence from the T7 pBluescript primer located the other PstI cloning site at nucleotide #4527 of the "ecouncc" *gid/unc* genes, and continued into *unc*B. Both miniTn10-*tet* insertions #57 was genetically linked by P1 transduction to a marker at minute 84.5, near the *gid/unc* operons at min. 84.2. Insertion # 58 was also found to be linked to minute 84.5 by P1 transduction. Insertion #58 was not cloned, however, and so the point of insertion is unknown.

MiniTn10-*tet* insertions #57 was found to dramatically increase miniTn7-*lac* papillation, while insertion #58 decreases miniTn7-*lac* papillation (see Figure 5-4, Chapter 5). MiniTn10-*lac* papillation was decreased in mutant #57, while mutant #58 did not affect miniTn10-*lac* papillation (see Figure 5-5, Chapter 5). MiniTn10-*tet* insertion #57 shows a 5 fold reduction of Tn7 transposition to pOX38*gen*, while miniTn7-*lac* transposition is reduced 2 fold as measured in the mating out assay.

Mutant 57

Sequences Obtained:

T3 Primer
From pOH56 - p57D2-T3:
GAATTCCTGCAGCCGAAGTGAATTCGTCACCTGACTGCGCCGCTTTCCGTGAAGAGTGGTGAAGATCT
CGTGCGTGTCCGGAATGACTTATGAAAATTAACCACGCTGAGGCGTTGCCcTGCGTGACAGACGACAGG
GenBank Match - >**ECOUNCC** (GenBank) E.coli unc operon encoding the eight subunits of ATP synthase.

OH2 Primer
No Sequence

OH3 Primer
No Sequence

T7 Primer
From pOH56 - p57D2-T7:
CTGCAGGCTCAACTCTTTCGTGAAGCCGCCGATGCCTTTCATTTTGATGCTGTAGAACAGAATCAGGATAAT
ACGCCAGTGCcATAGACAGCGTTAGCTTCACGTCGCAGAGCAACCACA
GenBank Match - >**ECOUNCC** (GenBank) E.coli unc operon encoding the eight subunits of ATP synthase.

***gid/unc* Sequences**

gidA Coding Sequence = 407-2293
gidB Coding Sequence = 2357-2980
uncI Coding Sequence = 3585-3977
uncB Coding Sequence = 3986-4810
uncE Coding Sequence = 4848-5087
Located at min. 84.2

Mutant #57:
6-7kb PstI/SacI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion somewhere between gidA and uncB coding sequence.
Both PstI sites located (1882 and 4527).

***gidA, gidB, uncI, uncB, uncE*:**

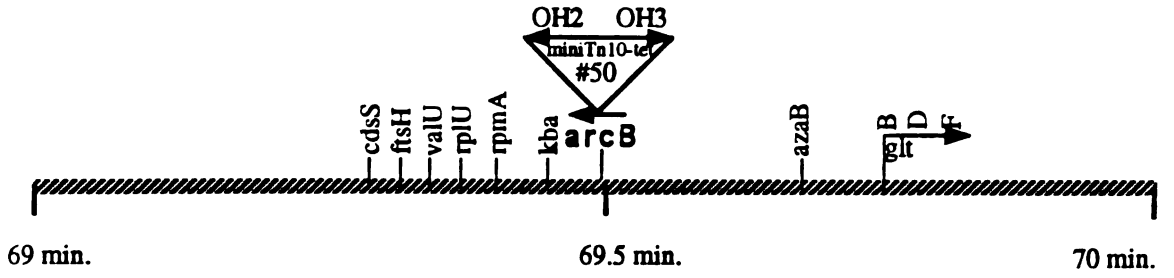
C1 (1f): |>u 1>+++++ ecouncc (14526 bases)++++++>u 14526>|

```
      1870        1880        1890        1900        1910        1920
AACCCCGTCG GCGGAAGCTG CAGCCGAAGT GAATGCTCAC CTGACTGCGC CGCTTTCCCG
                  ::: :::::::::: :::: :::: :::::::::: :::::::: :
   p57D2-T3>GAATTCCTG CAGCCGAAGT GAATCGTCAC CTGACTGCGC CGCTTTCC G
                     PstI
      1930        1940        1950        1960        1970        1980
TGAAGCCAGT GGTGAAGATC TGCTGCGTCC GGAAATGACT TATGAAAAAT TAACCACGCT
::::::  :: :::::::::: :::::::::: :::: ::::: :::::::: : ::::::::::
TGAAGA  GT GGTGAAGATC TCGTGCGTCC GGAA TGACT TATGAAAA T TAACCACGCT
                                                      p57D2-T3>

      1990        2000        2010        2020        2030        2040
GACGCCGTTT GCCCCTGCGT TGACAGACGA ACAGGCGGCG GAACAGGTTG AGATTCAGGT
:: ::::::  :::: :::::  :::::::::  ::::
GA GGCGTT  GCCc TGCGT  GACAGACGA  CAGG p57D2-T3

      2050        2060        2070        2080        2090        2100
TAAATACGAA GGTTATATCG CGCGCCAGCA AGATGAGATC GAAAAGCAGC TGCGTAACGA

      2110        2120        2130        2140        2150        2160
GAACACCCTG CTACCCGCGA CACTGGATTA CCGCCAGGTA TCCGGTCTTT CTAACGAAGT

      2170        2180        2190        2200        2210        2220
GATCGCCAAA CTTAACGATC ACAAACCAGC CTCTATCGGC CAAGCTTCGC GTATTTCTGG

      2230        2240        2250        2260        2270        2280
CGTCACGCCT GCGGCCATCT CCATTCTGCT GGTGTGGCTG AAAAAACAGG GTATGCTGCG

      2290        2300        2310        2320        2330        2340
TCGTAGCGCA TAACGCATTA AAAATGCCTG GTAAGCACCC GCTTACCAGG CAACGCATCA

      2350        2360        2370        2380        2390        2400
AGAACAGGTA ATCACCGTGC TCAACAAACT CTCCTTACTG CTGAAAGACG CAGGTATTTC

      2410        2420        2430        2440        2450        2460
GCTTACCGAT CACCAGAAAA ACCAGCTTAT TGCCTACGTG AATATGCTGC ATAAATGGAA

      2470        2480        2490        2500        2510        2520
CAAAGCGTAC AACCTGACTT CGGTCCGCGA TCCTAATGAG ATGCTGGTAC GCCATATTCT
```

```
      2530        2540        2550        2560        2570        2580
CGATAGCATT GTGGTGGCAC CGTATCTGCA AGGTGAACGG TTTATCGATG TCGGCACCGG

      2590        2600        2610        2620        2630        2640
ACCAGGACTG CCAGGCATTC CACTCTCTAT CGTGCGTCCT GAAGCCCATT TCACTCTGTT

      2650        2660        2670        2680        2690        2700
GGATAGCCTT GGTAAACGCG TGCGTTTCCT TCGTCAGGTG CAACATGAGC TTAAACTGGA

      2710        2720        2730        2740        2750        2760
GAATATTGAA CCAGTACAGA GCAGGGTAGA AGAGTTTCCT TCAGAGCCGC CATTTGATGG

      2770        2780        2790        2800        2810        2820
CGTAATTAGC CGCGCTTTTG CCTCTCTGAA CGATATGGTG AGCTGGTGCC ACCATCTTCC

      2830        2840        2850        2860        2870        2880
TGGTGAGCAA GGCCGTTTCT ACGCGCTGAA AGGGCAAATG CCGGAAGATG AAATCGCTTT

      2890        2900        2910        2920        2930        2940
GTTGCCCGAA GAATATCAGG TCGAATCAGT GGTTAAACTT CAGGTTCCAG CCCTGGATGG

      2950        2960        2970        2980        2990        3000
CGAACGTCAT CTGGTGGTGA TTAAAGCAAA TAAAATTTAA TTTTTATCAA AAAAATCATA

      3010        3020        3030        3040        3050        3060
AAAAATTGAC CGGTTAGACT GTTAACAACA ACCAGGTTTT CTACTGATAT AACTGGTTAC

      3070        3080        3090        3100        3110        3120
ATTTAACGCC ACGTTCACTC TTTTGCATCA ACAAGATAAC GTGGCTTTTT TTGGTAAGCA

      3130        3140        3150        3160        3170        3180
GAAAATAAGT CATTAGTGAA AATATCAGTC TGCTAAAAAT CGGCGCTAAG AACCATCATT

      3190        3200        3210        3220        3230        3240
GGCTGTTAAA ACATTATTAA AAATGTCAAT GGGTGGTTTT TGTTGTGTAA ATGTCATTTA

      3250        3260        3270        3280        3290        3300
TTAAAACAGT ATCTGTTTTT AGACTGAAAT ATCATAAACT TGCAAAGGCA TCATTTGCCA

      3310        3320        3330        3340        3350        3360
AGTAAATAAA TATGCTGTGC GCGAACATGC GCAATATGTG ATCTGAAGCA CGCTTTATCA

      3370        3380        3390        3400        3410        3420
CCAGTGTTTA CGCGTTATTT ACAGTTTTTC ATGATCGAAC AGGGTTAGCA GAAAAGTCGC

      3430        3440        3450        3460        3470        3480
AATTGTATGC ACTGGAAAAA TATTTAAACA TTTATTCACC TTTTGGCTAC TTATTGTTTG

      3490        3500        3510        3520        3530        3540
AAATCACGGG GGCGCACCGT ATAATTTGAC CGCTTTTTGA TGCTTGACTC TAAGCCTTAA

      3550        3560        3570        3580        3590        3600
AGAAAGTTTT ATACGACACG CGGCATACCT CGAAGGGAGC AGGAGTGAAA AACGTGATGT

      3610        3620        3630        3640        3650        3660
CTGTGTCGCT CGTGAGTCGA AACGTTGCTC GGAAGCTTCT GCTCGTTCAG TTACTGGTGG
```

```
      3670        3680        3690        3700        3710        3720
TGATAGCAAG TGGATTGCTG TTCAGCCTCA AAGACCCCTT CTGGGGCGTC TCTGCAATAA

      3730        3740        3750        3760        3770        3780
GCGGGGGCCT GGCAGTCTTT CTGCCTAACG TTTTGTTTAT GATATTTGCC TGGCGTCACC

      3790        3800        3810        3820        3830        3840
AGGCGCATAC ACCAGCGAAA GGCCGGGTGG CCTGGACATT CGCATTTGGC GAAGCTTTCA

      3850        3860        3870        3880        3890        3900
AAGTTCTGGC GATGTTGGTG TTACTGGTGG TGGCGTTGGC GGTTTTAAAG GCGGTATTCT

      3910        3920        3930        3940        3950        3960
TGCCGCTGAT CGTTACGTGG GTTTTGGTGC TGGTGGTTCA GATACTGGCA CCGGCTGTAA

      3970        3980        3990        4000        4010        4020
TTAACAACAA AGGGTAAAAG GCATCATGGC TTCAGAAAAT ATGACGCCGC AGGATTACAT

      4030        4040        4050        4060        4070        4080
AGGACACCAC CTGAATAACC TTCAGCTGGA CCTGCGTACA TTCTCGCTGG TGGATCCACA

      4090        4100        4110        4120        4130        4140
AAACCCCCCA GCCACCTTCT GGACAATCAA TATTGACTCC ATGTTCTTCT CGGTGGTGCT

      4150        4160        4170        4180        4190        4200
GGGTCTGTTG TTCCTGGTTT TATTCCGTAG CGTAGCCAAA AAGGCGACCA GCGGTGTGCC

      4210        4220        4230        4240        4250        4260
AGGTAAGTTT CAGACCGCGA TTGAGCTGGT GATCGGCTTT GTTAATGGTA GCGTGAAAGA

      4270        4280        4290        4300        4310        4320
CATGTACCAT GGCAAAAGCA AGCTGATTGC TCCGCTGGCC CTGACGATCT TCGTCTGGGT

      4330        4340        4350        4360        4370        4380
ATTCCTGATG AACCTGATGG ATTTACTGCC TATCGACCTG CTGCCGTACA TTGCTGAACA

      4390        4400        4410        4420        4430        4440
TGTACTGGGT CTGCCTGCAC TGCGTGTGGT TCCGTCTGCG GACGTGAACG TAACGCTGTC
                          :::::: :   :::::: :: ::::::: ::::::::::
                          TGTGGT T GCTCTGCG AC GTGAAGC TAACGCTGTC
                                                          <p57D2-T7

      4450        4460        4470        4480        4490        4500
TATGGCACTG GGCGTATTTA TCCTGATTCT GTTCTACAGC ATCAAAATGA AAGGCATCGG
:::::::::: : :::::: : :::::::::: :::::::::: :::::::::: ::::::::::
TATGGCACTG G CGTATT A TCCTGATTCT GTTCTACAGC ATCAAAATGA AAGGCATCGG
                                                          <p57D2-T7

      4510        4520  PstI 4530        4540        4550        4560
CGGCTTCACG AAAGAGTTGA CGCTGCAGCC GTTCAATCAC TGGGCGTTCA TTCCTGTCAA
:::::::::: :::::::::: ::::::::
CGGCTTCACG AAAGAGTTGA GCCTGCAG<p57D2-T7

      4570        4580        4590        4600        4610        4620
CTTAATCCTT GAAGGGGTAA GCCTGCTGTC CAAACCAGTT TCACTCGGTT TGCGACTGTT
```

*arc*B Mutant #50



Insertion #50 in *arc*B coding sequence.

Known Genes Near *arc*B

Gene		Function
cdsS		Stability of CDP-diglyceride synthetase activity
ftsH		Cell division
valU		Valine tRNA 1 (tandemly triplicated gene)
rplU		L21 subunit of 50S ribosomal complex
rpmA		L27 subunit of 50S ribosomal complex
kba		Ketose-bis-phosphate adolase
arcB		negative regulatory gene of genes in aerobic pathways
azaB		resistance or sensitivity to azaserine
glt	B	glutamate synthase, large subunit
	D	glutamate synthase, small subunit
	F	unknown - regulatory gene?

***arc*B - Mutant #50:**

MiniTn10-*tet* insertion mutant #50 was found to interrupt *arc*B. *arc*B codes for the periplasmic membrane-bound sensor for a classical two-component regulatory system (Iuchi et al., 1989; Iuchi et al., 1990). ArcB phosphorylates ArcA which represses aerobic metabolism genes under anaerobic growth conditions (Iuchi and EC., 1992). Phosphorylated ArcA also turns on a number of anaerobically expressed genes (Iuchi et al., 1989).

MiniTn10-*tet* insertion #50 is within the coding sequence of *arc*B. Sequence flanking each end of miniTn10-*tet* insertion #50 (OH2 and OH3 primers) matched *arc*B sequence. Sequence from the OH2 and OH3 primer ends of miniTn10-*tet* insertion #50 indicated that insertion lies between nucleotides #720 and #735 of the "ecoarcb" *arc*B gene locus. With *arc*B coding sequence stretching between nucleotides #24 and #2360, miniTn10-*tet* insertion #50 lies within the coding sequence of *arc*B. MiniTn10-*tet* insertion #50 was also genetically linked by P1 transduction to a marker at minute 70, near *arc*B at min. 69.5.

Mutant #50 decreases miniTn7-*lac* and decreases miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). As measured by the mating-out assay, however, mutant #50 produces a 2.5 fold increase of Tn7 transposition to pOX38*gen*, and a 1.8 fold increase in miniTn7-*lac* transposition.

Mutant 50

Sequences Obtained:

T3 Primer
From pOH70 - 50HT3:
GGCGAATCTGCCTTGAATTTCAGGTAGCGCAGGTTTTTGGACGCCACTGGCAGCGTCGGGCGCTCCAGCCTG
TACCACCTGATGAGCCACTGTTTATGGCACTACTTTTTTCATGTTGCGGGTTCCGATCCATTGCGGGATGCG
CATCAGCGGACAGACAATTGCGTCAGTCTTATCAACGTCTGGTAACATTTCGTGAGCTGGAATGTCGTTTAT
AGTGACA
GenBank Match - NONE

OH2 Primer
From pOH70 - 50H1OH2:
CCATCTGTTCATAGGTCAGTGACACATTAGTA
GenBank Match - >**ECOARCB** (GenBank) E. coli arcB gene for ArcB membrane protein

OH3 Primer
From pOH70 - 50H1OH3:
AAAGCCTGCTTTGAAATCGCTAAAGTGCGTACTAGCATC
GenBank Match - >**ECOARCB** (GenBank) E. coli arcB gene for ArcB membrane protein

T7 Primer
From pOH70 - 50HT7:
CCAAAACGCTGGCCCTCACTTCGCGCGACAGTTAATCCAGCTGGTGTTGTTGCATAAACGGCTCACCGCCTA
ACTGATACATCTGCCGTAAAATCCACGCCTGACGGCTACGCACGTAGCCGATGGCGAGGAGACTTTGAACGA
GenBank Match - NONE

***arcB* Sequence**

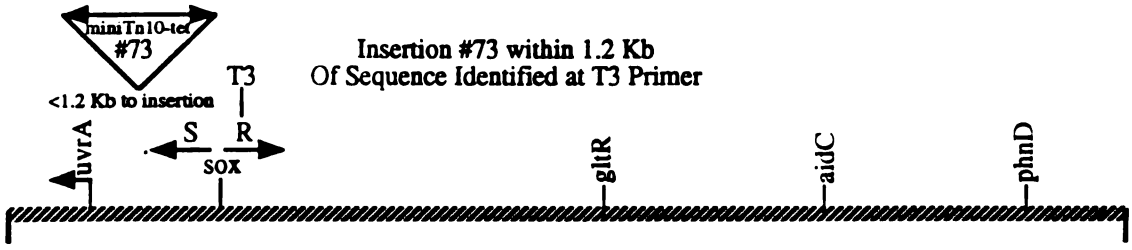
arcB Coding Sequence = 24-2360
Located at min. 69.5

6kb PstI insertion in pK19 plasmid.
MiniTn10-*tet* nsertion in *arc*B coding sequence between #720 and 735.
One PstI cloning site.

***arcB*:**

```
        10          20          30          40          50          60
GGTTGTCGTG AAGGAATTCC CTAATGAAGC AAATTCGTCT GCTGGCGCAG TATTATGTTG

        70          80          90         100         110         120
ACCTGATGAT GAAGTTAGGT CTGGTGCGCT TCTCAATGTT GCTGGCGCTG GCCCTCGTCG

       130         140         150         160         170         180
TTCTTGCCAT TGTGGTACAA ATGGCGGTAA CCATGGTGCT GCATGGTCAG GTCGAAAGCA

       190         200         210         220         230         240
TTGATGTTAT TCGTTCTATC TTCTTTGGTT TGCTGATTAC GCCGTGGGCG GTCTACTTTC

       250         260         270         280         290         300
TATCGGTGGT CGTCGAGCAA CTGGAGGAGT CACGACAACG TCTGTCACGG CTGGTGCAAA

       310         320         330         340         350         360
AACTGGAGGA GATGCGCGAG CGCGATTTGA GCCTCAACGT TCAGTTAAAA GATAATATTG

       370         380         390         400         410         420
CCCAGCTAAA TCAGGAAATT GCCGTTCGTG AAAAAGCGGA AGCAGAACTG CAGGAAACCT
                                                       PstI

       430         440         450         460         470         480
TCGGCCAACT GAAAATTGAA ATCAAAGAGC GCGAAGAGAC ACAAATTCAG CTCGAGCAGC

       490         500         510         520         530         540
AATCCTCATT CTTACGTTCC TTCCTTGATG CTTCACCCGA CCTGGTTTTT TATCGTAACG

       550         560         570         580         590         600
AAGATAAAGA GTTTTCCGGC TGTAACCGCG CGATGGAGCT GCTGACCGGA AAAAGCGAAA

       610         620         630         640         650         660
AACAACTGGT TCACCTGAAA CCTGCTGATG TTTACTCACC GGAAGCCGCC GCAAAAGTCA

       670         680         690         700         710         720
TTGAAACCGA TGAAAAAGTG TTCCGTCATA ATGTGTCACT GACCTATGAA CAGTGGCTGG
                          ::::: :::::::::: :::::::::: :::::::
                          TACTA ATGTGTCACT GACCTATGAA CAGATGG<50H10H2

       730         740         750         760         770         780
ATTACCCGGA CGGGCGCAAA GCCTGCTTTG AAATCCGTAA AGTGCCGTAC TACGACCGCG
               ::: :::::::::: :::::::::: ::::: :::: :::::::
          50H10H3>AAA GCCTGCTTTG AAATCGCTAA AGTGC GTAC TAGCATC

       790         800         810         820         830         840
TGGGTAAACG TCACGGTTTG ATGGGCTTTG GTCGCGACAT TACCGAGCGT AAGCGGTATC
```

*sox*RS
Mutant #73



Insertion #73 near soxR.

Known Genes Near *sox*RS

Gene		Function
uvrA		Repair of U.V. damage to DNA; excision nuclease
sox	R	Global regulatory resonse to oxidative stress; sensor
	S	Activator of oxidative stress regulon
gltA		Regulatory gene for glutamate permease
aidC		Induced by alkating agents
phnD		Carbon-phosphorus lyase

***sox*RS - Mutant #73:**

Mutant #73 was found near *sox*RS. The *sox*R gene codes for a sensor protein involved in global response to oxidative stress, while *sox*S codes for the activator of this oxidative stress regulon (Wu and Weiss, 1991).

The approximate location of miniTn10-*tet* insertion #73 was determined by sequencing into the one end of the cloned miniTn10-*tet* containing chromosomal DNA from the cloning vector plasmid (pBluescript). Sequence from the T3 pBluescript primer matched the sequence of *sox*R, and located one KpnI cloning site at nucleotide #1003 of the "ecosoxrs" *sox*RS genes for mutant #73. Sequence flanking the site of miniTn10-*tet* insertion was not obtained, however with miniTn10-*tet* 3 Kb long, the approximately 4.2 Kb KpnI insert size of the miniTn10-*tet* insertion #73 clone indicates that insertion was within 1.2 Kb of the located KpnI site. MiniTn10-*tet* insertion #73 was also found to be genetically linked by P1 transduction to a marker at minute 92.5, near the *sox*RS genes at min. 92.1. *uvr*A is the only other known gene within this region. *uvr*A codes for an excision endonuclease that was identified though its involvement in repair of UV damaged DNA (Witkin, 1972).

MiniTn10-*tet* insertion #73 was found to decrease both miniTn7-*lac*, and miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). Mutant #73 decreases in both Tn7 and miniTn7-*lac* transposition to pOX38*gen* (100 fold decrease of Tn7 transposition, while miniTn7-*lac* transposition is only slightly decreased 1.3 fold) - as measured in the mating out assay.

Mutant 73

Sequences Obtained:

T3 Primer
From pOH92 - 73IT3:
ACGCAACGGGCAATCACTGCGCGAAAGGCAGCCACAACCAATACATCCGTCC
GenBank Match - >**ECOSOXRS** (GenBank) E.coli SoxR and SoxS protein (soxR, soxS) genes, complete cds.

From pOH92 - 73IT3.top:
TCTTCTCGCATTGGGACGAAAGCTGTTTCACTCTTTCGCACTTACGTATGCCTTCAC
GenBank Match - >**ECOSOXRS** (GenBank) E.coli SoxR and SoxS protein (soxR, soxS) genes, complete cds.

OH2 Primer
No Sequence

OH3 Primer
No Sequence

T7 Primer
From pOH92 - 73IT7:
GTCATCGTGCACGACACCAATTACTGGCGTTGCGGGCATTATCTTTTTTTTAGTTCTCTTC
GenBank Match - NONE

From pOH92 - 73IT7.top:
AAATCAGAAGTGAATAATCAGCTCCGAACCTTTGCTCAACTGGCACTGGATAATCGAGCTGGTCA
GenBank Match - NONE

***soxRS* Sequence**

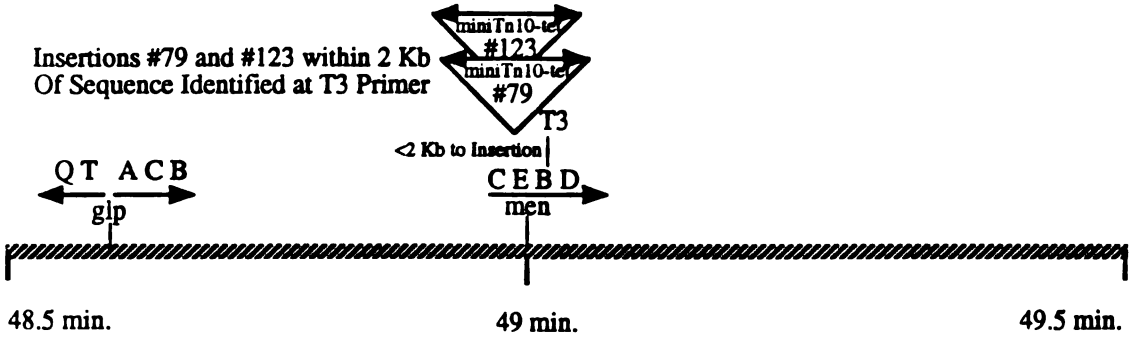
soxS Coding Sequence = 164-487
soxR Coding Sequence = 573-1037
Located at min. 92.1

Mutant #73:
4.2kb PstI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion not located.
One KpnI site (at #1003) located.

***soxRS*:**

```
C1 ( 1f): |>u 1>+++++ ecosoxrs (1099 bases)+++++>u 1099>|

       610         620         630         640         650         660
CGCTGCTAAC CCCCGGCGAA GTGGCGAAAC GCAGCGGTGT GGCGGTATCG GCGCTGCATT

       670         680         690         700         710         720
TCTATGAAAG TAAAGGGTTG ATTACCAGTA TCCGTAACAG CGGCAATCAG CGGCGATATA

       730         740         750         760         770         780
AACGTGATGT GTTGCGATAT GTTGCAATTA TCAAAATTGC TCAGCGTATT GGCATTCCGC

       790         800         810         820         830         840
TGGCGACCAT TGGTGAAGCG TTTGGCGTGT TGCCCGAAGG GCATACGTTA AGTGCGAAAG
                            ::      :::::  ::::::: : ::::::::::
                            GT      GAAGG  CATACGT A AGTGCGAAAG
                                                    <73IT3.top

       850         860         870         880         890         900
AGTGGAAACA GCTTTCGTCC CAATGGCGAG AAGAGTTGGA TCGGCGCATT CATACCTTAG
:::: ::::: :::::::::: ::::: :::: ::::
AGTG AAACA GCTTTCGTCC CAATG CGAG AAGA<73IT3.top

       910         920         930         940         950         960
TGGCGCTGCG TGACGAACTG GACGGATGTA TTGGTTGTGG CTGCCTTTCG CGCAGTGATT
                  : :::::::::: :::::::::: :::::::::: ::::::::::
                  G GACGGATGTA TTGGTTGTGG CTGCCTTTCG CGCAGTGATT<73IT3

       970         980         990        1000        1010        1020
GCCCGTTGCG TAACCCGGGC GACCGCTTAG GAGAAGAAGG TACCGGCGCA CGCTTGCTGG
:::::::::: :                     KpnI 1003
GCCCGTTGCG T<73IT3

      1030        1040        1050        1060        1070        1080
AAGATGAACA AAACTAAAGC GCCACAAGGG CGCTTTAGTT TGTTTTCCGG TCTTTGTCTT
```

*men*B
Mutants #79 and #123



Insertions #79 and #123 near *men*B.

Known Genes Near *men*B

Gene		Function	
glp	Q	Glcerol-3-phosphate diesterase	
	T	sn-Glycerol-3-phophate permease	
	A	Glycerol-3-phosphate dehydrogenase	anaerobic - large subunit
	C		anaerobic - small subunit
	B		anaerobic - membrane anchor
men	C	Conversion of chorismate to o-succinylbenzoate	
	E	o-succinylbenzoate-CoA synthetase	
	B	1;4-Dihydroxy-2-naphthoate synthase	
	D	Menaquinone biosynthesis	

***men*B - Mutants #123 and 79:**

Two of the miniTn10-*tet* generated Tn7 transposition mutants of E. *coli*, mutants #123 and #79, were found near *men*B. *men*B encodes 1,4-dihydroxy-2-naphthoate synthase - involved in the biosynthesis of the electron carrier menaquinone (vitamin K_2) (Sharma et al., 1992) and thus in the electron transport chains catalyzing the reduction of fumarate. *men* mutants fail to grow anaerobically on componds such as glycerol, lactate, and format; and are unable to grow on fermentable sugars unless uracil is added (Newton et al., 1971; H. Taber, 1980).

The location of miniTn10-*tet* insertions #123 and #79 were determined to be near the *men* B gene by sequencing into the one end of the cloned miniTn10-*tet* containing chromosomal DNA from the cloning vector plasmid (pBluescript). Sequence from the T3 pBluescript primer for both mutant #123 and #79 located one KpnI cloning site at nucleotide #972 of the "ecodhnasyn" *men*B gene. MiniTn10-*tet* insertions #123 was found to be weakly genetically linked by P1 transduction to a marker at minute 48.5, and miniTn10-*tet* insertions #79 was weakly linked to a marker at minute 49.5 - both near the *men*B gene at min. 49.

Mutant #123 was found to increase miniTn7-*lac* papillation, while mutant #79 decreased miniTn7-*lac* papillation (Figure 5-4). Mutant #123 increased miniTn10-*lac* papillation, while mutant #171 decreased miniTn10-*lac* papillation (Figure 5-5).

Mutant 79

Sequences Obtained:

T3 Primer

From pOH99 - 7.18a.79i.27.t3:
AGCCATGTAGGAAGCGCCCCAGCCGCCGTCGAAGGAACCGACTTTCGGGCCAGTCTGACCGAAGATGGCATT
ATCTGCCGCGATAGTCAGGTCGCACATCATGT
GenBank Match - >**ECODHNASYN** (GenBank) E.coli DHNA synthase (menB) gene, complete cds.

OH2 Primer

From pOH100 - 7.17s.79h.29.oh2:
TCCACCTTAACTTAATGATTTTTACCAAAATCATTAGGGGATTCATCAGGGCATAAAGCTCTGACGGTAAAA
CGCGTATCACGGCGGCAAC
GenBank Match - NONE

From pOH102 - 7.18a.79h.30.oh2:
ATTTACACCCAGGCGAGTGGCCTGTGATGTTGATTGCCATCCTGGCGCTGGTTTTTTGTTCACTACAATGCGA
CCGGCGTCTGGACCTTCAACTATGAAGAGCTGCTGAATACGCCAATGTCCAGTGGTGTGGAATACCTGTTAA
TGCTGGGCTTCTTCATCGCCTTCGCAGTCAAAATGCCGGTGGT
GenBank Match - NONE

From pOH99 - 7.18a.79i.27.oh2:
AGTGGCCAGTGATGTTGATTGCCATCCTGGCGCTGGTTTTTTGTTCACTACAATGCGACCGGCGTCTGGACCT
TCAACTATGAAGAGCTGCTGAATACGCCAATGTCAGTGGTGTGGAATACCTGTTAATGCTGGGCTTCTTCAT
CGCC
GenBank Match - NONE

From pOH100 - 7.18l.79h.29.oh2:
GAGTGGCCTGTGATGTTGATTGCCATCCTGGCGCTGGTTTTTTGTTCACTACAATGCGACCGGCGTCTGGACC
TTCAACTATGAAGAGCTGCTGAATACGCCAATGTCAGTGGTGTGGAATACCTGTTAATGCTGGGCTTCTTCA
TCGCCTTCGCAGTCAAA
GenBank Match - NONE

From pOH100 - 7.18l.79h.29.oh2b:
TTCTTCATTTACACCCAGGGCGAGTGGCC
GenBank Match - NONE

From pOH102 - 7.18l.79h.30.oh2:
ATTTACACCCAGGCGAGTGGCCTGTGATGTTGATTGCCATCCTGGCGCTGGTTTTTTGTTCACTACAATGCGA
CCGGCGTCTGGACCTTCAACTATGAAGAGCTGCTGAATACGCCAATGTCCAGTGGTGTGGAATACCTGTTAA
T
GenBank Match - NONE

From pOH102 - 7.28b.79h.oh2:
GTCTGGACCTTCAACTATAAGAGCTGCTGAATACGCCAATGTCCAGTGGTGTGGAATACTGTTAATGCTGGG
CTTCTTCT
GenBank Match - NONE

From pOH102 - 7.28b.79h.oh2b:
CAGGCGAGTGGCTGTGATGTTGATTGCATCT
GenBank Match - NONE

OH3 Primer

From pOH100 - 7.17s.79h.29.oh3:
GGGATCATATGACAAGATGTGTATCCACCTTAACTTAATGATTTTTACCAAAATCATTAGGGGATTCATCAG
CTTTATGCCCCCACAG
GenBank Match - NONE

From pOH102 - 7.18a.79h.30.oh3:
GGCACCAGCATCATTTCCCAGAAGAAGAAGAACAGGAACATGTCGATGGCAAGGAACACGCCGATAACGCCG
CCCAGGATCCACATCAGGTTGAGGTGGAAGAAGCCCTGATATTTTGGTTGAGGTGGAAGAA
GenBank Match - NONE

From pOH100 - 7.18l.79h.29.oh3:
GGTACATCGGGCACCAGCATCATTTCCCAGAAGAAGAAGAACAGGAACATGTCGATGGCAAGGAACACGCCG
ATAACGCCGCCCAGGATCCACATCAGGTTGAGGTGGAATGAAGCCCTGATATTTTTCGATCTCTTTCACGAC
ATAGTA
GenBank Match - NONE

From pOH99 - 7.18a.79i.27.oh3:
CCAGAAGAAGAAGAACAGGAACATCGTCCCGATGGCAAGGAACACGCCGATAACGCCGCCCAGGATCCACA
GenBank Match - NONE

From pOH102 - 7.18l.79h.30.oh3:
GGCACCAGCATCATTTCCCAGAAGAAGAAGAACAGGAACAGTCGATGGCAAGGAACACGCCGATAACGCCGC
CCAGGATCCACATCAGGTTGATGGTGGAAGAA
GenBank Match - NONE

From pOH102 - 7.28b.79h.oh3:
GATCCACATCAGGTTGAGGTGGAAGAAGCCCTGATATTTTTCGATCTCTTTCACGAACATAGTACCGCCAGC
ACACCGAC
GenBank Match - NONE

T7 Primer

From pOH100 - 7.18l.79h.29.t7:
CCTGGCTGTGGCTGGGTAAACGTACTCTGGTGACCTCCATCGCCAACAGTGCGCCGGGCCGTCTGCTGGGCA
CCTGGTGGTACAACGCCTGGGGATTTGACTGGCTGTATGACAAAGTGTTCGTCAAGCCGTTCCTGGGTATTG
CCTGGTTGCTGAAACCGATC
GenBank Match - NONE

Mutant 123

Sequences Obtained:

Forward Primer
From pOH104 - 7.17a.123i.69.f:
GCCTGCAGGTCGACTCTAGAGGACCCCGGGTACCAAGAAAGGTTGCGATCACGGACAGTGCGGAGCCTGTAC
CGTGCTGGTCAA
GenBank Match - NONE

From pOH131 - 123if:
TCGGTACCGTTGGTAATCTTGCCGTCGaGACTGCGACTGCTCAGGATCAAACCCGACTGCCGAGGCAATCAT
TTCGCG
GenBank Match - NONE

From pOH104 - 7.191.123i.69.f:
CTTTATCAAGCATGATGGCTTCAGTGCGGCTACTGCACCTCCGGGCAAATTTGCTCATCAGTAGCGGTGCTA
AAAGAGATTCAGGACGGCATTCCAGTCACGTCACGGTCGATTG
GenBank Match - NONE

From pOH131 - 123.47.fbl:
ATTGTCACAGAACCCGCAGAAACGGGAAACTGATCGCCGGTGACGCACTGCTCA
GenBank Match - NONE

T3 Primer
From pOH103 - 7.18a.123i.21.t3:
GCCATGTAGGAAGCGCCCCAGCCGCCGTCGAAGGAACCGACTTTCGGGCCAGTCTGACCGAAGATGGCATTA
TCTGCCGCGATAGTCAGGTCGCACATCATGTGCAGAACGTGACCGCCGCCGATGGAGTAGCCAGCCC
GenBank Match - >**ECODHNASYN** (GenBank) E.coli DHNA synthase (menB) gene, complete cds.

OH2 Primer
From pOH103 - 7.18a.123i.21.oh2:
CAGCAGCTCTTCATAGTTGAAGGTCCAGACGCCGGTCGCATTGTAGTGAACAAAAACCAGCGCCAGGATGGC
AATCAACATCACCAGGCCACTCGCCTGGGTGTAAATGAAGAACTTGGTTGCCGCCGTGATACGCGTT
GenBank Match - NONE

OH3 Primer
No Sequence

Reverse Primer
From pOH131 - 123ir:
GGGTACCAAGAAAGGTTGCGATCACGGACAGTGCGGcGCTGTACCGTGCTGGTCAATGGTCGCAGGCTTAAT
GCTGTGACGC
GenBank Match - NONE

***menB* Sequence**

menB Coding Sequence = 357-1214
Located at min. 49

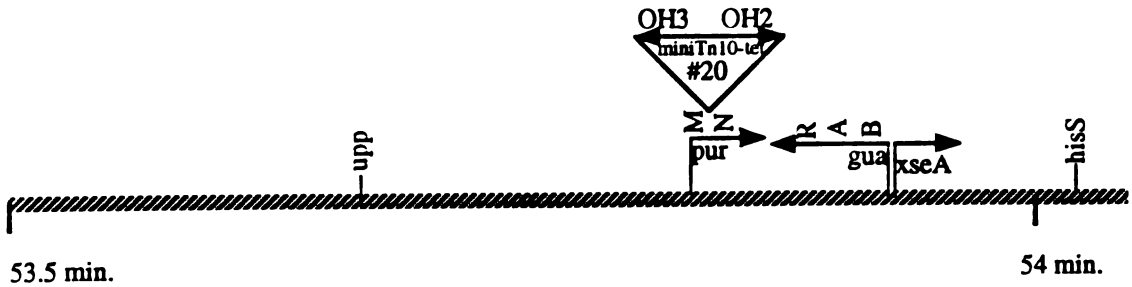
Mutant #123:
>20kb KpnI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion not located.
One KpnI site (at #972) located.

Mutant #79:
5kb KpnI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion not located.
One KpnI site (at #972) located.

***menB*:**

```
C1 ( 1f): |>u 1>++++++ ecodhnasyn (1349 bases)++++++>u 1349>|

       730        740        750        760        770        780
TTGTCGCGAT GGTGGCTGGC TACTCCATCG GCGGCGGTCA CGTTCTGCAC ATGATGTGCG
             ::::::: ::::::::::: ::::::::::: :::::::<7.18a.123i.21.t3
           GGGCTGGC TACTCCATCG GCGGCGGTCA CGTTCTGCAC ATGATGTGCG
                                                  AC ATGATGTGCG
                                                  <7.18a.79i.27.t3

       790        800        810        820        830        840
ACCTGACTAT CGCGGCAGAT AATGCCATCT TCGGTCAGAC TGGCCCGAAA GTCGGTTCCT
::::::::::: ::::::::::: ::::::::::: ::::::::::: :::::::<7.18a.123i.21.t3
ACCTGACTAT CGCGGCAGAT AATGCCATCT TCGGTCAGAC TGGCCCGAAA GTCGGTTCCT
ACCTGACTAT CGCGGCAGAT AATGCCATCT TCGGTCAGAC TGGCCCGAAA GTCGGTTCCT
                                                  <7.18a.79i.27.t3

       850        860        870        880        890        900
TCGACGGCGG CTGGGGCGCT TCCTACATGG CTCGCATCGT CGGGCAGAAA AAAGCGCGTG
::::::::::: ::::::::::: ::::::::::: :
TCGACGGCGG CTGGGGCGCT TCCTACATGG C<7.18a.123i.21.t3
TCGACGGCGG CTGGGGCGCT TCCTACATGG CT<7.18a.79i.27.t3

       910        920        930        940        950        960
AAATCTGGTT CCTGTGCCGT CAGTACGACG CAAAACAGGC GCTGGATATG GGCCTTGTGA

       970        980        990       1000       1010       1020
ACACCGTggt accGCTGGCG GATCTGGAAA AAGAAACCGT CCGTTGGTGC CGCGAAATGC
       KpnI 972

      1030       1040       1050       1060       1070       1080
TGCAAAACAG CCCGATGGCG CTGCGCTGCC TGAAAGCTGC ACTGAACGCC GACTGTGACG

      1090       1100       1110       1120       1130       1140
GGCAGGCGGG GCTGCAGGAG CTGGCGGGCA ACGCCACCAT GCTGTTCTAC ATGACGGAAG
```

*pur*MN Mutant #20



Insertion #20 between *pur*M and *pur*N.

Known Genes Near *pur*MN

Gene		Function
upp		Uricil phosphoribosyltransferase
pur	M	Phosphoribosyaminoimidazole synthetase
	N	5'-phosphoribosyglycinamide transformylase
gua	A	GMP synthetase
	B	IMP synthetase
	R	Regulatory gene
xseA		Exonuclease VII - Large subunit
hisS		Histidinyl-tRNA synthetase

***pur*MN - Mutant #20:**

MiniTn10-*tet* insertion #20 was found to lay between *pur*M and *pur*N at minute 54. *pur*M and N code for phosphoribosylaminoimidazole synthetase and 5'-phosphoribosyglycinamide transformylase. Both are involved in purine metabolism (Smith and 3d., 1987).

Sequence from the OH2 and OH3 primer ends of miniTn10-*tet* insertion #20 indicated that insertion lies at nucleotide #1800 of the "ecopurmn" *pur*MN gene locus. With *pur*M coding sequence between nucleotides #720 and #1817, and *pur*N coding sequence between nucleotides #1817 and #2455, miniTn10-*tet* insertion #20 lies in the end of *pur*M and just before *pur*N. MiniTn10-*tet* insertion #20 was genetically linked by P1 transduction to a marker at minute 54, near *pur*MN at min. 53.75.

MiniTn10-*tet* insertion #20 dramatically decreases miniTn7-*lac* papillation; however, it increases miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). As measured by the mating-out assay, miniTn10-*tet* insertion #20 produces a 2.1 fold increase of Tn7 transposition to pOX38*gen*, while miniTn7-*lac* transposition is conversely decreased 1.7 fold.

Mutant 20

Sequences Obtained:

Forward Primer

From pOH61 - 20.35.fb:
CTCTGGCAGAAGCTGGTTGCAGTGCGTTAAAGTCGGCATTGGCCTGGCTCTATCTGTACAACTCGTATCGTG
ACTGCGTCGGTGT
GenBank Match - >**ECOGUAB** (GenBank) E.coli guaBA operon: guaB and guaA genes coding for IMP dehydrogenase and GMP synthetase.

From pOH61 - 20.35.ft:
ATCGTTATCGCTGATGCGTATTCGCTTCTCGCGACATG
GenBank Match - NONE

From pOH61 - 20hf:
CTCTGGCAGAAGCTGGTTGCAGTGCGGTTAAAGTCGGCATTGCCTGCTCTATCTGTAC
GenBank Match - >**ECOGUAB** (GenBank) E.coli guaBA operon: guaB and guaA genes coding for IMP dehydrogenase and GMP synthetase.

OH2 Primer

From pOH130 - 20Ib2:
CGGCGCGTGGTTATCGAATAATAATGAATATGGTGGTTATTTCCGGCAACGAAGTAATTTACAGGCAATTAT
GACACCTGTAAAACCAACAAAATTAAAGGACGATAGGCGTTTT
GenBank Match - >**ECOPURMN** (GenBank) E.coli purM gene encoding synthetase, and purN gene,-5-aminoimidazole

OH3 Primer

From pOH130 - 20Ib3:
CGCGTTGTTCGGAATCAGAGGCTTTGATGATGCCGATTTTCCACGCGTTTTCACCGTTGGCATTGAGCAGGG
CGAGGGCTTTGTCCACTTCCGGAGCAGGCAGGGCAATAATCATCCCGACGCCGCAGTTGAAGGTG
GenBank Match - >**ECOPURMN** (GenBank) E.coli purM gene encoding synthetase, and purN gene,-5-aminoimidazole

Reverse Primer

From pOH61 - 20.35.rb:
GCGACGAGGGGATTTATCGTGTATTGGCATAGGGTTATTCAGGTT
GenBank Match - none

From pOH61 - 20.35.rt:
TTGACCGTATCGCTGAACAATATGTCGATCGAGTCAGTACC
GenBank Match - none

purMN Sequence

purM Coding Sequence = 720-1817
purN Coding Sequence = 1817-2455
Located at min. 53.75

Mutant #20:
>12kb PstI insertion in pK184 plasmid.
8kb KpnI insertion in pK194 plasmid.
"Forward" end of cloned chromosomal DNA ends in guaB.
MiniTn10-*tet* insertion in purMN Coding Sequence
One PstI cloning site located.

ecopurmn:

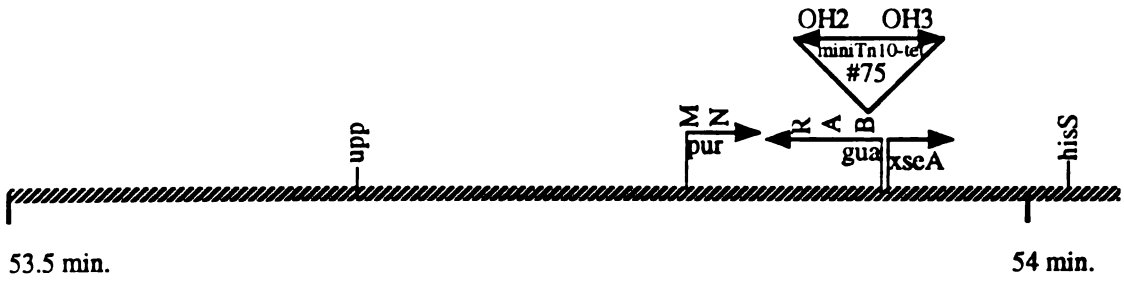
```
      1630        1640        1650        1660        1670        1680
ACTGGCTGCA AACGGCAGGT AACGTTGAGC ACCATGAAAT GTATCGCACC TTCAACTGCG
                                                    :::: ::::::::::
                                                    CACC TTCAACTGCG<20Ib3

      1690        1700        1710        1720        1730        1740
GCGTCGGGAT GATTATTGCC CTGCCTGCTC CGGAAGTGGA CAAAGCCCTC GCCCTGCTCA
:::::::::: :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
GCGTCGGGAT GATTATTGCC CTGCCTGCTC CGGAAGTGGA CAAAGCCCTC GCCCTGCTCA<20Ib3

      1750        1760        1770        1780        1790        1800
ATGCCAACGG TGAAAACGCG TGGAAAATCG GTATCATCAA AGCCTCTGAT TCCGAACAAC
:::::::::: :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
ATGCCAACGG TGAAAACGCG TGGAAAATCG GCATCATCAA AGCCTCTGAT TCCGAACAAC<20Ib3
                                                                 :
                                                               CGGC
```

miniTn10-*tet*-20Ib2>

<20Ib3-miniTn10-*tet*

```
GCGT
::::  1810        1820        1830        1840        1850        1860
GCGTGGTTAT CGAATAATGA ATATTGTGGT GCTTATTTCC GGCAACGGAA GTAATTTACA
:::::::::: :::::::::: ::::   ::: : :::::::: ::::::: :: ::::::::::
GCGTGGTTAT CGAATAATGA ATAT   GGTGG TTATTTCC GGCAACG AA GTAATTTACA
20Ib2>

      1870        1880        1890        1900        1910        1920
GGCAATTATT GACGCCTGTA AAACCAACAA AATTAAAGGC ACCGTACGGG CAGTTTTCAG
:::::::::  :::::::::: :::::::::: :::::::::  ::::::  :::    :::::
GGCAATTAT  GACACCTGTA AAACCAACAA AATTAAAGG  ACGATA GGC   GTTTT
20Ib2>

      1930        1940        1950        1960        1970        1980
CAATAAGGCC GACGCGTTCG GCCTTGAACG CGCCCGCCAG GCGGGTATTG CAACGCATAC
```

*gua*AB Mutant #75



Insertion #75 in *gua*B coding sequence.

Known Genes Near *gua*AB

Gene		Function
upp		Uricil phosphoribosyltransferase
pur	M	Phosphoribosyaminoimidazole synthetase
	N	5'-phosphoribosyglycinamide transformylase
gua	A	GMP synthetase
	B	IMP synthetase
	R	Regulatory gene
xseA		Exonuclease VII - Large subunit
hisS		Histidinyl-tRNA synthetase

***guaB* - Mutant #75:**

MiniTn10-*tet* insertion #75 lies in *guaB* at minute 54. The *guaAB* genes are GMP synthetase and IMP synthetase (Tiedeman and Smith, 1985; Tiedeman et al., 1985).

Sequence from the OH2 and OH3 primer ends of miniTn10-*tet* insertion #75 indicated that insertion lies between nucleotides #275 and #375 of the "ecoguab" *guaAB* gene locus. With *guaB* coding sequence between nucleotides #311 and #1777, miniTn10-*tet* insertion #75 lies in the start of *guaB*. MiniTn10-*tet* insertion #75 was genetically linked by P1 transduction to a marker at minute 54, near *guaB* at min. 53.75. *mic*A and *nup*G sequence from T7 and T3 primers was also obtained, however *mic*A and *nup*G lie near minute 64 on the E. *coli* chromosome (Bachmann, 1990), far from theP1 confirmed location of miniTn10-*tet* insertion #75 near minute 54, in *guaB*.

Mutant #75 dramatically decreases miniTn7-*lac* papillation. Mutant #75 also decreases miniTn10-*lac* papillation(see Chapter 5, Figures 5-4 and 5-5). As measured by the mating-out assay, miniTn10-*tet* insertion #75 decreases transposition to pOX38*gen* 5 fold, while slightly increasing Tn7 transposition.

Mutant 75

Sequences Obtained:

T3 Primer
From pOH67 - 7.18l.75h.10.t3:
AACCTTGGTTTTTTTGTTACCTGTGGCAAATAATTGCAAACATTCCCAATTTGCAAAACGAAATTGTGATGT
GGATAACATTTTTNCCCCTGAGCATCGTCAGGG
GenBank Match - >**ECONUPGP** (GenBank) E.coli nupG gene promoter

OH2 Primer
From pOH67 - 7.18a.75h.10.oh2:
CTGCCGAATACTTGCTGACCTCAGCACCCAGCTGACGAAAACTATTCGTCTGAATATCCCTATGCGTTCCGC
AGCAATGGATACGTAACGGAAG
GenBank Match - >**ECOGUAB** (GenBank) E.coli guaBA operon: guaB and guaA genes coding for IMP dehydrogenase and GMP synthetase.

OH3 Primer
From pOH67 - 7.18l.75h.10.oh3:
GCCGCGGCATTATACAGAGCGTAACCGATTGCATCTACCCCTTNTTGCAAAAAATGCTTGCTATCCCCGAAG
CGGGTTACTATCGACTGAATAACCT
GenBank Match - >**ECOGUAB** (GenBank) E.coli guaBA operon: guaB and guaA genes coding for IMP dehydrogenase and GMP synthetase.

T7 Primer
From pOH67 - 7.18l.75h.10.t7:
CTGCACCATGCGCAATGAATTGTCCAGGAATCATTTT
GenBank Match - >**ECOMICA** (GenBank) E.coli A/G-specific adenine glycosylase (micA) gene

From pOH67 - 7.18l.75h.10.t7t:
CAGGTTCGTTAAACCTTCTTCATGCGCAGAAGCCA
GenBank Match - >**ECOMICA** (GenBank) E.coli A/G-specific adenine glycosylase (micA) gene

***guaAB* Sequence**

guaB Coding Sequence = 311-1777
guaA Coding Sequence = 1846-3423
Located at min. 53.75

Mutant #75:
12kb PstI insertion in pBluescript plasmid.
12kb KpnI insertion in pK194 plasmid.
Insertion at start of guaB coding sequence.

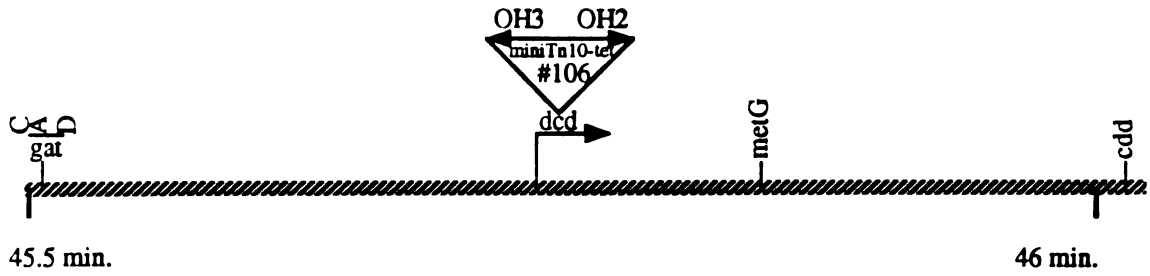
Mutant #20:
>12kb PstI insertion in pK184 plasmid.
8kb KpnI insertion in pK194 plasmid.
"Forward" end of cloned chromosomal DNA ends in guaB.
MiniTn10-*tet* insertion in purMN Coding Sequence
One PstI cloning site located.

ECOGUAB:

```
        10         20         30         40         50         60
TAAAGTACCA GTGACCGGAA GCTGGTTGCG TGAAATTAGA AATTTCGCCG CTGATCCAAA

        70         80         90        100        110        120
CCTGTCCCAT CTCATGCTCA AGCAGCAGAC GAACCGTTTG ATTCAGGCGA CTAACGGTAA

       130        140        150        160        170        180
AAATTGCAGG GGATTGAGAA GGTAACATGT GAGCGAGATC AAATTCTAAA TCAGCAGGTT
                                                      :::::
                                                      AGGTT

       190        200        210        220        230        240
ATTCAGTCGA TAGTAACCCG CCCTTCGGGG ATAGCAAGCA TTTTTTGCAA AAAGGGGTAG
:::::::::: ::::::::::   :::::::: :::::::::: :::::::::: ::::::::::
ATTCAGTCGA TAGTAACCCG   CTTCGGGG ATAGCAAGCA TTTTTTGCAA NAAGGGGTAG
                                              7.181.75h.10.oh3

       250        260        270        280        290        300
ATGCAATCGG TTACGCTCTG TATAATGCCG CGGCAATATT TATTAACCAC TCTGGTCGAG
:::::::::: :::::::::: :::::::::: ::::
ATGCAATCGG TTACGCTCTG TATAATGCCG CGGC
                                  <7.181.75h.10.oh3

       310        320        330        340        350        360
ATATTGCCCA TGCTACGTAT CGCTAAAGAA GCTCTGACGT TTGACGACGT TCTCCTCGTT


       370        380        390        400        410        420
CCTGCTCACT CTACCGTTCT GCCGAATACT GCTGACCTCA GCACCCAGCT GACGAAAACT
                  :: :::::::::: :::::::::: :::::::::: ::::::::::
                  CT GCCGAATACTTGCTGACCTCA GCACCCAGCT GACGAAAACT
7.18a75h.10.oh2>
```

```
       430         440         450         460         470         480
ATTCGTCTGA ATATCCCTAT GCTTTCCGCA GCAATGGATA CCGTAACGGA AGCGCGCCTG
:::::::::: :::::::::: :::::::::: :::::::
ATTCGTCTGA ATATCCCTAT GCGTTCCGCA GCAATGG
7.18a75h.10.oh2

       490         500         510         520         530         540
GCTATTGCTC TGGCTCAGGA AGGCGGTATC GGCTTTATCC ACAAAAACAT GTCCATTGAA

       550         560         570         580         590         600
CGCCAGGCAG AAGAAGTTCG CCGTGTGAAA AAACACGAAT CTGGTGTGGT GACTGATCCG

       610         620         630         640         650         660
CAGACTGTTC TGCCAACCAC GACGCTGCGC GAAGTGAAAG AACTGACCGA GCGTAACGGT

       670         680         690         700         710         720
TTTGCGGGCT ATCCGGTCGT TACCGAAGAA AACGAACTGG TGGGTATTAT CACCGGTCGT

       730         740         750         760         770         780
GACGTGCGTT TTGTTACCGA CCTGAACCAG CCGGTTAGCG TTTACATGAC GCCGAAAGAG

       790         800         810         820         830         840
CGTCTGGTCA CCGTGCGTGA AGGTGAAGCC CGTGAAGTGG TGCTGGCAAA AATGCACGAA

       850         860         870         880         890         900
AAACGCGTTG AAAAAGCGCT GGTGGTTGAT GACGAATTCC ACCTGATCGG CATGATCACC

       910         920         930         940         950         960
GTGAAAGACT TCCAGAAAGC GGAAGCTAAA CCGAACGCCT GTAAAGACGA GCAAGGCCGT

       970         980         990        1000        1010        1020
CTGCGTGTTG GTGCAGCGGT TGGCGCAGGT GCGGGTAACG AAGAGCGTGT TGACGCGCTG

      1030        1040        1050        1060        1070        1080
GTTGCCGCAG GCGTTGACGT TCTGCTGATC GACTCCTCCC ACGGTCACTC AGAAGGTGTA

      1090        1100        1110        1120        1130        1140
CTGCAACGTA TCCGTGAAAC CCGTGCTAAA TATCCGGATC TGCAAATTAT CGGCGGCAAC

      1150        1160        1170        1180        1190        1200
GTGGCAACAG CTGCAGGTGC ACGCGCTCTG GCAGAAGCTG GTTGCAGTGC GGTTAAAGTC
            PstI          ::::: :::::::::: :::::::::: ::::::::::
                  20.35.fb>CTCTG GCAGAAGCTG GTTGCAGTGC GGTTAAAGTC
                      20hf>CTCTG GCAGAAGCTG GTTGCAGTGC GGTTAAAGTC

      1210        1220        1230        1240        1250        1260
GGCATTGGCC CTGGCTCTAT CTGTACAACT CGTATCGTGA CTGGCGTCGG TGTTCCGCAG
::::::::::  ::::::::: :::::::::: :::::::::: ::: :::::: :::
GGCATTGGCC  TGGCTCTAT CTGTACAACT CGTATCGTGA CTG CGTCGG TGT  20.35.fb
GGCATT GCC  TG CTCTAT CTGTAC                               20hf
```

dcd
Mutant #106



Insertion #106 in *dcd* coding sequence.

Known Genes Near *dcd*

Gene		Function
gat	C	Regulatory gene
	A	Galacitol-specific enzyme II of phosphotransferase system
	D	Galacitol-1-phosphate dehydrogenase
dcd		2'-deoxycytidine 5'-triphosphate deaminase
metG		methionyl-tRNA synthase
cdd		Deoxycytidine triphosphate deaminase

***dcd* - Mutant #106:**

MiniTn10-*tet* insertion mutant #106 interrupts *dcd*. *dcd* codes for 2'-deoxycytidine 5'-triphosphate deaminase (Wang and Weiss, 1992). *dcd* was isolated as a mutation suppressing *dut* (dUTPase) mutations, and as a mutation sensitizing E. *coli* to U.V. irradiation (Wang and Weiss, 1992).

MiniTn10-*tet* insertion #106 is within the coding sequence of *dcd*. Sequence flanking each end of miniTn10-*tet* insertion #106 (OH2 and OH3 primers) matched *dcd* sequence. Sequence from the OH2 and OH3 end primers indicated that insertion lies at nucleotide #642 of the "ecodcda" *dcd* gene locus. With *dcd* coding sequence stretching between nucleotides #64 and #675, miniTn10-*tet* insertion #106 lies within coding sequence, near the end of *dcd*. An open reading frame of undetermined function lies down-stream of *dcd*, stretching from nucleotide #697 to #1298 (Wang and Weiss, 1992). *att*λ sequence from the Reverse primer of another plasmid was obtained, however *att*λ lies near minute 17 on the E. *coli* chromosome (Bachmann, 1990), far from *dcd* at minute 46.

Mutant #106 decreases both miniTn7-*lac*, but has no effect on miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). As measured by the mating-out assay, mutant #106 produces a 1.6 fold increase of Tn7 transposition to pOX38*gen*, and a 3 fold decrease in miniTn7-*lac* transposition.

Mutant 106

Sequences Obtained:

Forward Primer

From pOH76 - 106.40.fb:
GGTACCCGGGGATCCTCTAGAGTCGACCTGCAGCCCTGATTAATGATTCCGGGCCACATGCTGCATTAAACA
TATCCACCGGGCTACGTGGC
GenBank Match - NONE

From pOH76 - 106.40.ft:
GGAGTAAGACCATAACGACTGCG
GenBank Match - NONE

From pOH76 - 106hf:
GGTACCCGGGGATCTCTAGAGTCGACCTGCAGCCCTGATTAATGATTCCGGGCCACACGCTGCATTAAACAT
AT
GenBank Match - NONE

From pOH76 - 106hf.2:
GGATCTCTAGAGTCGACCTGCAGCCCTGATTAATGATTCCGGGCCACACGCTGCATTAAACATATCCACCGG
GCTACGTGGCGGGAATGGAACTTCAGCCCCCCACTTCACGAATGCGGAAACAAC
GenBank Match - NONE

OH2 Primer

From pOH74 - 106HOH2:
AATTTTTACCAAAATCATTAGGGGATTCATCAGGGCGCGGTCACGAGCCGATTCGATAAAGACTAATTCAGG
CCCATTGAGGATACATGAGACGATTCT
GenBank Match - >**ECODCDA** (GenBank) E.coli deoxycytidine triphosphate deaminase (dcd) gene, cds and ORF, 5' end.

From pOH74 - 106HOH2.top:
TTATCTCGGTTAGTGTTGCTGGTGATCGATGATCGCGACTATATGGTCAGCAGTGCTGCACGTAGCGTATCA
TGCAGCTCGACGGCACTGCGTG
GenBank Match - >**ECODCDA** (GenBank) E.coli deoxycytidine triphosphate deaminase (dcd) gene, cds and ORF, 5' end.

From pOH77 - 106Hb2:
ATCTGGGCGCGGTAGCCAGCCGAATCGATAAAGACTAATTCAGGCCCATTGAGGATACCATGAGAGCATTTC
TGAGCAGCTCGATGATA
GenBank Match - >**ECODCDA** (GenBank) E.coli deoxycytidine triphosphate deaminase (dcd) gene, cds and ORF, 5' end.

From pOH75 - 106Hb2.0:
GAGCCGAATCGATAAAGACTAATTCAGGCCCATTGAGGATACATGAGACGATTTCTG
GenBank Match - >**ECODCDA** (GenBank) E.coli deoxycytidine triphosphate deaminase (dcd) gene, cds and ORF, 5' end.

From pOH74 - 106HbA:
GGGGATTCATCAGGGCGCGGTAGCCAGCCGAATCGATAATGACTAATTCAGGCCCATTGAGGATACCATGAG
ACGATTTCTGACGACGCTGATGATACTCCTGGTCGAGCTGGTGGCGGTATCTGCGTTAGTGTTGCTGGTGAT
CGAATGATT
GenBank Match - >**ECODCDA** (GenBank) E.coli deoxycytidine triphosphate deaminase (dcd) gene, cds and ORF, 5' end.

OH3 Primer

From pOH74 - 106HOH3:
GTGTATCCACCTTAACTTAATGATTTTTACCAAAATCATTAGGGGATTCATCAGTACCGCGCCCTGCTGGTT
GCGATATTG
GenBank Match - >**ECODCDA** (GenBank) E.coli deoxycytidine triphosphate deaminase (dcd) gene, cds and ORF, 5' end.

From pOH75 - 106Hb3.0:
TACCGCGCCCTGCTGGTTGCGATATTTCGCATCTCACGGCGGTTGTAAGGTGCACCGCCGGGCGAAAG
GenBank Match - >**ECODCDA** (GenBank) E.coli deoxycytidine triphosphate deaminase (dcd) gene, cds and ORF, 5' end.

Reverse Primer

From pOH76 - 106.40.rb:
GTCAGGGATGCAAAATAGTGTTGAGCATCGAAATTCTGCGCTTCTTTTGCCGACAGAATCGGGCGAGAAGAG
GTACCAGGCGCG
GenBank Match - NONE

From pOH76 - 106.40.rbl:
GCGTCACGGTCACGCCAAAAGCCAATGCCAGCGCCAGACGGAACTGAAATGTGTTCACA
GenBank Match - >**ECOLAMATT** (GenBank) E.coli ATT site for lambda prophage integration.

From pOH76 - 106.40.rml:
AAAAAATGAATCCGTTGAAGCCTGCTTTTTTATACTAACTTGAGCGAAACGGGAGT
GenBank Match - >**ECOLAMATT** (GenBank) E.coli ATT site for lambda prophage integration.

From pOH76 - 106.40.rt:
CAGACGTGAAACTGAAAATGTGTTCACAGGTTGCTCCGGCTATGAATAGAAAAATGATC
GenBank Match - >**ECOLAMATT** (GenBank) E.coli ATT site for lambda prophage integration.

From pOH76 - 106hr:
GTGTCAGGGATGCAAAATAGTGTTGAGCATCGAAATTCTGCGCTTCTTTTGCCGACAGAATCGGGCGAGAAG
AGGTACCAGGCGCGGTTTGATCA
GenBank Match - NONE

From pOH76 - 106hr.2:
AGCTTGCATGCCTGCAGCACCTGGTGTCAGGGATGCAAAATAGTGTTGAGCATCGAAATTATG
GenBank Match - NONE

***dcdA* Sequence**

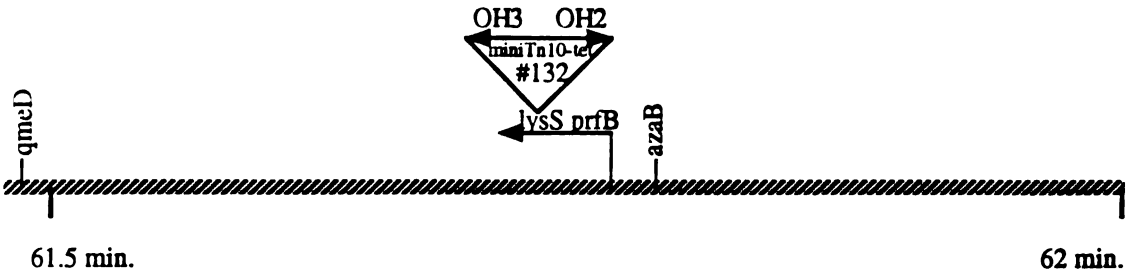
dcdA Coding Sequence = 94-675
ORF in dcd Operon Sequence = 697->1298
Located at min. 46

Mutant #106:
>12kb PstI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion at #645 in dcd coding sequence.
Neither PstI sites located.

***dcdA*:**

```
C1 ( 1f): |>u 1>+++++ ecodcda (1298 bases)+++++>u 1298>|

       490        500        510        520        530        540
GGCTGGTCTG GTTGCATTGT GCTGGAGTTC TACAACTCCG GTAAGCTGCC GCTGGCGCTG

                                                          <106Hb3.0
                                            C TTT CG CCC GGCGGTGC A
       550        560        570        580 ::: :: 590 :::::::600
CGTCCGGGCA TGTTAATTGG TGCGCTGAGC TTTGAGCCGC TTTCCGGCCC GGCGGTGCGA

CCTTACAACC GCCGTGA GA TGCGAAATAT CGCAACCAGC AGGGCGCGGT A<106Hb3.0
                  <106HOH3CAATAT CGCAACCAGC AGGGCGCGGT ACTGATGAAT
       610        620       ::630 :::::::640 :::::::650 :      660
CCTTACAACC GCCGTGAAGA TGCGAAATAT CGCAACCAGC AGGGCGCGGT AGCCAGCCGA
                                              ::::::::::   : : ::::
    106HOH2>AATTTTTACCAAAATCATTAGGGGATTCATC AGGGCGCGGT CACGAGCCGATTCG
                                106Hb2>ATC TGGGCGCGGT AGCCAGCCGA
                           106HbA>GGGGATTCATC AGGGCGCGGT AGCCAGCCGA

CCCCTAATGATTTTGGTAAAAATCATTAAGTTAAGGTGGATACAC<106HOH3

       670        680        690        700        710        720
ATCGATAAAG ACTAATTCAG GCCCATTGAG GATACCATGA GACGATTTCT GACGACGCTG
::    ::::::::::::::  :::::::::: ::::: :::: ::::::: ::
ATTCGATAAAGACTAATTCA  GCCCATTGAG GATAC ATGA GACGATT CT 106HOH2
AT CGATAAAGACTAATTCAG GCCCATTGAG GATACCATGA GAGCATTTCT GAGCAGCTCG106Hb2>
ATCGATAATG ACTAATTCAG GCCCATTGAG GATACCATGA GACGATTTCT GACGACGCTG106HbA>

       730        740        750        760        770        780
ATGATACTCC TGGTCGTGCT GGTGGCCGGG TTATCTGCGT TAGTGTTGCT GGTGAATCCG
                                :::::: :: :::::::::: ::::: :: :
                         106HOH2.top>TTATCTCGGT TAGTGTTGCT GGTGA TC G
ATGATA 106Hb2
ATGATACTCC TGGTCGAGCT GGTGGC GG  T ATCTGCGT TAGTGTTGCT GGTGA TC G106HbA>

       790        800        810        820        830        840
AATGATTTCC GCGACTATAT GGTCAAGCAA GTTGCTGCAC GTAGCGGTTA TCAATTGCAG
: ::::  :  :::::::::: ::::: :::  :: ::::::: :::::: : : ::: : ::::
A TGAT  C  GCGACTATAT GGTCA GCA  GT GCTGCAC GTAGCG T A TCA T GCAG
AATGATT 106HbA                                          106HOH2.top>

       850        860        870        880        890        900
CTCGACGGGC CACTGCGTTG GCACGTCTGG CCGCAGCTTA GTATCCTCTC CGGGCGAATG
:::::::: :  ::::::: :
CTCGACGG C  ACTGCGT G
```

lysS
Mutant #132



Insertion #132 in *lysS* coding sequence.

Known Genes Near *lysS*

Gene	Function
qmeD	Unspecified membrane defect; tolerance to glycine
lysS	Lysyl tRNA synthetase
prfB	protein release factor 2
glyU	glycine tRNA synthetase

***lysS* - Mutant #132:**

MiniTn10-*tet* insertion mutant #132 was found to interrupt *lysS*. *lysS* codes for the E. *coli*'s major lysyl tRNA synthetase (Emmerich and Hirshfield, 1987). *lysS* (also known as *herC*) is part of a two gene (*prfB-lysS*) operon. *lysS* activity may modulate LRP (leucine responsive regulatory protein), which is know to have a global regulatory role and is known to regulate a number of recombination systems (Ernsting et al., 1992; Ito et al., 1993; Newman et al., 1992).

MiniTn10-*tet* insertion #132 is within the coding sequence of *lysS*. Sequence flanking each end of miniTn10-*tet* insertion #132 (OH2 and OH3 primers) matched *lysS* sequence. Sequence from the OH2 and OH3 end primers indicated that insertion lies between nucleotides #876 and #893 of the "ecoherc" *lysS* gene locus. With *lysS* coding sequence stretching between nucleotides #229 and #1746, miniTn10-*tet* insertion #132 lies within the coding sequence of *lysS*.

Mutant #132 decreases both miniTn7-*lac* and miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). As measured by the mating-out assay, mutant #132 produces a 1.7 fold decrease of Tn7 transposition to pOX38*gen*, and a 1.4 fold decrease in miniTn7-*lac* transposition.

Mutant 132

Sequences Obtained:

T3 Primer
From pOH71 - 132HT3:
CTCAAGTTAGCGGATTATCCTGAAGNGCTAATTTCAAAGAAGGTAGCGACATNCGAAGACGGTGTCTCTCTC
TATCAGATTGATCCTGCGT
GENBANK MATCH - NONE

OH2 Primer
From pOH71 - 132HOH2:
GCCAGGATCACTGCATATCGGCGTTTCAACTTCCATAAAGCCGCGGTTCACCATGAACTGGCGAATACAGAG
AGGACTGCGAGCGCACTTAAAGGTGTT
GENBANK MATCH - >**ECOHERC** (GenBank) E.coli peptide chain release factor 2 (prfB) gene, 3' end, protein gene

OH3 Primer
From pOH71 - 132HOH3:
GTTTATACCCACCATAACGCGCTGATCTCGACATGTACCTGCGTAT
GENBANK MATCH - >**ECOHERC** (GenBank) E.coli peptide chain release factor 2 (prfB) gene, 3' end, protein gene

T7 Primer
No Sequence

***lysS* (*herC*) Sequence**

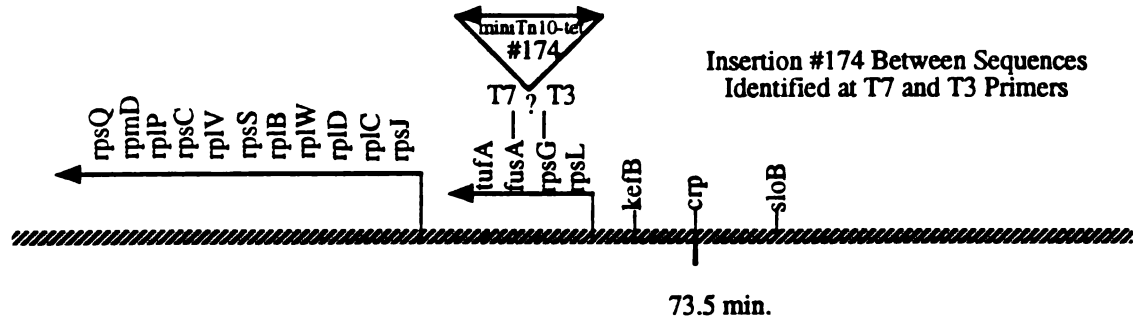
lysS Coding Sequence = 229-1746
Located at min. 61.7

8.5kb PstI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion in Coding Sequence
No PstI cloning site on sequence.

***lysS*:**

```
(ecoherc)
       670        680        690        700        710        720
CTGCTGACCA AAGCACTGCG TCCGCTGCCG GATAAATTCC ACGGCTTGCA GGATCAGGAA

       730        740        750        760        770        780
GCGCGCTATC GTCAGCGTTA TCTCGATCTC ATCTCCAACG ATGAATCCCG CAACACCTTT
                                                      :::::::::
                                                      AACACCTTT
                                                        <132HOH2

       790        800        810        820        830        840
AAAGTGCGCT CGCAGATCCT CTCTGGTATT CGCCAGTTCA TGGTGAACCG CGGCTTTATG
 ::::::::: ::::: :::: :::: ::::: :::::::::: :::::::::: ::::::::::
 AAGTGCGCT CGCAG TCCT CTCT GTATT CGCCAGTTCA TGGTGAACCG CGGCTTTATG
                                                        <132HOH2

       850        860        870        880        890        900
GAAGTTGAAA CGCCGATGAT GCAGGTGATC CCTGGCGGTG CCGCTGCGCG TCCGTTTATC
:::::::::: :::::::: :: :::: ::::: : ::::             132HOH3>GTTTAT
GAAGTTGAAA CGCCGAT AT GCAG TGATC C TGGC<132HOH2

       910        920        930        940        950        960
ACCCACCATA ACGCGCTGGA TCTCGACATG TACCTGCGTA TCGCGCCGGA ACTGTACCTC
:::::::::: :::::::: : :::::::::: :::::::::: :
ACCCACCATA ACGCGCTG A TCTCGACATG TACCTGCGTA T
132HOH3>

      970        980        990       1000       1010       1020
AAGCGTCTGG TGGTTGGTGG CTTCGAGCGT GTATTCGAAA TCAACCGTAA CTTCCGTAAC

     1030       1040       1050       1060       1070       1080
GAAGGTATTT CCGTACGTCA TAACCCAGAG TTCACCATGA TGGAACTCTA CATGGCTTAC

     1090       1100       1110       1120       1130       1140
GCAGATTACA AAGATCTGAT CGAGCTGACC GAATCGCTGT TCCGTACTCT GGCACAGGAT

     1150       1160       1170       1180       1190       1200
ATTCTCGGTA AGACGGAAGT GACCTACGGC GACGTGACGC TGGACTTCGG TAAACCGTTC
```

*rps*L-*tuf*A Mutant #174



Insertion #174 between *rps*G and *fus*A.

Known Genes Near *rps*L-*tuf*A

Gene	Function	
rpsQ	Ribosomal protein, small	S10 of 30S
rpmC	Ribosomal protein, large	L3 of 50S
rplP	Ribosomal protein, large	L4 of 50S
rpsC	Ribosomal protein, small	S3 of 30S
rplV	Ribosomal protein, large	L22 of 50S
rpsS	Ribosomal protein, small	S19 of 30S
rplB	Ribosomal protein, large	L2 of 50S
rplW	Ribosomal protein, large	L23 of 50S
rplD	Ribosomal protein, large	L4 of 50S
rplC	Ribosomal protein, large	L3of 50S
rpsJ	Ribosomal protein, small	S10 of 30S
tufA	Protein chain elongation factor EF-tu	
fusA	far; Protein chain elongation factor EF-G	
rpsL	Ribosomal protein, small	3S S12-subunit
rpsG	Ribosomal protein, small	3S S7-subunit
kefB	NEM-activatable K^+/H^+ antiporter	
crp	Cyclic AMP receptor protein	
sloB	slow growth rate; tolerance to nalidixic acid	

***rpsL/fusA* - Mutant #174:**

MiniTn10-*tet* insertion mutant #174 was found to interrupt the *rpsL* - *tufA* operon. The *rps* genes code for components of the 30S and 50S ribosomal protein complexes . *fusA* codes for protein chain elongation factor EF-G (Zengel et al., 1984), while *tufA* codes for protein chain elongation factor EF-Tu (Post and Nomura, 1980).

MiniTn10-*tet* insertion #174 is located somewhere between the adjoining genes *rpsG* and *fusA*. The location of miniTn10-*tet* insertion #174 was determined by sequencing into the each end of the cloned miniTn10-*tet* containing chromosomal DNA from the cloning vector plasmid (pBluescript). Sequence from the T3 pBluescript primer matched *rpsL* and *rpsG*, while sequence from the T7 pBluescript primer matched *fusA*. MiniTn10-*tet* insertion #174 was genetically linked by P1 transduction to a marker at minute 74, near the *gid* - *unc* operon at min. 73.5. Though DNA sequence flanking insertion #174 (from both the OH2 and OH3 primers) was obtained, this sequence did not match any sequences from GenBank. GenBank sequence between of the region between *rpsG* and *fusG* is broken into different files, which may account for the inability to find a sequence match in this region.

MiniTn10-*tet* insertion #174 was found to decrease miniTn7-*lac* papillation (see Figures 4 , Chapter 5), however no effect on miniTn10-*lac* papillation was observed (Figure 5-5). MiniTn10-*tet* insertion #174 shows a 4.2 fold increase of Tn7 transposition to pOX38*gen*, while miniTn7-*lac* transposition is unaffected - as measured in the mating out assay.

Mutant 174

Sequences Obtained:

T3 Primer

From pOH78 - 174.2.t3bs:
ACTTCGAAACCTGTTAGTCAGACGAACACGGCATACTTTACGCAGCCGAGTTCGTTTTCTAGGAGTGGTAGT
ATATACACA
GenBank Match - >**ECOSTR6** (GenBank) Part of the E. coli STR operon including the gene (str or ribosomal protein S12 and the beginning of the gene (rpsG) for S7.

From pOH84 - 174.24.t3b:
CGCGGCGAGACTTAACTTCTACAGTCGGGCGCACGTTTCGAGAGCTACTT
GenBank Match - >**ECOSTR6** (GenBank) Part of the E. coli STR operon including the gene (str or ribosomal protein S12 and the beginning of the gene (rpsG) for S7.

From pOH78 - 6.5.92-174.2.t3.2:
GTCACTTCGAAACCGTTAGTCAGACGAACACGGCATACTTTACGCAGC
GenBank Match - >**ECOSTR6** (GenBank) Part of the E. coli STR operon including the gene (str or ribosomal protein S12 and the beginning of the gene (rpsG) for S7.

From pOH78 - 6.5.92-174.t3.2:
TGATAAGTAGAACCCACCAACCGCGGCGAGACTTAACTTCTACAGTCGGGCGCACGTTTTCGAGAG
GenBank Match - >**ECOSTR6** (GenBank) Part of the E. coli STR operon including the gene (str or ribosomal protein S12 and the beginning of the gene (rpsG) for S7.

From pOH78 - 6.5.921-174.1.t3.2:
AGCGCGCTGTATACGATAGATTCAGCAGTAGATTTTTTACCATCTACCATCAGGATATTTACAAATTTAGCC
AGCAGTTCTGATCCGAACTTCGGATCCGGCAGAATTTTACNTGACCAATGACGCGACGA
GenBank Match - >**ECOSTR6** (GenBank) Part of the E. coli STR operon including the gene (str or ribosomal protein S12 and the beginning of the gene (rpsG) for S7.

From pOH84 - 174ib3:
ACGCTGACCAATGACGCTCGACGTGGCATGGAAATACTCCGTTGTTAATTCAGGATTGTCAAAACTCTACGA
GTTTAGTTTGACATTTAG
GenBank Match - >**ECOSTR6** (GenBank) Part of the E. coli STR operon including the gene (str or ribosomal protein S12 and the beginning of the gene (rpsG) for S7.

Forward Primer

From pOH85 - 174.42.fb:
TGAAATCACTGCCGGCTTGAACGACGAGCAGCGTCGTAGG
GenBank Match - NONE

OH2 Primer

From pOH82 - 174.22.o2t:
ATTGCCACTTTTAAATTGTTGGGTCTGTACGACATTGAACAGTGCTGGGTTTGTGCGGCTTCACTGCGCGAA
CCGGTTAGATCCGCAGACACC
GenBank Match - NONE

From pOH84 - 174.24.o2t:
CCACTTTTAAATTGTTGGGTCTGTACGACATTGAACAGTGCTGGGTTGTGCGGCTTCACTGCGCGA
GenBank Match - NONE

From pOH81 - 174HOH2:
GGCAACTTCGCATTAACTGACGATCTGTCTTCTTTATAGCTGATGGCGTTT
GenBank Match - NONE

From pOH81 - 174HOH2.top:
CACTTGCCACTTTAATGTGGTCTGTAGCACATTGACA
GenBank Match - NONE

From pOH84 - 174Ib2:
CTGGCAACTTCCGCATTAACTGAtCTGGCTGTCTTCTTTATAGCTGATGGCGTTTTTCAGCTGCTGCCAGGA
CAAAAGCCCGATGCAGTGCTGGCGCGTGATTACATTCGGACTTTTAAATTGTT
GenBank Match - NONE

OH3 Primer

From pOH82 - 174.22.o3t:
ACAGGTCAGCGAGGCTTCCGCCAGCGCCCCAAGTCCGCTTAAGGTAAATCCCTGCTGAAGGTTTGACGAAGC
CAGCCCAGTCTTCGGC
GenBank Match - NONE

From pOH84 - 174.24.o3b:
GGTGCAGTAGAAAAAACAAACGCAATTCGTTTCATCAG
GenBank Match - NONE

From pOH81 - 174HOH3:
GGGGATTCATCAGAGTAAAGCATCTAAACCTTCCCGGCCTGCGCTGTAC
GenBank Match - NONE

From pOH81 - 174HOH3.top:
TGTCACACCTCAGCGAGGCTTCGCAGCGCCCAGTCGCTAGTAATCTGCTGAGTTGACGAGCAGCCAGTCTCG
CTCGTTCATCACACGCACGCTAATGCTGCGCTA
GenBank Match - NONE

From pOH80 - 174Hb3:
CCAAAATCATCAGGGGATTCATCAGATAAAGCATCTAAACCTTCCCGGCCTGCGGCTGTACATGAGGTGCAG
TAGAAAAAACAAACGCAATTCATCAGAACTGTCCCACCCTGT
GenBank Match - NONE

From pOH83 - 174Ib3:
AGTAAAGCATCTAAACCTTCCCGGCCTGCGGCTGTACCATGAGGTGCAGTAGAAAAAACAAACGCAATTGCT
TTCATCAGAACTGTACCACCCTGTCACAGGTCAGCGAGGCTTCCGCAGCG
GenBank Match - NONE

T7 Primer

From pOH84 - 174.24.t7b:
GAAGACGATCACGTCGTGGTTGATGGTAACCACATGCTGGCCGGTCAGAACTGAA
GenBank Match - NONE

From pOH84 - 174.24.t7t:
GAGTCGTGCGCGTCATGCACGATCACGT
GenBank Match - NONE

From pOH78 - 6.5.92-174.1.t7.2:
GGTTGATGGAACCACATGCTGGCCGGTCAGAACCTGAAATTCAACGTTGAAGTTGTGGCGATTCCGGA
GenBank Match - >**ECOSTRA** (GenBank) E. coli fus gene coding for elongation factor G (fusA).

From pOH78 - 6.5.921-174.1.t7.02:
CACGATCACCACCACGATCACGACCACGACGGTTGCTGCGGCGGTCATGGCCACGATCACGGTCAT
GenBank Match - NONE

Reverse Primer

From pOH85 - 174.42.rb:
ACCTAATAAGTAGAACCAACGCGGCGAGACTTAACTTCTACAGTCGGGCGCACGTTTTCGAGAGCTACTTCG
ATGCT
GenBank Match - >**ECOSTR6** (GenBank) Part of the E. coli STR operon including the gene (str or ribosomal protein S12 and the beginning of the gene (rpsG) for S7.

From pOH85 - 174.42.rt:
TTCAGCAGTAGATTTTTTTACCATCTACCATCAGGATATTTACAA
GenBank Match - >**ECOSTR6** (GenBank) Part of the E. coli STR operon including the gene (str or ribosomal protein S12 and the beginning of the gene (rpsG) for S7.

***rpsL/rpsG* Sequences**

str1 (rpsL) Coding Sequence = 130-504
str6 (rpsG) Coding Sequence = 601-843
Located at min. 73.5

Mutant #174:
7kb PstI insertion in pBluescript plasmid.
7kb KpnI insertion in pK184 plasmid.
MiniTn10-*tet* insertion between str1 (rpsL), and strA (fusA).
One PstI site (at #353) located.

***rpsL/rpsG*:**

rpsL, mut# 174
revcomp:
C1 (1f): |>u 843>----- ecostr6 (843 bases)----->u 1>|

```
                                        174.42.rb>ACCTAATAAGTAGA
                                  6.5.92-174.t3.2>TGATAAGTAGAACCC

       834         824         814         804         794         784
ACCAACGCGG CGAGACTTAA CTTCTACAGT CGGGCGCACG TTTTCGAGAG CTACTTCGAA
174.24.t3b>:::::::::: :::::::::: :::::::::: ::: :::::: ::::::
     CGCGG CGAGACTTAA CTTCTACAGT CGGGCGCACG TTT CGAGAG CTACTT
ACCAACCGCGGCGAGACTTAA CTTCTACAGT CGGGCGCACG TTTTCGAGAG174.42.rb
ACCAACGCGG CGAGACTTAA CTTCTACAGT CGGGCGCACG TTTTCGAGAG CTACTTCGA
                                                    6.5.92-174.t3.2>

       774         764         754         744         734         724
TGCTTCCAGT TCAGATTTAC CAGAGCGCTG AGCCAGGGTC TCCAGCGCGC TGTATACGAT
TGCT 6.5.92-174.t3.2                                 ::::::: ::::::::::
                                  6.5.921-174.1.t3.2>AGCGCGC TGTATACGAT

       714         704         694         684         674         664
AGATTCAGCA GTAGATTTTT TACCATCTAC CATCAGGATA TTTACAAATT TAGCCAGCAG
:::::::::: :::::::::: :::::::::: :::::::::: ::::6.5.921-174.1.t3.2>
AGATTCAGCA GTAGATTTTT TACCATCTAC CATCAGGATA TTTACAAATT TAGCCAGCAG
   TTCAGCA GTAGATTTTT TACCATCTAC CATCAGGATA TTTACAA
174.42.rt>

       654         644         634         624         614         604
TTCTGATCCG AACTTCGGAT CCGGCAGAAT TTTACGCTGA CCAATGACGC GACGACGTGG
:::::::::: :::::::::: :::::::174ib3>ACGCTGA CCAATGACGC T CGACGTGG
TTCTGATCCG AACTTCGGAT CCGGCAGAAT TTTACNTGAC C AATGACGC GACGA
                                                    6.5.921-174.1.t3.2

       594         584         574         564         554         544
CATGGAAATA CTCCGTTGTT AATTCAGGAT TGTCCAAAAC TCTACGAGTT TAGTTTGACA
:::::::::: :::::::::: :::::::::: :::: ::::: :::::::::: ::::::::::
CATGGAAATA CTCCGTTGTT AATTCAGGAT TGTC AAAAC TCTACGAGTT TAGTTTGACA
                                                          174ib3>

       534         524         514         504         494         484
TTTAAGTTAA AACGTTTGGC CTTACTTAAC GGAGAACCAT TAAGCCTTAG GACGCTTCAC
:::: :
TTTA G  174ib3
```

```
       474         464         454         444         434         424
GCCATACTTG GAACGAGCCT GCTTACGGTC TTTAACGCCG GAGCAGTCAA GCGCACCACG

       414         404         394         384         374         364
TACGGTGTGG TAACGAACAC CCGGGAGGTC TTTAACACGA CCGCCACGGA TCAGGATCAC

       354         344         334         324         314         304
GGAGTGCTCC TGCAGGTTGT GACCTTCACC ACCGATGTAG GAAGTCACTT CGAAACCGTT
       PstI 353                                         :::: ::::::: :::
                                                174.2.t3bs>ACTT CGAAACCTGTT
                                 6.5.92-174.2.t3.2>GTCACTT CGAAACCGTT


       294         284         274         264         254         244
AGTCAGACGA ACACGGCATA CTTTACGCAG CGCGGAGTTC GGTTTTTTAG GAGTGGTAGT
:::::::::: :::::::::: :::::::::: : : :::::: : :::::::: ::::::::::
AGTCAGACGA ACACGGCATA CTTTACGCAG C C GAGTTC G TTTTCTAG GAGTGGTAGT
AGTCAGACGA ACACGGCATA CTTTACGCAG C                          174.2.t3bs>
                            6.5.92-174.2.t3.2


       234         224         214         204         194         184
ATATACACGA GTACATACGC CACGTTTTTG CGGGCATGCT TCCAGCGCAG GCACGTTGCT
:::::::: :
ATATACAC A  174.2.t3bs

       174         164         154         144         134         124
TTTCGCAACT TTGCGAGCAC GTGGTTTGCG TACCAGCTGG TTAACTGTTG CCATTAAATA

       114         104          94          84          74          64
GCTCCTGGTT TTAGCTTTTG CTTCGTAAAC ACGTAATAAA ACGTCCTCAC ACAATATGAG

        54          44          34          24          14           4
GACGCCGAAT TTTAGGGCGA TGCCGAAAAG GTGTCAAGAA ATATACAACG ATCCCGCCAT


CAT
```

***fusA* Sequence**

strA (fusA) Coding Sequence = 31-2076
Located at min. 73.5

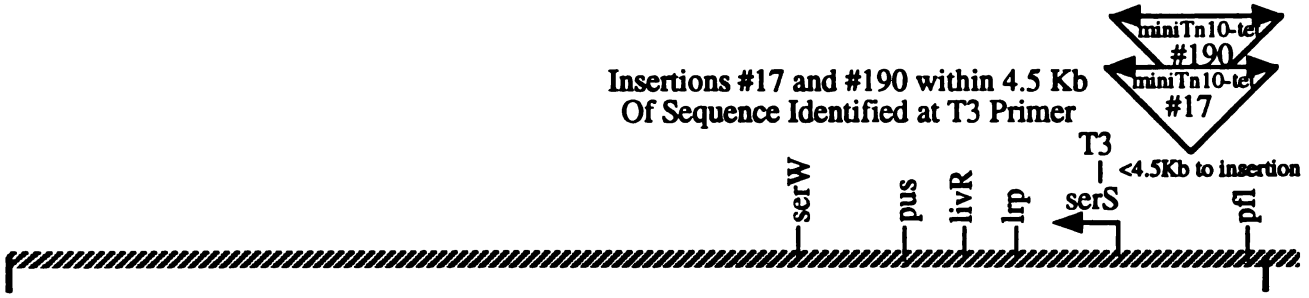
Mutant #174:
7kb PstI insertion in pBluescript plasmid.
7kb kpnI insertion in pK184 plasmid.
MiniTn10-*tet* insertion between str1 (rpsG), and str1 (fusA).

***fusA*:**

C1 (1f): |>u 1>+++++ ecostra (2076 bases)+++++>u 2076>|

```
      1270        1280        1290        1300        1310        1320
TTCCCTGAGC CGGTAATCTC CATCGCAGTT GAACCGAAAA CCAAAGCTGA CCAGGAAAAA

      1330        1340        1350        1360        1370        1380
ATGGGTCTGG CTCTGGGCCG TCTGGCTAAA GAAGACCCGT CTTTCCGTGT ATGGACTGAC

      1390        1400        1410        1420        1430        1440
GAAGAATCTA ACCAGACCAT CATCGCGGGT ATGGGCGAAC TGCACCTCGA CATCATCGTT
                                                    : :: :  ::: :
                           6.5.92-174.1.t7.2>GGTTGATGGAACCACATGCTG

      1450        1460        1470        1480        1490        1500
GACCGTATGA AGCGTGAATT CAACGTTGAA GCGAACGTAG GTAAACCGCA GGTTGCTTAC
: :::   :: : : :::::: :::::::::: :
G CCGGTCGA ACC TGAATT CAACGTTGAA GTTGTGGCGATTCCGGA  6.5.92-174.1.t7.2

      1510        1520        1530        1540        1550        1560
CGTGAAACTA TCCGCCAGAA AGTTACCGAT GTTGAAGGTA AACACGCGAA ACAGTCTGGT

      1570        1580        1590        1600        1610        1620
GGTCGTGGTC AGTATGGTCA TGTTGTTATC GACATGTACC CGCTGGAGCC GGGTTCAAAC

      1630        1640        1650        1660        1670        1680
CCGAAAGGCT ACGAGTTCAT CAACGACATT AAAGGTGGTG TAATCCCTGG CGAATACATC
```

*ser*S
Mutants #17 and #190



Insertions #17 and #190 near serS.

Known Genes Near *ser*S

Gene	Function
serW	Serine tRNA 5
pus	Effect of suppressors on relB mutation
livR	High affinity branched chain amino acid transport - regulatory gene
lrp	leucine-reponsive regulatory protein
serS	ser yl-tRNA synthetase
pfl	pyruvate formate-lyase

***ser*S - Mutants #17 and #190:**

Two of the miniTn10-*tet* generated Tn7 transposition mutants of E. *coli*, mutants #17 and #190, were found near *ser*S. The *ser*S gene codes for seryl-tRNA synthetase (Hartlein et al., 1987).

MiniTn10-*tet* insertions #17 and #190 are located clockwise of *ser*S. The location of miniTn10-*tet* insertions #17 and #190 were determined by sequencing into the one end of the cloned miniTn10-*tet* containing chromosomal DNA from the cloning vector plasmid (pBluescript). Sequence from the T3 pBluescript primer located one PstI cloning site at nucleotide #660 of the "ecosers" *ser*S gene for both mutants #17 and #190. Both miniTn10-*tet* insertions #17, and #190 were genetically linked by P1 transduction to a marker at minute 20, near the *ser*S gene at min. 19.9.

MiniTn10-*tet* insertions #17 was found to decrease miniTn7-*lac* papillation (see Figures 4, Chapter 5). MiniTn10-*tet* insertions #190 increased miniTn7-*lac* papillation. MiniTn10-*lac* papillation was unaffected by either mutant #17 or #190 (Figures 5). MiniTn10-*tet* insertion #17 shows a slight increase in both Tn7 and miniTn7-*lac* transposition to pOX38*gen* (1.7 fold increase of Tn7, while miniTn7-*lac* transposition is increased 1.8 fold) - as measured in the mating out assay. Mutant #190, however, shows a dramatic 13 fold increase in Tn7 transposition, and a 5.2 fold increase in miniTn7-*lac* transposition.

Mutant 17

Sequences Obtained:

T3 Primer
From pOH88 - 17HT3:
GGCTCGGCTTTTGCTGCATCCAGCTCTTCGCCCAGTTTGTTCACTTCCAGACGTAAAGGCTCGATATCTTCC
CCGCGCGCTTTCGCCTGGCCAATGGATTTCGATCGGgAGTTACGCTCCGCTTGCAGGTTTCGTTTGACCTGC
AATAC
GenBank Match - >**ECOSERS** (GenBank) E. coli serS gene for seryl-tRNA synthetase.

OH2 Primer
From pOH88 - 17HOH2:
TGATTAAAGCGAATATCCGACCCGTATCGCCTTTACCGTATCAGTAAGATTGACTCACGTACCATTCTTGAT
CAGGCTGGCGC
GenBank Match - NONE

From pOH88 - 17OH2
AGAACTCAGCGTCCATCGGTTGATGTTATTACTTGGTCTGATTAAAGCGAATATCCGACCCGTATCGAATTT
ACCTGTATCAGTAAGATTGCACTCAGT
GenBank Match - NONE

OH3 Primer
From pOH88 - 17HOH3:
AGTTGCCAGTACGAGGTGGATACCGCGGCACGGCTTTTTGCGCCAGACGTGCTATCAGCTC
GenBank Match - NONE

T7 Primer
From pOH88 - 17HT7:
CCCTGCGCCAGAACAACCGGTGGCAGGTAACGCCTGGCAAGCCGAAGAGCAGCAATCCACTTTTGCTCCACA
GTCTACATACCAGACTGAGCAAACTTATCAGCAGCCAGCCGCTCAGGAGCCGTTGTACCAACAGCCGCAACC
GenBank Match - NONE

***serS* Sequence**

serS Coding Sequence = 337-1629
Located at min. 19.9

Mutant #190:
7.5kb PstI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion not located.
One PstI site (at #660) located.

Mutant #17:
7.5kb PstI insertion in pBluescript plasmid.
MiniTn10-*tet* insertion not located.
One PstI site (at #660) located.

***serS*:**

```
C1 ( 1f): |>u 1>+++++ ecosers (1854 bases)+++++>u 1854>|

       370         380         390         400         410         420
AATGAGCCAG ACGCAGTCGC TGAAAAACTG GCACGCCGGG GCTTTAAGCT GGATGTAGAT
                                             :::::: ::   ::::::::
                                             CTTTAA CT   ATGTAGAT
                                                   <6.5.921-190.8.t3.2


                                                                <6.5.921-190.8.t3.2
AAGCTGGGGC CTCTTGAAGA GCGTCGTAAA GTATTGCAGG TCAAAACGGA AAACCTGCAA
:::::::430 :::::::440 :::::::450 :::::::460 :::::::470 :::::::480
AAGCTGGGCG CTCTTGAAGA GCGTCGTAAA GTATTGCAGG TCAAAACGGA AAACCTGCAA
                                  :::::::::: ::::: :: : :: :::::::
                                  GTATTGCAGG TCAAA CG A AA CCTGCAA<17HT3


GCGGAGCGTA ACTCCCGATC GAAATCCA<6.5.921-190.8.t3.2
:::::::490 :::::::500 :::::::510         520         530         540
GCGGAGCGTA ACTCCCGATC GAAATCCATT GGCCAGGCGA AAGCGCGCGG GGAAGATATC
:::::::::: :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
GCGGAGCGTA ACTCCCGATC GAAATCCATT GGCCAGGCGA AAGCGCGCGG GGAAGATATC<17HT3

                                                          <6.5.92-190.8.t3.2
      TTAC GTCTGGAAGT GAACAAACTG GGCGAAGAGC TGGATGCAGC AAAAGCCGAG
      :550 :::::::560 :::::::570 :::::::580 :::::::590 :::::::600
GAGCCTTTAC GTCTGGAAGT GAACAAACTG GGCGAAGAGC TGGATGCAGC AAAAGCCGAG
:::::::::: :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
GAGCCTTTAC GTCTGGAAGT GAACAAACTG GGCGAAGAGC TGGATGCAGC AAAAGCCGAG<17HT3

       610         620
CTGGATGCTT TACAGGCTGA AAT<6.5.92-190.8.t3.2
:::::::610 :::::::620 :::    630         640         650         660
CTGGATGCTT TACAGGCTGA AATTCGCGAT ATCGCGCTGA CCATCCCTAA CCTGCCTGCA
:                                                       PstI 660
CC<17HT3

       670         680         690         700         710         720
GATGAAGTGC CGGTAGGTAA AGACGAAAAT GACAACGTTG AAGTCAGCCG CTGGGGTACC
```

Mutant 190

Sequences Obtained:

T3 Primer
From pOH91 - 6.5.921-190.8.t3.2:
TGGATTTCGATCGGGAGTTACGCTCCGCTTGCAGGTTTTCCGTTTTGACCTGCAATACTTTACGACGCTCTT
CAAGAGGCCCCAGCTTATCTACATAGTTAAAG
GenBank Match - >**ECOSERS** (GenBank) E. coli serS gene for seryl-tRNA synthetase.

From pOH91 - 6.5.92-190.8.t3.2
ATTTCAGCCTGTAAAGCATCCAGCTCGGCTTTTGCTGCATCCAGCTCTTCGCCCAGTTTGTTCACTTCCAGA
CGTAA
GenBank Match - >**ECOSERS** (GenBank) E. coli serS gene for seryl-tRNA synthetase.

OH2 Primer
From pOH91 - 6.5.921-190.oh2.2:
GTCATCATCAGGTCGGCAAATTGTCAACCAACACCACAATGTATGGTTCTTTTTTCAGCACCGGATGCTGGG
CATCATACTGTCACC
GenBank Match - NONE

OH3 Primer
From pOH91 - 190.8.o3b1:
TTTACCGTATCCAGTAAGATTGACTCACGTACCATTCTTGATCAGGCTGGCGCGGAATCACTGCTGGGTATG
GGGGATATGCTCTACTCTGGGCCGAACTC
GenBank Match - NONE

From pOH89 - 190HOH3:
AGAACTCAGCGTCCATCGGTTGATGTTATTACTGGTCTGATTAAACGGATAACGACCGTATCC
GenBank Match - NONE

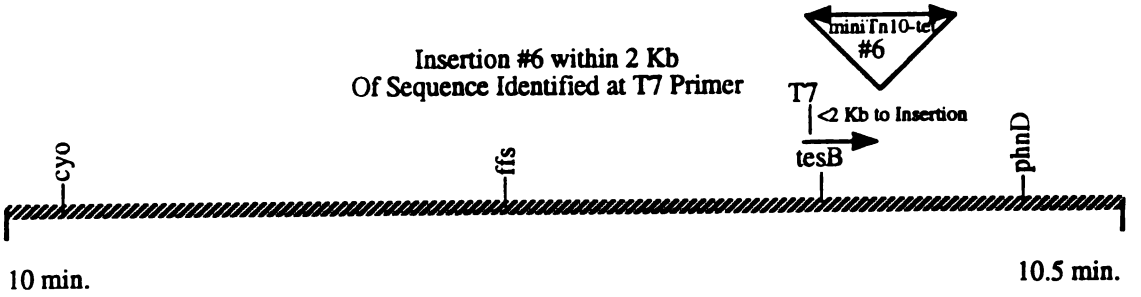
From pOH91 - 6.5.921-190.oh3.2:
TTTACCGTATCCAGTAAGATTGACTCACGTACCATTCTTGATCAGGCTGGCGCGGAATCACTGCTGGGTATG
GGGGATATGCTCTACTCTGGGCCGAACTCCACGTTGCCGGTACGTGTCCATGGTGCTTTTGTTCGCGATCAG
GAAGTTCATGCGTGGTGC
GenBank Match - NONE

T7 Primer
From pOH89 - 190HT7:
CATAATATTGCAGGAACACCGGTATTCTCCTGCGCAACAGAGTAAATACTGACGAGCAGTGCCGCGATTGTT
CATACGCATGACATCTCAG
GenBank Match - NONE

From pOH91 - 6.5.921-190.8.t7.2:
TTACCGCATGACAATCCTTCCCAGCAGTAAAGCCCGGCCCAAGCGCGATTGTTACTGGTGCATATCTG
GenBank Match - NONE

From pOH91 - 6.5.92-190.8.t7.2:
ATGCCCCATAATATTGCCAGGAACACCGGTATTCTCCTGCGCACAAC
GenBank Match - NONE

*tes*B
Mutant #6



Insertion #6 near tesB.

Known Genes Near *tes*B

Gene	Function
cyo	Cytochrome-o-terminal oxidase complex
ffs	4.5S RNA
tesB	Thioesterase II
nusB	Transcription termination

***tes*B - Mutant #6:**

Sequence form mutant #6 indicates that it lies near *tes*B. The *tes*B gene codes for thioesterase II (Naggert et al., 1991).

The location of miniTn10-*tet* insertion #6 was determined by sequencing into the one end of the cloned miniTn10-*tet* containing chromosomal DNA from the cloning vector plasmid (pBluescript). Sequence from the T7 pBluescript primer located one KpnI cloning site at nucleotide #15 of the "ecotesb" *tes*B gene for mutant #6. The 4-5 Kb KpnI insert size of the miniTn10-*tet* insertion #73 clone indicates that the insertion was within 1-2 Kb of the located KpnI site.

Sequence from mutant #6 indicated that the miniTn10-*tet* insertion lay near *tes*B at minute 10.4 on the E. *coli* chromosome. P1 transduction of a kanamycin marker expected to be at minute 10.5 into a strain containing miniTn10-*tet* insertion #6 showed no loss of the tetracycline resistance marker. This suggests that the one of the two markers are not in the expected location.

MiniTn10-*tet* insertion #6 was found to decrease miniTn7-*lac* papillation, though no effect is seen on miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). Mutant #6 decreases in Tn7 miniTn7-*lac* transposition to pOX38*gen* 3 fold, and conversely increase Tn7 transposition 2.6 fold - as measured in the mating out assay.

Mutant 6

Sequences Obtained:

T3 Primer
From pOH96 - 6IT3:
GGCAGTAATCGATGGACTGGCGGTGATCAGGTTTTCTCCGACTTCTAACGTTGTAATAAC
GenBank Match - NONE

From pOH96 - 6IT3.top:
GCAGAGGAGATCGGGACTTTTGCGCACGTCAATAGCAAACTAACTGATGCGCTACTGAGTGCTGACCGTGCA
GCTCTCGTTGCGATGTGATTGCACGGATGCGACACTGTCAATCTTTCGAAGCGCGAGTATCAGTACTCACGC
TAGCAGC
GenBank Match - NONE

OH2 Primer
From pOH128 - 6H1OH2:
TGACCTGCTGACCATTACCTTTAACGGACTGTCGAAAACGTACCGCGTTGCAGGCTTCCGTCAGGGTGGATG
GTGGTTGC
GenBank Match - NONE

OH3 Primer
From pOH128 - 6H1OH3:
CGGCGAAGATAATGAGATTATGCTGACGTGCAATCTCCACAATCTCCATTAAAGCTCTTGGAATATACG
GenBank Match - NONE

T7 Primer
From pOH128 - 6H1T7:
GGACTTCCACGGCAGGAGTGGCGATAACAGCAAAAAAGGTCAAGATTCATGTCCCATTGAAAATCACGAAAT
TAGAGA
GenBank Match - NONE

From pOH96 - 6IT7:
GCAGCCGGTGCTGGCGTCTGAATATCCGCGCTTTTATCTGCGG
GenBank Match - >**ECOTESB** (GenBank) E.coli thioesterase II (tesB) gene,
complete cds.

***tesB* Sequence**

tesB Coding Sequence = 387-1247
Located at min. 10.4

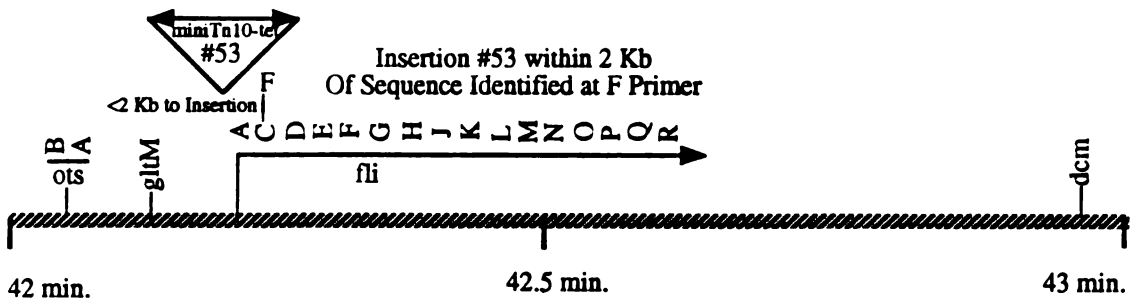
Mutant #6:
>11kb PstI insertion in pK194 plasmid.
4-5kb kpnI insertion in pK194 plasmid.
MiniTn10-*tet* insertion not located.
One KpnI site (at #15) located.

***tesB*:**

C1 (1f): |>u 1>+++++ ecotesb (1455 bases)+++++>u 1455>|

```
        10          20          30          40          50          60
GGATCCAGAC GGTACCGGAG ACATTCGGTT GCTGGATAGC TGGTTGTTGT GTTGCTGAAA
           KpnI
        70          80          90         100         110         120
TAGACGTATT TGCAGCCGGT GCTGGCGTCT GAATATCCGC GCTTTTATCT GCGCAAGCCG
           ::::::::: ::::::::: ::::::::: ::::::::: ::::
      6IT7>GCAGCCGGT GCTGGCGTCT GAATATCCGC GCTTTTATCT GCGG


       130         140         150         160         170         180
CCAACGCAAT CGCAACCGCT AAACCACTGG CCATGTGCAC GAGTTTCATT CATTTCTCCT

       190         200         210         220         230         240
TATTATCAAT GCACCAGCGG GCTAACTTTC CTCGCCGGAA GAGTGGTTAA CAAAATAGTA

       250         260         270         280         290         300
ACGTCAACAA GTGTGGCACA CATCACGCAT TTCTGCCTGT AATTAGCCCG TAATTCAGAC

       310         320         330         340         350         360
CATTGCACCC ATCGGACCAG TAGCAAATTT GCGTTATACT CAACTCACTT TGGCTTGCTG

       370         380         390         400         410         420
CGGCAGCTTT GTTACTGGAG AGTTATATGA GTCAGGCGCT AAAAAATTTA CTGACATTGT

       430         440         450         460         470         480
TAAATCTGGA AAAAATTGAG GAAGGACTCT TTCGCGGCCA GAGTGAAGAT TTAGGTTTAC

       490         500         510         520         530         540
GCCAGGTGTT TGGCGGCCAG GTCGTGGGTC AGGCCTTGTA TGCTGCAAAA GAGACCGTCC
```

fliC Mutant #53



Insertion #53 near *fliC* (*hag*A).

Known Genes Near *fliC* (*hag*A)

Gene		Function
ots	A B	Trehalose phoshphate synthase production
gltM		Level of glutamyl-tRNA synthetase activity
fli	A-R	Flagellar synthesis
dcm		DNA cytosine methylase

***fli*C- Mutant # 53:**

Sequence form mutant #53 indicates that it lies near *fli*C. *fli*C(or *hag*A) encodes a flagellar filament structural protein (Kuwajima et al., 1986).

The location of miniTn10-*tet* insertion #53 was determined by sequencing into the one end of the cloned miniTn10-*tet* containing chromosomal DNA from the cloning vector plasmid (p194). Sequence from the Forward p194 primer located one PstI cloning site at nucleotide #504 of the "ecohaga" *fli*C gene for mutant #53. MiniTn10-*tet* insertion #53 was. The 5 Kb PstI insert size of the miniTn10-*tet* insertion #53 clone indicates that the insertion was within 2 Kb of the located PstI site.

MiniTn10-*tet* insertion #53 was found to decrease miniTn7-*lac* papillation, though no effect is seen on miniTn10-*lac* papillation (see Figures 5-4 and 5-5, Chapter 5). Mutant #53 decreases both Tn7 (2 fold) and miniTn7-*lac* (5 fold) transposition to pOX38*gen* as measured in the mating out assay.

Mutant 53

Sequences Obtained:

T3 Primer
No Sequence

Forward Primer
From pOH106 - 53,381.ftb:
GCGTAATACTTCCCGTCGTTATCACCACCGG
GenBank Match - >**ECOHAGA** (GenBank) E.coli hag gene encoding minumum-size flagellin.

From pOH106 - 53.38.fb:
GGTACCCGGGGATCCTCTAGAGTCGACCTGTAGTATTATCAATCTGAACAGGTGTACGCCTGAAGAGATAGT
TGTAGCTTTAGTAGTATTTGCATCAGTACAGTGCATTGCG
GenBank Match - >**ECOHAGA** (GenBank) E.coli hag gene encoding minumum-size flagellin.

From pOH106 - 53.38.ft:
CTGTTACTGCGTAATACTTCCATCGTTAACACCACCG
GenBank Match - >**ECOHAGA** (GenBank) E.coli hag gene encoding minumum-size flagellin.

From pOH106 - 53.381.fb:
TGCCGTTGCTCCAGTCGCCATTGTCACTGTATTA
GenBank Match - >**ECOHAGA** (GenBank) E.coli hag gene encoding minumum-size flagellin.

From pOH106 - 53.381.ftt:
CGCATAGTAATCATTACCATTATCAGTATAAACACCCTCAATTGAAGCTGGGTTAGTTCGCAGTATCAGTGG
CTGCTTCGTAGAAAGGGTA
GenBank Match - >**ECOHAGA** (GenBank) E.coli hag gene encoding minumum-size flagellin.

From pOH106 - 53hf:
GGTACCCGGGATCCTCTAGAGTCGACCTGCAGTATTATCAATCTGAACAGGTGTACGCCTGAAGTGATAGTT
GTAGCTTT
GenBank Match - >**ECOHAGA** (GenBank) E.coli hag gene encoding minumum-size flagellin.

OH2 Primer
From pOH105 - 53.39.o2t:
ATTTATGTATATTGAACAGCATTCTCGCTATCAAAATAAAGCTAATAACATCCAATTAAGATATGATGATAG
CAGTTTCT
GenBank Match - NONE

OH3 Primer
No Sequence

T7 Primer
From pOH108 - 53ibt7:
cgggtataaacaggcaggaaattaatagcaatgagtcatcgtgcacgacaccaattactggcnttgcgggca
ttatcttt
GenBank Match - NONE

Reverse Primer

From pOH106 - 53.38.r.b:
TCAGCCCAATGAATGCAGCAAGACCATAACGTCTGCG
GenBank Match - NONE

From pOH106 - 53.38.r.t:
TATATTCATATCTACCCCTGCTTGTACCATTATGTTATACACCTCTTCAGGAGTATTCATAAAACAAGGCAA
ATGTAAAC
GenBank Match - NONE

From pOH106 - 53.381.rb:
TCAGCCCAATGAATGCAGCAAGACCATAACGTCTGCGGT
GenBank Match - NONE

From pOH106 - 53hr:
GATTCCGGGCCACACGCTGCATTAAACATATCCACCGGGCTACGTGGCGGCAATGGAACTCAGCCCCCACTT
CACGAATA
GenBank Match - NONE

fliC (hagA) Sequence

fliC Coding Sequence = 1-876
Located at min. 42.6

Mutant #53:
5kb PstI insertion in Pnk194 plasmid.
3.5kb kpnI insertion in Pnk plasmid.
MiniTn10-*tet* insertion not located.
One PstI site (at #504) located.

***fliC*:**

```
C1 ( 1f): |>u 867>----- ecohaga (867 bases)----->u 1>|

       558         548         538         528         518         508
ATTACCCTTG GAATCCTGCA GTTTTACTAA GCTAACAGCA CCAAGGTTGG CAGTTGCGGA

                                  53.38.fb>GGTACCCGGGGATCCTCTAGAGTCG

  PstI498         488         478         468         458         448
ACctgcagTA TTATCAATCT GAACAGGTGT ACCGCCTGAA GTGATAGTTG TAGCTTTAGT
:::::::::: :::::::::: :::::::::: :: ::::::: :::::::::: ::::::::::
ACctgcagTA TTATCAATCT GAACAGGTGT AC GCCTGAA GAGATAGTTG TAGCTTTAGT
                                                        53.38.fb>

       438         428         418         408         398         388
AGTATTTGCA TCAGTTACAG TTGCATTTGC CGTTGCTCCA GTCGCCATTG TCACTGTACC
:::::::::: ::::: :::: : ::::: :: :
AGTATTTGCA TCAGT ACAG T GCATT GC G  53.38.fb
                  53.381.fb>TGC CGTTGCTCCA GTCGCCATTG TCACTGTATTA


       378         368         358         348         338         328
ATCATTAGCA ACTGTTACTG CGTAATACTT CCCATCGTTA TCACCACCGG TGATTTTCGC
                   : :::::::::: :::::::::: :::::::53.381.ftt>CGC
          53,381.ftb>G CGTAATACTT CCCGTCGTTA TCACCACCGG
   53.38.ft>CTGTTACTG CGTAATACTT CC ATCGTTA ACACCACCG


       318         308         298         288         278         268
ATAGTAATCA TTACCATTAT CAGTATAAAC ACCCTCAATT GAAGCTGGGT TAGTTCCGCC
:::::::::: :::::::::: :::::::::: :::::::::: :::::::::: :::::: : :
ATAGTAATCA TTACCATTAT CAGTATAAAC ACCCTCAATT GAAGCTGGGT TAGTTC G C
                                                        53.381.ftt>

       258         248         238         228         218         208
AGTATCAGTG GCTGCTTCCG TAGAAAGGGT AATTCCAGTA AGTTTAATAT TGTTTGTGGT
:::::::::: :::::::: : :::::::::: :
AGTATCAGTG GCTGCTTC G TAGAAAGGGT A 53.381.ftt

       198         188         178         168         158         148
GGTAGCACCA AAAGCAGTTA CTGGAGCACT AGTGGTAACT GTATCGTTAT TTTTAACGCT
```

Identity of Mutants #61, 62, 118 , 171, and #49 Remain Unknown:

Mutants #61, 62, 118 , and 171:

Sequence from mutants #61, 62, 118 , and 171 found no matches within the GenBank data base. Mutants #118 and 62 were sequenced from both ends of the miniTn10-*tet* insertion, and in from one of the cloned outside ends, while mutant #61 was sequence from all but the OH3 primer.

No miniTn7-*lac* or miniTn10-*lac* papillation is observed in mutant #118, while very little papillation is observed from mutant #62 (see Figures 5-4 and 5-5, Chapter 5). Mutants #171 and 61 increase miniTn7-*lac* papillation.

Mutant #49:

Mutant #49 was not cloned. Mutant #49 decreased miniTn7-*lac* papillation, and shows a 5 fold decrease in miniTn7-*lac* transposition, and a 1.7 fold decrease in Tn7 transposition to pOX38*gen* - as measured in the mating out assay.

Mutant 61

Sequences Obtained:

Forward Primer
From pOH111 - 7.17a.61i.3.f:
TACCGTTGGTAATCTTGCCGTCCNNACTGCGACTGCTCAGGATCAAAACCCGACTGCCGAGGCAA
GenBank Match - NONE

From pOH110 - 7.28b.61i.2.f:
GGCTTCAGTGCGGCTACGCACCTCGG
GenBank Match - NONE

From pOH111 - 7.28b.61i.3.f:
CCTTCGGGTACGTTCGGTAACTTCGCCGCTCTG
GenBank Match - NONE

From pOH113 - 61.33.f:
GATGCCTCGAGCCCTGATTAATGATTCCGGGCCACACGCTGCATTAAACATATCCACCGGGCTACGTG
GenBank Match - NONE

From pOH113 - 61.331.fb:
CACGAACACATCACGGTCGCGTCATTCGTACATGTCGATGCATCTGTCATGTAAGACGAATCTGCGTTACAG
ATCTCGAGCATGGACGT
GenBank Match - NONE

From pOH113 - 61.331.ft:
AAATATATTCATATCTACCCTGCTTGTACCATTATGTTATACACTCTCAGAGTATCATAAACAGCAATGTAA
GACTGTATG
GenBank Match - NONE

From pOH113 - 61hf:
CGATGCCTCGAGCCCTGATTAATGATTCCGGGCCACACGTGCATTAAACATATCCACCGGGCTACGTGGCGG
CAATGGAAC
GenBank Match - NONE

OH2 Primer
From pOH109 - 61H192:
TAGGTACTCTTAAAATTTTCTTGTTGATGATTTTTATTTTCCATGATAGATTTAAAATAACATACCGTCAGT
ATGTTTATGGTATCATGATGATGATGTGGTCGTGACAATCTTAAGAACATTTAGGTTATTTTAGT
GenBank Match - NONE

OH3 Primer
No Sequence

Reverse Primer
From pOH111 - 7.17a.61i.3.r:
GGGTACCAAGAAAGGTTGCGATCACGGACAGTGC
GenBank Match - NONE

From pOH113 - 61.331.rtt:
AATATATTCATATCTACCCTGCTTGTACCATTATGTTATACACCTCTTCAGGAGTATTCATAAAACAAGGCA
AATGTAAAGAACTGTATTGTTTTGTATAACAAGATAGTTTCTAATCGCAATGAATATAAGCTCATCATTTCT
CCTATTTTTATATTAGAGTGACAGAGATTGCAGG
GenBank Match - NONE

Mutant 62

Sequences Obtained:

Forward Primer
From pOH115 - 62.2.fb:
CTCGGACCAAGAAAGGTTGCGATCACGGACAGTGCGG
GenBank Match - NONE

From pOH118 - 7.17s.62.9.f
ATGCCTGCAGGTCGACTCTAGAGGACCCCGGGTACCATATCGAAAACCACAA
GenBank Match - NONE

OH2 Primer
From pOH114 - 62.3s.o2b1:
ATGATGTGGTCGTGACAATCTTAAGAACATTTAGGTTATTTTATGTATATTGAACAGCATTCTCGCTATCAA
AATAAAGA
GenBank Match - NONE

From pOH114 - 6.5.921-62.3s.oh2.2:
TGATGTGGTCGTGACAATCTTAAGAACATTTAGGTTATTTTATGTATATTGAACAGCATTCTCGCTATCAAA
ATAAACTC
GenBank Match - NONE

OH3 Primer
From pOH114 - 6.5.921-62.3s.oh3.2:
GAAAATAACGATGCTTGCCTTGAGCTACTACTGTGATTAGCTGGCAATCTAATAATTTAGATAAATGACTGC
TCGCCGTG
GenBank Match - NONE

Reverse Primer
No Sequence

Mutant 118

Sequences Obtained:

Forward Primer
From pOH123 - 118.34.f.t.2:
CCATAAAAACTTGATGGGGACATCCAGTTTTTCTTTTCACGAACACATC
GenBank Match - NONE

From pOH123 - 118.34.fb:
TGCCTGCAGATTCATGAGAGATAAGGTCTTCTTGCTCTGTTGGCATCAGGTCGGGCAACAGGATATCGCAAT
ACTTAGCA
GenBank Match - NONE

From pOH123 - 118.34.ft:
GATGTCGCATGTTGATTGATCATGTGTTACTGCGTCAGCATAAA
GenBank Match - NONE

From pOH123 - 118.34l.ftb:
TGTGATTGATCAATGTGTTACTGGCGTCAGACCATAAAAACTTGATGGGGACATCCAG
GenBank Match - NONE

From pOH123 - 118hf:
GCCTCGAGATTCATGAGAGATAAGGTCTTCTTGCTCTGAAGGCATCAGGTCGGGCAACAGGATATCGCAATA
CTTAGCCC
GenBank Match - NONE

OH2 Primer
From pOH123 - 118HOH2:
CAATATTCGTGCTGAGTTAACCAGTGATTGATAGGTACTCTTAAAATTTTCTTGTTGATGATTTTTATTTTC
CATGATAT
GenBank Match - NONE

From pOH123 - 118HOH2.top:
GAACATTTAGGTTATTTTATGTATATGACAGCATCTCGCTCTCAAATAAGCT
GenBank Match - NONE

OH3 Primer
From pOH123 - 118HOH3:
AGCTAAATGATCATAGCAAGTACGTGCTTTTCGTAAATGCACTGGCGTGGAAACTTTGGCATGTACGCCATG
GTTTAAGC
GenBank Match - NONE

From pOH123 - 118HOH3.top:
CTTGAGCTACTACTGTGATTAGCTGGCATCTATATTTAGATAATGACTGCT
GenBank Match - NONE

Reverse Primer
No Sequence

Mutant 171

Sequences Obtained:

Forward Primer
From pOH125 - 7.191.171h.59.f:
TACCGTTGGTAATCTTGCCGTCGCAAACTGCGACTGCTCAGGATCAAACCCGACTGCCGAGGCAATCATTTC
GCGAACGTTCATACAGGCGGCGTAAACCGAGGTGGAGGTATTCGCGCCCATTGTCACCAGAACCGCAGAAAC
CGGAA
GenBank Match - NONE

From pOH126 - 7.28b.171i.60.f:
CCATCAGGCGGCCTTTATAAGCATGATGGCTTCAGTGCGCTACGCACCTCGGCAA
GenBank Match - NONE

From pOH125 - 7.17a.171h.59.f
CAGCACACACAGCGAGCATACCCCAATCCCGAACTTCTCCGTCGCGCTATGCACGCCGACCTCCACAAAA
GenBank Match - NONE

From pOH125 - 7.28c.171h.59.f:
AATCCCCTAAGTAGTCCCGTATTTCGAGACTGCCATTTTGCGCATAGTGCCGCCGTTGGT
GenBank Match - NONE

OH2 Primer
No Sequence

OH3 Primer
No Sequence

Reverse Primer
From pOH125 - 7.28c.171h.59.r:
CTGCCTTGACCGCGTGCCGTCGTACAACTGGGACC
GenBank Match - NONE

Chapter 6: Summary and Perspectives

The goal of this thesis was to identify *Escherichia coli* components involved in Tn7 transposition. Host components participate in the recombination of many mobile elements (Berg and Howe, 1989). Including host components in transposition enables transposons to tie transposition to favorable host environment conditions and take advantage of existing tools within a cell.

Historically, host components of transposition have been identified by either directly testing host components known to be involved in other recombination systems, or through naive genetic and biochemical deconstruction of transposition. In this thesis, we have both tested mutants known to affect other bacterial transposons, and screened for new E. *coli* mutants affecting Tn7 transposition.

The tools used for the investigation of nature limit and shape what we learn. Our standard assay for Tn7 transposition (Mating-Out) involved determining the fraction of conjugal plasmids in a population onto which Tn7 has hopped (Waddell and Craig, 1988). This assay of transposition frequency is cumbersome, and unsuited to genetic screens for rare mutations. In the second Chapter of this thesis, "A Visual Assay for Tn7 Transposition", I reported the construction and characterization of a genetically tractable assay for Tn7 transposition.

The transposition assay developed and characterized in Chapter Two is based on the ability of a transposition event to move a genetically hidden, or unexpressed, function to an exposed or expressed site. MiniTn7-*lac* was constructed to carry promoterless *lac*ZYA genes between the ends of Tn7. Transposition of the miniTn7-*lac* from a non-transcribed to a transcribed genetic region results in a phenotypic transition from Lac^- to a Lac^+. On MacConkey Lactose indicator plates, red Lac^+ papillae are easily distinguished against the white Lac^- colony, and the rate of colony papillation is related to the rate of transposition . Thus the transposition activity within each bacterial colony on a plate can be visually assessed by scoring the rate of red papillae formation.

We showed in Chapter Two that Lac$^+$ miniTn7-*lac* papillation faithfully represents miniTn7-*lac*, i.e. Tn7, transposition in that: 1) MiniTn7-*lac* transposes in a manner characteristic of Tn7, with *tns*ABC+D being required for transposition to *att*Tn7 targets, and *tns*ABC+E required for transposition to non-*att*Tn7 targets. 2) Papillation requires the same *tns* transposition functions as Tn7 and miniTn7-*lac* transposition. 3) Formation of Lac$^+$ papillae generally involves the translocation of miniTn7-*lac* to a new genetic location.

The development of a papillation assay for Tn7 transposition enabled us to address questions about Tn7 transposition that were inaccessible with previous transposition assays. A particularly intriguing aspect of Tn7 transposition is its insertion orientation specificity. Previous characterization of Tn7 transposition to *att*Tn7 found that insertion in *att*Tn7 always occurred with the left end of Tn7 adjacent to *glm*S. In Chapter Three of this thesis we tested the extent of transposition orientation specificity.

To examine the orientation of large numbers of Tn7 insertions in *att*Tn7, we took advantage of unique properties miniTn7-*lac* presents. Unidirectional transcription of *lac* in from the left end of miniTn7-*lac*, and what was found in Chapter Three to be unidirectional transcription across *att*Tn7 from *glm*S, was combined to form a sensitive assay of miniTn7-*lac* orientation in *att*Tn7. Transposition of miniTn7-*lac* to *att*Tn7 with it's right end adjacent to *glm*S results in a Lac$^-$ phenotype, while insertion with the left end of miniTn7-*lac* adjacent to *glm*S results in a Lac$^+$ phenotype. Chapter Three, "Orientation Specificity of Tn7 Transposition to *att*Tn7", reports the utilization of miniTn7-*lac*'s ability to report insertion orientation in *att*Tn7 to expose a low frequency of opposite orientation miniTn7-*lac* insertion.

Orientation specificity is an important component to the understanding of Tn7 transposition. The mechanism of transposition does not involve homologous pairing between the transposon and target, and so has no DNA inherent mechanism of determining relative orientation. The ability of Tn7 to transpose to *att*Tn7 in a nearly

orientation-specific manner implies *att*Tn7's orientation, relative to the Tn7 ends, is distinguished in the process of transposition.

Tn7 transposition to *att*Tn7 involves the conjunction of two non-equivalent transposon ends (Gosti-Testu, 1982; Lichtenstein, 1982; Lichtenstein and Brenner, 1982; Arciszewska et al., 1989; Arciszewska et al., 1991; Arciszewska and Craig, 1991; Tang et al., 1991), an asymmetric target site (McKown et al., 1988; Gringauz et al., 1988; Quadri et al., 1989; Bainton et al., 1993), and four transposon-encoded proteins, TnsA+B+C and either TnsD or TnsE (Rogers et al., 1986; Waddell and Craig, 1988). The synaptic complex built of these asymmetric components must form a structure competent to perform transposition. If the synaptic complex was functional in only one form, opposite orientation transposition would be mechanistically excluded. The work of Chapter Three shows that transposition orientation is not an absolute, but a preference, thus constraining the possible models for the synaptic transposition complex.

The identification of mutants affecting transposition orientation would aid our understanding of transposition. Orientation mutants could provide clue's to how the synaptic complex is formed and indicate where transposition orientation can be generated, and lost . MiniTn7-*lac* is specifically designed to ease the identification of transposition mutants. MiniTn7-*lac*'s ability to report transposition orientation could be readily employed for the identification of transposition orientation mutants.

The identification of mutations affecting Tn7 transposition was the goal that miniTn7-*lac* was designed to help us attain. Of particular interest to this thesis are E. *coli* mutations that alter Tn7 transposition activity. Previously defined interactions of other bacterial transposons with their E. *coli* host were reviewed in Chapter One, the introduction to this thesis. The similarity of host components involved with the different bacterial transposons is striking. Chapter Four of this thesis, "Characterization of the Effect of Mutations in *dam*, *hns*, *fis*, and IHF's *hip* and *him*A on Tn7 Transposition"

directly examined effects on Tn7 transposition of mutations in four E. *coli* components known to affect other transposons and recombination systems.

dam was shown to increase miniTn7-*lac* transposition (as measured by both the mating-out assay and papillation), reminiscent of the effect of *dam* on other bacterial transposons that move *via* a conservative transposition mechanism. Loss of *dam* function was also seen to increase intact Tn7 transposition, however attempts to reproduce this result have been unsuccessful (Bob DeBoy and N. Craig, personal communication). Thus the relevance of *dam* regulation to the transposition of intact Tn7 is remains to be settled (see Discussion of Chapter 4).

Regulation of transposition by DAM is one of the best understood examples of host-transposon interactions (reviewed in Chapter One of this thesis). DAM has been shown to regulate IS10 transposition though controlling both expression and activity of IS10 transposase. DNA adenine methyltransferase acts at two GATC sequences in IS10. One DAM site is near the outside end in the -10 region of the pIN transposase promoter. The other DAM site lies within the inside end transposase binding site. DAM methylation is thought to decreases both transposase end binding activity and transposase transcription (Roberts et al., 1985).

The site of DAM action on Tn7 is unknown; however there is only one DAM site within the *cis*-acting end sequences known to be important for Tn7 transposition (Arciszewska et al., 1989; Hauer and Shapiro, 1984; McKown et al., 1988; Ouartsi et al., 1985; Rogers et al., 1986; Smith and Jones, 1984; Waddell and Craig, 1988). A GATC sequence defining a DAM site lies within a TnsB binding site in the right end of the transposon. We hypothesized that adenine methylation at this **R**40 - GATC site alters TnsB binding to the end of Tn7 relative to binding in the un- or hemimethylated state, such that synaptic complex formation is inhibited and/or *tns*AB transcription is repressed.

To directly test whether methylation at the **R**40 - GATC is the site of DAM regulation of Tn7, a Tn7 mutant altered at this site is currently being tested by Bob

DeBoy in Dr. Craig's lab. Tn7 altered at the R40 - GATC, such that it is no longer recognized as a DAM site, should be insensitive to the *dam* $^{+/-}$ genotype of the cell. An *in vitro* assay for TnsB binding has also been developed in Dr. Craig's lab, and can also be used to directly examine the effect of methylation on TnsB binding.

The -35 region of Tn7's predicted *tns*AB promoter lies within the inner-most TnsB binding site (Smith and Jones, 1986; Gay et al., 1986). The TnsB binding sites within the right end of Tn7 lie head to tail in a slightly overlapping manner, raising the possibility of communication through the TnsB binding sites. Altered TnsB binding at the R40 - GATC may thus affect TnsB binding over the predicted -35 region of the *tns*AB promoter and subsequently *tns*AB transcription. Separating the effects of DAM on TnsB binding/transposase activity from transcriptional effects will be very interesting, and slightly more challenging than identifying whether R40 - GATC is the site of DAM regulation of Tn7. The use of reporter fusions to the *tns*AB promoter, along with the measurement of miniTn7 transposition in *dam*$^{+/-}$ strains - as *tns*AB are produced from a heterologous promoter - should help separate the effects of DAM on TnsB binding/transposase activity from *tns*AB transcription.

In Chapter Four, we also looked at the involvement of histone-like proteins in Tn7 transposition. We found that mutation of a number of the histone-like proteins known to be involved in other transposition systems also effect Tn7 transposition. Mutation of *hns*, *fis*, and the two IHF subunits *him*A and *hip*, were all shown to decrease the transposition of Tn7 elements. By both mating-out and papillation assays, a null allele of *hns* was shown to decrease intact Tn7 transposition. Null alleles of *fis* and IHF's *hip* or *him*A were shown to decrease miniTn7-*lac* transposition as measured by papillation.

The molecular mechanisms for the involvement of histone-like proteins in Tn7 transposition may be more difficult to elucidate than the mechanism of DAM regulation, with the particular challenge being the determination of direct vs. indirect action on Tn7. IHF has been shown to alter the binding of transposase to transposon end for γδ and IS10

(Roberts et al., 1987; Wiater and Grindley, 1988), while FIS has been found to bind the ends of Mu and Tn5 (Alazard et al., 1992; Betermier et al., 1989; Weinreich, 1992). IHF and FIS have also been shown to alter transposon transcription by altering the DNA binding of transcription regulators (Krause and Higgins, 1986; Kleckner, 1989).

A possible clue to the mechanism of FIS involvement in Tn7 transposition lies with the observation that miniTn7-*lac* is affected to a far greater extent by a *fis*⁻ mutation than intact Tn7. The combination of *fis* mutation and the impaired right end of miniTn7-*lac* may reveal a synthetic transposition phenotype that is hidden when intact Tn7 is assayed. In light of this possible synthetic interaction of *fis* mutation and an impaired Tn7 end, we noted in Chapter 2 that the crippling of miniTn7-*lac* may be suppressed by over-expression of the *tns* genes from a plasmid. If FIS acts to increase the binding of TnsB to the end of Tn7, then one might see a greater effect of FIS on an element that begins with reduced TnsB binding, as we see in the case of miniTn7-*lac* vs. intact Tn7. This would imply that overexpression of TnsB would supress the decreased transposition phenotype seen with miniTn7-*lac* in a wild-type stain background, however *fis*⁻ mutation would again reveal a deceased transposition phenotype.

As with DAM, understanding the molecular mechanisms of histone-like protein involvement in Tn7 transposition may come with study of TnsB binding to the ends of the transposon, and examination of transcription from the *tns* promoters (see Disscussion section of Chapter 4 for greater detail).

To extend the work of Chapter Four beyond genes known to affect other transposition and recombination systems, in Chapter Five miniTn7-*lac* was employed to search for new E. *coli* mutants which affect Tn7 transposition. MiniTn10-*tet* insertions in the E. *coli* chromosome were screened for altered miniTn7-*lac* papillation. Through a variety of secondary screens, twenty six chromosomal E. *coli* mutants which affect both miniTn7-*lac* and intact Tn7 transposition were isolated.

Sequence information from chromosomal DNA flanking the miniTn10-*tet* insertions was used to identify or localize the site of insertion. GenBank sequence matches were able to localize/identify the point of insertion for twenty one of the twenty five mutants which were sequenced. The ability to identify these insertion mutations attest to the value of large sequence data bases.

A variety of interesting mutants were identified in Chapter Five. *hns* was represented by two of the mutants isolated in the work described by Chapter Five. The involvement of *hns* in Tn7 transposition was examined in the previous Chapter of this thesis. *hns* is a histone-like protein with pleiotropic activities ranging from the control of genes responding to environmental osmotic conditions s (Higgins et al., 1988), to the control of a number of recombination systems (Falconi et al., 1991; Gama et al., 1992; Higgins et al., 1988; Lejeune and Danchin, 1990; Spears et al., 1986).

The conjunction of small histone-like proteins and regulatory molecules in the control of processes requiring the formation of complex, highly ordered DNA structures has been observed in a number of systems (Krause and Higgins, 1986; Thompson et al., 1987; Surette and Chaconas, 1989; Surette et al., 1989; Falconi et al., 1991; Gama et al., 1992; Ball and Johnson, 1991a; Falconi et al., 1991; Blomfield et al., 1993). H-NS, FIS, and IHF have been found to act together to facilitate and control DNA/protein complexes (Gama et al., 1992). The integration of a number of components in the construction of highly ordered DNA complexes could allow for combinatorial control in response to a wide variety of environmental signals.

One of the most intriguing specific environmental signals which regulate Tn7 transposition may be aerobic vs. anaerobic growth conditions. Five of the sixteen groups identified in Chapter Five were in or near genes which affect the choice between aerobic and anaerobic growth. The mechanism, and rational for growth condition control of Tn7 transposition was not explored in this thesis. The possibility that some of the *tns* promoters are part of the global aerobic/anaerobic regulons seems possible. Two of the

identified mutants (an insertion in *arc*B and one possibly in *sox*RS) are components of the global aerobic/anaerobic regulon control (Iuchi et al., 1990; Wu and Weiss, 1991).

There remain exciting possibilities for the elucidation of the roles in Tn7 transposition played by the E. *coli* components identified in this thesis. Investigation of the effects on the *tns* promoters of the identified mutants should be straight forward with the construction of *tns* promoter/reporter fusions, and the current development in Dr. Craig's lab of sensitive antibodies for the detection and measure of *tns* expression *in vivo*.

In vitro assays for the activities of the Tns proteins should help identify any direct roles played by the E. *coli* components identified in this thesis. In particular, the *in vitro* binding assay for TnsB binding to the ends of Tn7 may be used to examine possible roles of the histone-like proteins and DAM in the construction of synaptic transposition complexes and the control of the *tns*AB promoter.

A number of the mutants identified in Chapter Five of this thesis were involved in the production or regulatory sensing of various nutrients. Further use of miniTn7-*lac* for the investigation of Tn7 transposition under specific growth conditions or in the presence of growth media supplementation should prove interesting.

MiniTn7-*lac* has proven to be extremely useful for the identification of mutants affecting Tn7 transposition. MiniTn7-*lac* may be utilized to screen for other types of mutants affecting Tn7 transposition. MiniTn7-*lac* papillation has recently been used to identify *tns*C mutants able to carry out transposition in the absence of *tns*D or *tns*E (A. Stellwagen, personal communication). Chapter Two of this thesis detailed how miniTn7-*lac* has given us a sensitive assay for transposition orientation, and a way to screen for orientation affecting mutants. MiniTn7-*lac*, and the mutants it helps to identify should greatly advance our understanding of Tn7 transposition and transposition in general.

Materials and Methods

Media, Chemicals, and Enzymes:

LB broth and agar were prepared and used as described by (Miller, 1972) and (Foster et al., 1981). MacConkey Lactose agar was used as described by Miller (1972). Trimethoprim selection was on isosensitest agar (Oxoid). Bacteriological supplies were purchased from Difco. Antibiotic concentrations used were: carbenicillin (Cb), 100ug/ml; chloramphenicol (Cm), 30 ug/ml; gentamycin (Gen), 10ug/ml; kanamycin (Km), 100 ug/ml; nalidixic acid (Nal), 20 ug/ml; spectinomycin (Sp), 50 ug/ml; streptomycin (St), 50 ug/ml; tetracycline (Tc), 5 or 20 ug/ml; trimethoprim (Tp), 100 ug/ml. 2-aminopurine from Sigma was used at a concentration of 200ug/ml. DNA modifying enzymes were obtained from commercial sources and used as recommended by the manufacturer.

Bacterial Strains:

NLC28 is E. *coli* F$^-$ *ara*D139 Δ(*arg*F-*lac*)U169 *rps*L150 *rel*A1 *flb*B5301 *deo*C1 *pts*F25 *rbs*R (N. Craig, Johns Hopkins University). NLC51 is E. *coli* F$^-$ *ara*D139 Δ(*arg*F-*lac*) U169 *rps*L150 *rel*A1 *flb*B5301 *deo*C1 *pts*F25 *rbs*R *val*^{R} *rec*A56 (McKown et al., 1987). CW51 is E. *coli* F$^-$ *ara*$^-$ *arg*$^-$ *lac pro*XIII *nal*^{R} *rif*^{R} *rec*A56 (Waddell and Craig, 1988). RJ366 is E. *coli* F$^-$ *att*Tn7::Tn7 Δ(*lac-pro*) *ara*$^-$ *srl*$^-$ *str*^{R} *rec*A56 (R. Johnson, personal communication). RJ368 is E. *coli* F$^-$ *att*Tn7::Tn7 Δ(*lac-pro*) *ara*$^-$ *srl*$^-$ *str*^{R} *rec*A56 *fis*-767 (R. Johnson, personal communication). RZ201 is E. *coli* F$^-$ Δ(*lac-pro*) *ara*, *str* (CHS26 *str*^{R}) (R. Johnson, personal communication). *fis*767 was P1 transduced from RJ1529 which is RZ201 *fis*767. *fis*-985 was P1 transduced from RJ1806 which is MC1000 *fis*-985. MC1000 is E. *coli* F$^-$ *ara*D139, Δ*lac*74, Δ(*ara-leu*)1697, *gal*K2, *rps*L (R. Johnson, personal communication). *hip*-306 was P1 transduced from RW1892 which is E. *coli* F$^-$ *hip*-306, *hsd*R514 (r_K^-, m_K^+), *sup*E44, *sup*F58, Δ(*lac*IZY)6, *gal*K2, *gal*T22, *met*B1, *trp*R55, λ$^-$ (K. Kubo and N. Craig, personal communication). *him*A-825 was P1

transduced from RW1840 which is E. *coli* F⁻ *him*A-825, *hsd*R514 (r_K^-, m_K^+), *sup*E44, *sup*F58, Δ(*lac*IZY)6, *gal*K2, *gal*T22, *met*B1, *trp*R55, λ⁻ (K. Kubo and N. Craig, personal communication). NK7419 is E. *coli* F⁻ *trp*31 *his*1 *arg*G6 *ton*A2 Δ*lac*, *sup*E44, *xy*l7, *mt*l2, *met*B1, *tsx*, *leu*2, *pro*⁺, *str*-106 (Bolland and Kleckner, 1992). *dam*-13 was P1 transduced from GM2929 which is E. *coli* F⁻ *dam*-13::Tn9, *dcm*-6, *hsd*R2, McrA⁻, McrB⁻, *gal*K2, *gal*T22, *ara*-14, *lac*Y1, *xyl*-5, *thi*-1, *ton*A31, *rps*L136, *his*G4, *tsx*-78, *mtl*-1, *gln*V44, *leu*B6, *rfb*D1 (M.G. Marinus, personal communication). *hns*-205 was P1 transduced from GM230 *ara*D139 Δ(*arg*F- *lac*)U196 *rps*L150 *rel*A1 *deo*C1 *pts*F25 *rbs*R *flb*B5302 Φ(*pro*U-*lac*Z) hyb2 (λplac Mu15) *osm*Z205::Tn10 (*hns*-205) (Higgins et al., 1988). LE392 is E. *coli* F⁻ *hsd*R514 (r_K^-, m_K^+), *sup*E44, *sup*F58, Δ(*lac*IZY)6, *gal*K2, *gal*T22, *met*B1, *trp*R55, λ⁻ (Murray et al., 1977). ATH176 is E. *coli* F⁻ *thi*-1, *his*-4, *arg*E3, *rps*L31, *lac*BK1, φ80d*lac*Z4524::Tn7, *rec*D1903::mini*tet*, *att*Tn7::miniTn7-KmR199 (Hagemann and Craig, 1993). DH5α is E. *coli* F⁻ *end*A1, *hsd*R17, *sup*E44, *thi*-1, λ⁻, *rec*A1, *gyr*A96, *rel*A1, Δ(*arg*F-*lac*ZYA)U169, φ80d*lac*ZΔM15 (Hanahan, 1983). HB101 is E. *coli* F⁻ *hsd*S20 (r_K^-, m_K^-), *rec*A13, *ara*-14, *pro*A2, *lac*Y1, *gal*K2, *rps*L20 (Sm^r), *xyl*-5, *mtl*-1, *sup*E44, λ⁻ (Bolivar and Backman, 1979). JM109 is E. *coli* *rec*A1, *sup*E44, *end*A1, *hsd*R17, *thi*, *leu*, *rps*L, *lac*Y, *gal*K, *gal*T, *ara*, *ton*A, *thr*, *tsx*, Δ(*lac-pro*AB) F' [*tra*D36, *pro*AB⁺, *lac*ZΔM15] (Yanisch-Perron et al. 1985). MG1655 is E. *coli* F⁻ λ⁻ Prototroph (Singer et al., 1989).

All other strains in this work are derivatives of the above strains, as indicated in text and figures. P1 transduction and F' conjugation, for the construction of strain derivatives, were performed as described by Miller (1972).

Tn7 Derivatives:

The Tn7 derivative used in this thesis, Tn7S, contains an ISI element inserted near its antibiotic resistance determinants; the transposition properties of Tn7S are indistinguishable from those of the canonical Tn7 (Hauer and Shapiro, 1984). MiniTn7-

Cm is Tn7S::Tn9ΔPstI (Hauer and Shapiro, 1984) and contains approximately 1.9 kb of the left end of Tn7 and 537 bp of the right end of Tn7 flanking Tn9's chloramphenicol resistance determinant. MiniTn7-KmR199 (McKown et al., 1988) contains 166 bp of the left end of Tn7 and 199 bp of the right end of Tn7 flanking a segment encoding kanamycin resistance. MiniTn7-KmR70 contains 166 bp of the left end of Tn7 and 70 bp of the right end of Tn7 flanking a segment encoding kanamycin resistance (Arciszewska et al., 1989). MiniTn7-*lac* contains 166 bp of the left end of Tn7 and 70 bp of the right end of Tn7 flanking a segment encoding kanamycin resistance and promoterless *lac*ZYA genes situated such that *lac*Z is adjacent the left end of Tn7 (as described in Chapter 2 of this thesis).

Manipulation and Analysis of DNA:

Plasmid growth, isolation, transformation, and restriction enzyme analysis were performed as described by (Maniatis et al., 1982). Cloning procedures were as described by Maniatis *et al.* (1982) except that DNA fragments contained in slices excised from low-melting temperature agarose gels (Sea Plaque) were used directly in assembly of recombinant molecules as described by Struhl, 1983.

Plasmids:

tns plasmids:

pCW4 contains approximately 9 kb of Tn7 extending from the unique EcoRI site at position 9.0 through the right end of Tn7 and 165 bp of flanking *att*Tn7 sequence inserted into the EcoRI site of pACYC184 with attTn7 near vector Pcat (McKown et al., 1987). pCW4, pCW4::miniMu76 and pCW4::miniMu107 are Tc^R pACYC184 derivatives containing the *tns* genes (Waddell and Craig, 1988). pCW4 provides *tns*ABCDE, pCW4::miniMu76 provides functional *tns*ABC+*tns*D and pCW4::miniMu107 provides functional *tns*ABC + *tns*E (Waddell and Craig, 1988). pCW15: the PvuII-PvuII fragment

from pCW4 containing *tns*A, *tns*B and *tns*C was inserted into the Klenow treated SalI site of pACYC184 with *tns*C near vector P*tc* (Waddell and Craig, 1988).

F' plasmids:

pOX38*gen* (Johnson and Reznikoff, 1984) is a transfer proficient derivative of the conjugable plasmid F' and lacks gamma-delta or IS. pOX38*gen*-attTn7 (Arciszewska et al., 1989) is the same as pOX38*gen* except that it carries a transposition-defective, tetracycline resistant, derivative of Tnl0 that contains *att*Tn7 (LA223.1).

Construction of MiniTn7-*lac*:

pOHO and pOH1 were constructed as described in Chapter 2 of this thesis, with maps in Chapter 2, Figure 2. pRS415 (Simons et al., 1987), pRG0 (Gallager, 1989), pOHO (this work), pLA50 (Arciszewska et al., 1989) and pOH1 (this work) confer Cb^R. pRG0, pOHO, pLA50 and pOH1 also confer Km^R. To generate a Lac^- replicon containing miniTn7-*lac*, miniTn7-*lac*was transposed from pOH1 to an F' plasmid, producing pOX38*gen*::miniTn7-*lac*. After conjugation, Km^R F' plasmids were screened for Lac^- phenotype. To generate a Lac^- *att*Tn7::miniTn7-*lac*, pOX38*gen*::miniTn7-*lac* was introduced into NLC28 pCW4. After transposition of miniTn7-*lac* to *att*Tn7, *att*Tn7::miniTn7-*lac* was P1 transduced to NLC28*att*Tn7::miniTn7-Cm selecting for Km^R. P1 lysates of the Km^R/Cm^S transductants were used in subsequent strain construction for the transduction of *att*Tn7::miniTn7-*lac*.

Transposition Assays -

Papillation:

Both bacterial colonies, and patches, were examined for Lac$^+$ papillation. Colony papillation was examined after plating cells at a dilution in LB to achieve 100 colonies or less per plate on thickly poured MacConkey Lactose plates, supplemented with appropriate antibiotics. Papillation of patches was examined after spreading cells, from a colony grown the night before, on LB supplemented with the appropriate antibiotics. MacConkey Lactose plates were protected from light and desiccation for the extended incubations at 37°C or 30°C. Papillation was scored either graphically as the number of papillae per colony over time, reported photographically after an indicated incubation period, or reported qualitatively by *'s, with *** representing the degree of papillation in a wild type colony.

Mating Out:

Transposition frequencies were determined by a mating-out assay. In this assay, a Tn7 element transposes in a donor cell to a conjugable target plasmid and transposition is detected by identification of a Tn7 element-containing transconjugant (Waddell and Craig, 1988). For each strain tested, individual single colonies were inoculated into LB broth with the appropriate selective antibiotic. Cultures were grown with shaking aeration at 37°C overnight. The F' recipient was grown without antibiotics. F' donor cultures were diluted 1 to 50 into 3 ml of LB without antibiotics and grown with very gentle aeration at 37°C for 5 hours. F' recipient cultures were diluted 1 to 50 into 50 ml of LB without antibiotics and grown with vigorous shaking aeration at 37°C for 5 hours. F' donor cultures were removed from gentle aeration and incubated a further 5 min at 37°C for cells to aggregate and settle to the bottom of culture tube. 0.25 ml of LB + cells from the bottom of the F' donor culture (≈75% of the cells) were added to 0.75 ml of F' recipient culture. The recipient strain was always CW51 and donor strains were

derivatives of either NLC28 pOX38*gen* and NLC28 pOX38*gen*::*att*Tn7, or NLC51 pOX38*gen* and NLC51 pOX38*gen*::*att*Tn7. The mating mixture was incubated at 37°C with gentle aeration. After 60 min., the mixture was vortexed vigorously, placed on ice and aliquots plated. The total number of transconjugants was determined by selection of $Gen^R Nal^R$ colonies. The number of transconjugants that had acquired a Tn7 derivative was determined by selection of Tp^R/Nal^R, Cm^R/Nal^R or Km^R/Nal^R colonies for Tn7, miniTn7-Cm, or miniTn7-Km and miniTn7-*lac* transposition, respectively. The transposition frequency is expressed as the total number of Tn7 derivative-containing transconjugants divided by the total number of transconjugants.

Since the completion of these experiments, it has been found that this assay probably measures both translocation of the transposable element to the conjugal plasmid followed by conjugation of that plasmid to CW51 as intended, and a background frequency of F' donor strain mutation to nalidixic acid resistance (Bob DeBoy and Nancy Craig, personal communication). Thus, numbers reported as transposition frequencies as measured by the mating-out assay in this thesis may not reflect only transposition events. Spontaneous mutation to Nal^R occurs at a frequency of approximately 10^{-7} (Bob DeBoy and Nancy Craig, personal communication), thus significant in the measurement of miniTn7-*lac* transposition.

Southern Blot Hybridization Analysis:

Total chromosomal DNA (prepared as described by Ausubel *et al.* - Supplement 9, 1990) was digested with EcoRI and either HpaI or HindII, electrophoresed in 0.6% agarose, transferred to Nytran (0.45 micron, Schleicher and Schuell) in 10X SSC, and the DNA was covalently bound to the membrane by ultraviolet irradiation in a Stratalinker (from Stratagene). Filters were probed with the Tn7L oligonucleotide complementary to nucleotides L31-60 in the left end of Tn7. The Oligonucleotide was labeled at it's 5' end using T4 polynucleotide kinase and (γ-^{32}P)ATP (6000 Ci/mmol; ICN Radionucleotides).

A 4 hr prehybridization with 1.0 M NaCl, 1%SDS; was followed by hybridization at 55°C in 10% dextran sulfate, 1.0 M NaCl, 1.0% SDS, 0.01% salmon sperm DNA, 1 mM EDTA. Filters were washed at 65°C in 0.1xSSPE, 1% SDS over a period of two hours.

Mutagenesis With λ1098:

NLC28*att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* was infected with λ1098, in order to isolate E. *coli* mutants altered for Tn7 transposition. Phage lambda (λ1098) growth on LE392 was performed as described by Maniatis *et al.* (1982). λ1098 is cI857 P_{am} 80nin5, with a DNA segment carrying miniTn10-*tet* and transposase transcribed from a pTAC promoter cloned into λ between EcoRI sites #1 and #2 (Way et al., 1984). λ1098 infection of NLC28*att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* was performed as described by (Way et al., 1984) at an MOI of 0.1. λ1098 infected cells were plated directly on MacConkey Lactose Tetracycline, at a dilution titrated to achieve approximately 100 tetracycline resistant colonies per plate for optimal papillation.

Cloning Chromosomal MiniTn10-*tet*: Insertions:

Chromosomal DNA, from E. *coli* mutants generated by miniTn10-*tet* insertion, was prepared as describe by Ausubel *et al.* (1990 - Supplement 9). Chromosomal DNA and cloning vectors (pBluescript, pK184, and pK194) were digested with PstI or KpnI, as described by Maniatis *et al.* (1982). Chromosomal DNA/cloning vector ligations were performed as described by Maniatis *et al.* (1982), and transformed into highly competent DH5α (GIBCO - BRL). Colonies were selected on LB supplemented with Cb and Tc. Plasmids were isolated with STETL mini preps (from 5 ml cultures grown in LB + Cb and Tc) as describe by Ausubel *et al.* (1990 - Supplement 9). Isolated plasmids were checked for appropriate PstI or KpnI insertions.

Sequencing Cloned Chromosomal MiniTn10-*tet*: Insertions:

Sequence determination was performed by the method of (Sanger and Coulson, 1975). In order to sequence from either end of the transposable element into flanking chromosomal DNA, oligo-nucleotide primers unique to *tet*R (OH3) and *tet*A (OH2) were employed. To sequence in from both of the vector / chromosomal insert junctions, either the T7 and T3 primers for pBluescript clones, or the F(orward) and R(everse) primers for pK184 and pK194 clones were employed. T3 (cat # 300301), T7 (cat # 300302), Forward (cat # 300303) and Reverse (cat # 300304) primers for the sequencing reactions were obtained commercially from Stratagene. OH2 and OH3 primers were synthesized by the UCSF Biomolecular Resource Center. OH2 is 5'-GGTCACCAACGCTTTTCCCG-3', complementary to nucleotides #3382-3401 of GenBank "trn10tetr". OH3 is 5'-CCATTTTCAGTGATCCATTGC-3', complementary to nucleotides #639-659 of GenBank "trn10tetr".

P1 Linkage Analysis:

P1 linkage analysis was perfomed as described in Chapter 5 - Appendix, Table 5 Legend, with Km^R markers transduced from the following Km^R strains kindly provided by Carol Gross (Singer et al., 1989): 27.25 min. is CG18551 (zch-3117/Tn10Km), 25.27 min. is CG18544 (zcf-1314/Tn10Km), 54 min. is CG18631 (zff-3139/Tn10Km), 70 min. is CG18605 (zha-3168/Tn10Km), 84.5 min. is CG18599 (ilv-3164/Tn10Km), 74 min. is CG18556 (zhe-3172/Tn10Km), 20 min. is CG18528 (zbj-3110/Tn10Km), 92.5 min. is CG18630 (zjc-3181/Tn10Km), 10.5 min. is CG12107 (zba-3101/Tn10Km), 48.5 min. is CG12183 (zei-3143/Tn10Km), 49.5 min. is CG18552 (zej-3144/Tn10Km). All CG strains were Tn10Km insertion derivatives of MG1655.

Appendix B

The following section reports the OH# and derivation of strains referred to in this thesis.

Appendix B: E. coli Strains Used in Thesis

Chapter 1:

Introduction:.....Page.1

No Strains.

Chapter 2:

A Visual Assay for Tn7 Transposition.....Page.36

(For references to strains see Materials and Methods)

Construction of miniTn7-*lac*:

OH141 DH5 pRG0

OH142 DH5 pRS415

OH171 DH5 pOH0

OH168 DH5 pLA50

OH173 DH5 pOH1

OH161 JM109 pOH2

Table 2-1:

OH158 NLC51 *att*Tn7::Tn7 pOH1 pOX38*gen*

NLC51 *att*Tn7::Tn7 (OH170) transformed to Km^R with pOH1 from OH173
NLC51 *att*Tn7::Tn7 pOH1 mated with CW51 pOX38*gen* (OH148)
NLC51 *att*Tn7::Tn7 pOH1 pOX38*gen* transconjugate selected with Km + Gn

OH373 NLC51 *att*Tn7::Tn7 pOH0 pOX38*gen*

NLC51 *att*Tn7::Tn7 (OH170) transformed to Km^R with pOH0 from OH171
NLC51 *att*Tn7::Tn7 pOH0 mated with CW51 pOX38*gen* (OH148)
NLC51 *att*Tn7::Tn7 pOH0 pOX38*gen* transconjugate selected with Km + Gn

OH156 NLC51 *att*Tn7::Tn7 pOX38*gen*

NLC51 *att*Tn7::Tn7 (OH170) mated with CW51 pOX38*gen* (OH148)
NLC51 *att*Tn7::Tn7 pOX38*gen* transconjugate selected with Km + Gn

Table 2-2:

OH374 NLC28 *att*Tn7::miniTn7-Cm ϕ80d*lac*::Tn7 pOX38*gen*

NLC28 *att*Tn7::miniTn7-Cm transduced to Tp^R with ϕ80d*lac*::Tn7 from ATH176
NLC28 *att*Tn7::miniTn7-Cm ϕ80d*lac*::Tn7 (OH265) mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-Cm ϕ80d*lac*::Tn7 pOX38*gen* transconjugate selected with Tp + Gn

OH869 NLC28 *att*Tn7::miniTn7-KmR70 ϕ80d*lac*::Tn7 pOX38*gen*

NLC28 *att*Tn7::miniTn7-KmR70 transduced to Tp^R with ϕ80d*lac*::Tn7 from ATH176
NLC28 *att*Tn7::miniTn7-KmR70 ϕ80d*lac*::Tn7 (OH279) mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-KmR70 ϕ80d*lac*::Tn7 pOX38*gen* transconjugate selected with Tp + Gn

OH963 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transduced to Tp^R with ϕ80d*lac*::Tn7 from ATH176
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 (OH288) mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen* transconjugate selected with Tp + Gn

Table 2-3:

OH866 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} pOX38*gen*

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transformed to Tc^R with pCW4::miniMuΩ^{76E}
NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} pOX38*gen* transconjugate selected with Km + Gn

OH863 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} pOX38*gen-att*Tn7

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transformed to Tc^R with pCW4::miniMuΩ^{76E}
NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} mated with CW51 pOX38*gen-att* (LA 223.1)
NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} pOX38*gen-att* transconjucate selected with Km + Gn

OH865 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} pOX38*gen*

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transformed to Tc^R with pCW4::miniMuΩ^{107D}
NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} pOX38*gen* transconjugate selected with Km + Gn

OH864 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} pOX38*gen-att*Tn7

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transformed to Tc^R with pCW4::miniMuΩ^{107D}
NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} mated with CW51 pOX38*gen-att* (LA 223.1)
NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} pOX38*gen-att* transconjucate selected with Km + Gn

OH867 NLC28 *att*Tn7::miniTn7-*lac* pCW15 pOX38*gen*

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transformed to Cm^R with pCW15
NLC28 *att*Tn7::miniTn7-*lac* pCW15 mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* pCW15 pOX38*gen* transconjucate selected with Km + Gn

OH862 NLC28 *att*Tn7::miniTn7-*lac* pCW15 pOX38*gen-att*Tn7

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transformed to Cm^R with pCW15
NLC28 *att*Tn7::miniTn7-*lac* pCW15 mated with CW51 pOX38*gen-att* (LA 223.1)
NLC28 *att*Tn7::miniTn7-*lac* pCW15 pOX38*gen-att* transconjucate selected with Km + Gn

OH860 NLC28 *att*Tn7::miniTn7-*lac* pOX38*gen*

NLC28 *att*Tn7::miniTn7-*lac* (OH288) mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* pOX38*gen* transconjucate selected with Km + Gn

OH861 NLC28 *att*Tn7::miniTn7-*lac* pOX38*gen-att*Tn7

NLC28 *att*Tn7::miniTn7-*lac* (OH288) mated with CW51 pOX38*gen-att* (LA 223.1)
NLC28 *att*Tn7::miniTn7-*lac* pOX38*gen-att* transconjucate selected with Km + Gn

Figures 2-3 and 2-4:

OH857 NLC51 *att*Tn7::miniTn7-KmR199 pCW4 pOX38*gen*::miniTn7-*lac*

NLC51 *att*Tn7::miniTn7-KmR199 transformed to Tc^R with pCW4
NLC51 *att*Tn7::miniTn7-KmR199 pCW4 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC51 *att*Tn7::miniTn7-KmR199 pCW4 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH858 NLC51 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac*

NLC51 *att*Tn7::miniTn7-KmR199 transformed to Tc^R with pCW4::miniMuΩ^{107D}
NLC51 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{107D} mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC51 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH859 NLC51 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac*

NLC51 *att*Tn7::miniTn7-KmR199 transformed to Tc^R with pCW4::miniMuΩ^{76E}
NLC51 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{76E} mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC51 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH856 NLC51 *att*Tn7::miniTn7-KmR199 pCW15 pOX38*gen*::miniTn7-*lac*

NLC51 *att*Tn7::miniTn7-KmR199 transformed to Cm^R with pCW15
NLC51 *att*Tn7::miniTn7-KmR199 pCW15 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC51 *att*Tn7::miniTn7-KmR199 pCW15 pOX38*gen*::miniTn7-*lac* transconjugates selected with Cm + Gn

OH855 NLC51 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac*

NLC51 *att*Tn7::miniTn7-KmR199 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC51 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* transconjugates screened for Km^R, Rif^S

Figures 2-5 and 2-6:

OH189 NLC28 *att*Tn7::miniTn7-KmR199
pCW4 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transformed to Tc^R with pCW4
NLC28 *att*Tn7::miniTn7-KmR199 pCW4 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pCW4 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH190 NLC28 *att*Tn7::miniTn7-KmR199
pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 transformed to Tc^R with pCW4::miniMuΩ^{107D}
NLC28 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{107D} mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH191 NLC28 *att*Tn7::miniTn7-KmR199
pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transformed to Tc^R with pCW4::miniMuΩ^{76E}
NLC28 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{76E} mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH853 NLC28 *att*Tn7::miniTn7-KmR199
pCW15 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transformed to Cm^R with pCW15
NLC28 *att*Tn7::miniTn7-KmR199 pCW15 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pCW15 pOX38*gen*::miniTn7-*lac* transconjugates selected with Cm + Gn

OH851 NLC28 *att*Tn7::miniTn7-KmR199
pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* transconjugates screened for Km^R, Rif^S

Figures 2-7 and 2-8:

OH976 NLC28 *att*Tn7::miniTn7-*lac* pCW4
NLC28 *att*Tn7::miniTn7-*lac* transfomed to Tc^R with pCW4

OH977 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E}
NLC28 *att*Tn7::miniTn7-*lac* (OH288) transfomed to Tc^R with pCW4::miniMuΩ^{76E}

OH978 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D}
NLC28 *att*Tn7::miniTn7-*lac* (OH288) transfomed to Tc^R with pCW4::miniMuΩ^{107D}

OH979 NLC28 *att*Tn7::miniTn7-*lac* pCW15
NLC28 *att*Tn7::miniTn7-*lac* (OH288) transfomed to Cm^R with pCW15

OH288 NLC28 *att*Tn7::miniTn7-*lac*

Table 2-4:

OH851 NLC28 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac*
NLC28 *att*Tn7::miniTn7-KmR199 (OH13) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* transconjugates screened for Km^R, Rif^S

OH189 NLC28 *att*Tn7::miniTn7-KmR199 pCW4 pOX38*gen*::miniTn7-*lac*
NLC28 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* (OH851) transformed to Tc^R with pCW4

OH853 NLC28 *att*Tn7::miniTn7-KmR199 pCW15 pOX38*gen*::miniTn7-*lac*
NLC28 *att*Tn7::miniTn7-KmR199 pOX38*gen*::miniTn7-*lac* (OH851) transformed to Cm^R with pCW15

Chapter 3:

Figure 3-3:

OH232 NLC28

OH844 NLC28 pOX38*gen*::miniTn7-*lac*
NLC28 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 pOX38*gen*::miniTn7-*lac* transconjugates screened for Gn^R, Rif^S

OH79 NLC28 pCW4 pOX38*gen*::miniTn7-*lac*
NLC28 transformed to Tc^R with pCW4
NLC28 pCW4 (OH7) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 pCW4 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH124-127 NLC28 *att*Tn7::miniTn7-*lac* (Lac^-) pOX38*gen*::miniTn7-*lac*
(Lac^- cells from Slow Papillation colony type)

OH120-123 NLC28 *att*Tn7::miniTn7-*lac* (Lac^+) pOX38*gen*::miniTn7-*lac*
(Lac^+ cells from Fast Papillation colony type)

Figure 3-4:

OH880 NLC28 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac*
NLC28 transformed to Tc^R with pCW4::miniMuΩ^{76E}
NLC28 pCW4::miniMuΩ^{76E} (OH9) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

Data Not Shown (p87):

OH83 NLC28 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac*
NLC28 transformed to Tc^R with pCW4::miniMuΩ^{76E}
NLC28 pCW4::miniMuΩ^{76E} (OH9) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH81 NLC28 pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac*

NLC28 transformed to Tc^{R} with pCW4::miniMuΩ^{107D}
NLC28 pCW4::miniMuΩ^{107D} mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH846 NLC28 pCW15 pOX38*gen*::miniTn7-*lac*

NLC28 transformed to Cm^{R} with pCW15
NLC28 pCW15 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 pCW15 pOX38*gen*::miniTn7-*lac* transconjugates selected with Cm + Gn

OH844 NLC28 pOX38*gen*::miniTn7-*lac*

Chapter 4:

Effects of DAM, FIS, IHF and H-NS Mutations on Tn7.....121

Figures 4-1 and 4-2:

OH977 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E}

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transformed to Tc^{R} with pCW4::miniMuΩ^{76E}

OH969 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} *dam*-13

NLC28 *att*Tn7::miniTn7-*lac* (OH288) *dam*-13 transduced to Cm^{R} with *dam*-13 from GM2929 (OH 298)
NLC28 *att*Tn7::miniTn7-*lac* *dam*-13 (OH309) transformed to Tc^{R} with pCW4::miniMuΩ^{76E}

OH978 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D}

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transformed to Tc^{R} with pCW4::miniMuΩ^{107D}

OH970 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} *dam*-13

NLC28 *att*Tn7::miniTn7-*lac* *dam*-13 (OH309) transformed to Tc^{R} with pCW4::miniMuΩ^{107D}

Table 4-1:

OH349 NLC28 *att*Tn7::Tn7 pOX38*gen*

NLC28 *att*Tn7::Tn7 mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::Tn7 pOX38*gen* transconjugate selected with Tp + Gn

OH365 NLC28 *att*Tn7::Tn7 pOX38*gen* *dam*-13

NLC28 *att*Tn7::Tn7 transduced to Cm^R with *dam*-13 from GM2929 (OH 298)
NLC28 *att*Tn7::Tn7 *dam*-13 (OH326) mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::Tn7 pOX38*gen* *dam*-13 transconjugate selected with Tp + Gn

OH348 RZ201 *att*Tn7::Tn7 pOX38*gen*

RZ201 transduced to Tp^R with *att*Tn7::Tn7 from Anne Stellwagen's MH1 lysate
RZ201 *att*Tn7::Tn7 (OH334) was mated with CW51 pOX38*gen* (OH148)
RZ201 *att*Tn7::Tn7 pOX38*gen* transconjugate selected with Tp + Gn

OH360 RZ201 *att*Tn7::Tn7 pOX38*gen* *dam*-13

RZ201 *att*Tn7::Tn7 transduced to Cm^R with *dam*-13 from GM2929 (OH 298)
RZ201 *att*Tn7::Tn7 *dam*-13 (OH350) transformed to Tc^R with pCW4::miniMuΩ^{107D}

OH346 NK7419 *att*Tn7::Tn7 pOX38*gen*

NK7419 transduced to Tp^R with *att*Tn7::Tn7 from AS's MH1 *att*Tn7::Tn7 lysate
NK7419 *att*Tn7::Tn7 (OH312) was mated with CW51 pOX38*gen* (OH148)
NK7419 *att*Tn7::Tn7 pOX38*gen* transconjugate selected with Tp + Gn

OH371 NK7419 *att*Tn7::Tn7 pOX38*gen* *dam*-13

NK7419 *att*Tn7::Tn7 (OH312) transduced to Cm^R with *dam*-13 from GM2929 (OH 298)
NK7419 *att*Tn7::Tn7 *dam*-13 (OH343) mated with CW51 pOX38*gen* (OH148)
NK7419 *att*Tn7::Tn7 pOX38*gen* *dam*-13 transconjugates selected with Tp + Gn

Table 4-2:

OH963 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

NLC28 *att*Tn7::miniTn7-*lac* transduced to Tp^R with ϕ80d*lac*::Tn7 from ATH176
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 (OH265) mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen* transconjugate selected with Tp + Gn

OH974 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen dam*-13

NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 (OH265) transduced to Cm^R with *dam*-13 from GM2929 (OH 298)
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 *dam*-13 mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen dam*-13 transconjugate selected with Tp + Gn

OH958 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen-att*Tn7

NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 (OH265) mated with CW51 pOX38*gen-att* (LA 223.1)
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen-att* transconjugate selected with Tp + Gn

OH959 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen-att*Tn7 *dam*-13

NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 *dam*-13 mated with CW51 pOX38*gen-att* (LA 223.1)
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 CW51 pOX38*gen-att* *dam*-13 transconjugate selected with Tp + Gn

Figures 4-3 and 4-4:

OH977 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E}

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transformed to Tc^R with pCW4::miniMuΩ^{76E}

OH843 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} *fis*-985

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transduced to Sp^R with *fis*-985 from RJ1806
NLC28 *att*Tn7::miniTn7-*lac fis*-985 transformed to Tc^R with pCW4::miniMuΩ^{76E}

OH978 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D}

NLC28 *att*Tn7::miniTn7-*lac* transformed to Tc^R with pCW4::miniMuΩ^{107D}

OH841 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} *fis*-985

NLC28 *att*Tn7::miniTn7-*lac* transduced to Sp^R with *fis*-985 from RJ1806
NLC28 *att*Tn7::miniTn7-*lac fis*-985 transformed to Tc^R with pCW4::miniMuΩ^{107D}

Figure 4-5:

OH198 NLC28 *att*Tn7::miniTn7-Cm pCW4 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-Cm (OH12) transfomed to Tc^R with pCW4
NLC28 *att*Tn7::miniTn7-Cm pCW4 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-Cm pCW4 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH201 NLC28 *att*Tn7::miniTn7-Cm *fis*-767 pCW4 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-Cm (OH12) transduced to Km^R with *fis*-767 from RJ368 (OH236)
NLC28 *att*Tn7::miniTn7-Cm *fis*-767 (OH18) transfomed to Tc^R with pCW4
NLC28 *att*Tn7::miniTn7-Cm pCW4 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-Cm pCW4 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH199 NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-Cm (OH12) transfomed to Tc^R with pCW4::miniMuΩ^{107D}
NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{107D} mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH202 NLC28 *att*Tn7::miniTn7-Cm *fis*-767 pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-Cm (OH12) transduced to Km^R with *fis*-767 from RJ368 (OH236)
NLC28 *att*Tn7::miniTn7-Cm *fis*-767 (OH18) transfomed to Tc^R with pCW4::miniMuΩ^{107D}
NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{107D} mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH207 NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-Cm (OH12) transfomed to Tc^R with pCW4::miniMuΩ^{76E}
NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{76E} mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH203 NLC28 *att*Tn7::miniTn7-Cm *fis*-767 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-Cm (OH12) transduced to Km^R with *fis*-767 from RJ368 (OH236)
NLC28 *att*Tn7::miniTn7-Cm *fis*-767 (OH18) transfomed to Tc^R with pCW4::miniMuΩ^{76E}
NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{76E} mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-Cm pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH118 NLC28 *att*Tn7::miniTn7-Cm pACYC184 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-Cm (OH12) transfomed to Tc^R with pACYC184
NLC28 *att*Tn7::miniTn7-Cm pACYC184 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-Cm pACYC184 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH119 NLC28 *att*Tn7::miniTn7-Cm *fis*-767 pACYC184 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-Cm (OH12) transduced to Km^R with *fis*-767 from RJ368 (OH236)
NLC28 *att*Tn7::miniTn7-Cm *fis*-767 (OH18) transfomed to Tc^R with pACYC184
NLC28 *att*Tn7::miniTn7-Cm *fis*-767 pACYC184 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-Cm *fis*-767 pACYC184 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

Table 4-3:

OH963 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

NLC28 *att*Tn7::miniTn7-*lac* transduced to Tp^R with ϕ80d*lac*::Tn7 from ATH176
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen* transconjugate selected with Tp + Gn

OH980 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen fis*-985

NLC28 *att*Tn7::miniTn7-*lac* transduced to Sp^R with *fis*-985 from RJ1806 (OH840)
NLC28 *att*Tn7::miniTn7-*lac fis*-985 transduced to Tp^R with ϕ80d*lac*::Tn7 from ATH176
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 *fis*-985 mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen fis*-985 transconjugate selected with Tp + Gn

OH958 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen-att*Tn7

NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 (OH265) mated with CW51 pOX38*gen-att* (LA 223.1)

NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen-att* transconjugate selected with Tp + Gn

OH981 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen-att* *fis*-985

NLC28 *att*Tn7::miniTn7-*lac* transduced to Sp^R with *fis*-985 from RJ1806 (OH840)

NLC28 *att*Tn7::miniTn7-*lac fis*-985 transduced to Tp^R with ϕ80d*lac*::Tn7 from ATH176

NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 *fis*-985 mated with CW51 pOX38*gen-att* (LA 223.1)

NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 CW51 pOX38*gen-att* *fis*-985 transconjugate selected with Tp + Gn

Table 4-4:

OH103 NLC28 *att*Tn7::Tn7 pOX38*gen*

NLC28 *att*Tn7::Tn7 (OH14) mated with CW51 pOX38*gen* (OH148)

NLC28 *att*Tn7::Tn7 pOX38*gen* transconjugate selected with Tp + Gn

OH104 NLC28 *att*Tn7::Tn7 pOX38*gen* *fis*-767

NLC28 *att*Tn7::Tn7 (OH14) transduced to Km^R with *fis*-767 from RJ368 (OH236)

NLC28 *att*Tn7::Tn7 *fis*-767 (OH52) mated with CW51 pOX38*gen* (OH148)

NLC28 *att*Tn7::Tn7 pOX38*gen* *fis*-767 transconjugates selected with Tp + Gn

OH134 RJ366 *att*Tn7::Tn7 pOX38*gen*

RJ366 *att*Tn7::Tn7 (OH241) mated with CW51 pOX38*gen* (OH148)

RJ366 *att*Tn7::Tn7 pOX38*gen* transconjugates selected with Tp + Gn

OH135 RJ366 *att*Tn7::Tn7 pOX38*gen* *fis*-767 (RJ368)

RJ368 *att*Tn7::Tn7 (OH236) mated with CW51 pOX38*gen* (OH148)

RJ368 *att*Tn7::Tn7 pOX38*gen* transconjugates selected with Tp + Gn

OH105 NLC28 *att*Tn7::Tn7 pOX38*gen-att*Tn7

NLC28 *att*Tn7::Tn7 (OH14) mated with CW51 pOX38*gen-att* (LA223.1)

NLC28 *att*Tn7::Tn7 pOX38*gen-att* transconjugate selected with Tp + Gn

OH106 NLC28 *att*Tn7::Tn7 pOX38*gen-att*Tn7 *fis*-767

NLC28 *att*Tn7::Tn7 *fis*-767 (OH52) mated with CW51 pOX38*gen-att* (LA223.1)

NLC28 *att*Tn7::Tn7 pOX38*gen-att* *fis*-767 transconjugates selected with Tp + Gn

Figures 4-6 and 4-7:

OH977 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E}

OH966 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} *hns*-205
NLC28 *att*Tn7::miniTn7-*lac* (OH288) transduced to Tc^R with *hns*-205 from GM230 (OH324)
NLC28 *att*Tn7::miniTn7-*lac* *hns*-205 transformed to Sp^R with pCW4::miniMuΩ^{76E}

OH978 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D}

OH967 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} *hns*-205
NLC28 *att*Tn7::miniTn7-*lac* (OH288) transduced to Tc^R with *hns*-205 from GM230 (OH324)
NLC28 *att*Tn7::miniTn7-*lac* *hns*-205 transformed to Sp^R with pCW4::miniMuΩ^{107D}

Table 4-5:

OH348 RZ201 *att*Tn7::Tn7 pOX38*gen*

OH361 RZ201 *att*Tn7::Tn7 pOX38*gen* *hns*-205
RZ201 *att*Tn7::Tn7 (OH334) transduced to Tc^R with *hns*-205 from GM230 (OH324)
RZ201 *att*Tn7::Tn7 *hns*-205 (OH339) mated with CW51 pOX38*gen* (OH148)
RZ201 *att*Tn7::Tn7 pOX38*gen* *hns*-205 transconjugates selected with Tp + Gn

OH963 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH391 NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen* *hns*-43*
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 transduced to Tc^R with *hns*-43* from OH543
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 *hns*-43* mated with CW51 pOX38*gen* (OH148)
NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen* *hns*-43* transconjugate selected with Tp + Gn

Figures 4-8 and 4-9:

OH977 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E}

OH964 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} *hip*-306

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transduced to Cm^R with *hip*-306 from RW1892 (OH112)
NLC28 *att*Tn7::miniTn7-*lac* *hip*-306 transfomed to Tc^R with pCW4::miniMuΩ^{76E}

OH978 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D}

OH965 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} *hip*-306

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transduced to Cm^R with *hip*-306 from RW1892 (OH112)
NLC28 *att*Tn7::miniTn7-*lac* *hip*-306 transfomed to Tc^R with pCW4::miniMuΩ^{107D}

Figures 4-10 and 4-11:

OH977 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E}

OH848 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{76E} *him*A-825

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transduced to Tc^R with *him*A-825 from RW1840 (OH111)
NLC28 *att*Tn7::miniTn7-*lac* *hip*-306 transfomed to Sp^R with pCW4::miniMuΩ^{76E}

OH978 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D}

OH847 NLC28 *att*Tn7::miniTn7-*lac* pCW4::miniMuΩ^{107D} *him*A-825

NLC28 *att*Tn7::miniTn7-*lac* (OH288) transduced to Tc^R with *him*A-825 from RW1840 (OH111)
NLC28 *att*Tn7::miniTn7-*lac* *hip*-306 transfomed to Sp^R with pCW4::miniMuΩ^{107D}

Figure 4-12:

OH189 NLC28 *att*Tn7::miniTn7-KmR199
pCW4 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transformed to Tc^R with pCW4
NLC28 *att*Tn7::miniTn7-KmR199 pCW4 (OH186) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pCW4 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH100 NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306
pCW4 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transduced to Cm^R with *hip*-306 from RW1892 (OH112)
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 (OH46) transformed to Tc^R with pCW4
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pCW4 (OH184) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pCW4 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH190 NLC28 *att*Tn7::miniTn7-KmR199
pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transformed to Tc^R with pCW4::miniMuΩ^{107D}
NLC28 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{107D} (OH187) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH101 NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306
pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transduced to Cm^R with *hip*-306 from RW1892 (OH112)
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 (OH46) transformed to Tc^R with pCW4::miniMuΩ^{107D}
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pCW4::miniMuΩ^{107D} (OH101) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pCW4::miniMuΩ^{107D} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH191 NLC28 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transformed to Tc^R with pCW4::miniMuΩ^{107D}
NLC28 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{76E} (OH188) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH102 NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transduced to Cm^R with *hip*-306 from RW1892 (OH112)
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 (OH46) transformed to Tc^R with pCW4::miniMuΩ^{76E}
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pCW4::miniMuΩ^{76E} (OH185) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pCW4::miniMuΩ^{76E} pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH116 NLC28 *att*Tn7::miniTn7-KmR199 pACYC184 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transformed to Tc^R with pACYC184
NLC28 *att*Tn7::miniTn7-KmR199 pACYC184 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 pACYC184 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

OH117 NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pACYC184 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::miniTn7-KmR199 (OH13) transduced to Cm^R with *hip*-306 from RW1892 (OH112)
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 (OH57) transformed to Tc^R with pACYC184
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pACYC184 mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::miniTn7-KmR199 *hip*-306 pACYC184 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tc + Gn

Chapter 5:

New Host Mutants.....176

Mutagenisis:

OH378 NLC28 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

NLC51 *att*Tn7::Tn7 (OH170) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC51 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* (OH215) transconjugate selected with Tp + Gn
NLC51 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* mated to CW51 (OH146)
CW51 pOX38*gen*::miniTn7-*lac*, (::Tn7) (OW234) transconjugates selected with (Tp) + Km + Gn
(Later found that Tp selection did not work and strain is CW51 pOX38*gen*::miniTn7-*lac* (No Tn7)
NLC28 *att*Tn7::Tn7 was mated with CW51 pOX38*gen*::miniTn7-*lac* (OH234)
NLC28 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* transcongugate screened for Gn^R, Rif^S

OH218 LE392

Mutants:

OH501-695 NLC28::miniTn10-*tet*$^{\#1\text{-}195}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

NLC28 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* (OH378) infected with λ1098
NLC28::miniTn10-*tet*$^{\#1\text{-}195}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* selected on MacConkey Lactose + Tc

Mating-Out Test on Mutants:

NLC28::miniTn10-*tet*$^{\#x}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

NLC28 *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 (OH265) transduced to Tc^R from OH501-695
NLC28::miniTn10-*tet*$^{\#x}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 mated with CW51 pOX38*gen* (OH148)
NLC28::miniTn10-*tet*$^{\#x}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen* transconjugates selected with Tp + Gn

OH380 NLC28::miniTn10-*tet*$^{\#3}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH881 NLC28::miniTn10-*tet*$^{\#4}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH882 NLC28::miniTn10-*tet*$^{\#5}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH883 NLC28::miniTn10-*tet*$^{\#6}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH381 NLC28::miniTn10-*tet*$^{\#15}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH382	NLC28::miniTn10-*tet*$^{\#17}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH884	NLC28::miniTn10-*tet*$^{\#19}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH383	NLC28::miniTn10-*tet*$^{\#20}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH384	NLC28::miniTn10-*tet*$^{\#22}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH385	NLC28::miniTn10-*tet*$^{\#25}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH386	NLC28::miniTn10-*tet*$^{\#28}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH387	NLC28::miniTn10-*tet*$^{\#33}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH388	NLC28::miniTn10-*tet*$^{\#35}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH389	NLC28::miniTn10-*tet*$^{\#36}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH390	NLC28::miniTn10-*tet*$^{\#38}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH391	NLC28::miniTn10-*tet*$^{\#43}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH392	NLC28::miniTn10-*tet*$^{\#48}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH393	NLC28::miniTn10-*tet*$^{\#49}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH394	NLC28::miniTn10-*tet*$^{\#50}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH395	NLC28::miniTn10-*tet*$^{\#51}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH396	NLC28::miniTn10-*tet*$^{\#53}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH397	NLC28::miniTn10-*tet*$^{\#57}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH399	NLC28::miniTn10-*tet*$^{\#62}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH400	NLC28::miniTn10-*tet*$^{\#68}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH401	NLC28::miniTn10-*tet*$^{\#71}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH402	NLC28::miniTn10-*tet*$^{\#72}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*
OH403	NLC28::miniTn10-*tet*$^{\#73}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH404 NLC28::miniTn10-*tet*$^{\#74}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH405 NLC28::miniTn10-*tet*$^{\#75}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH406 NLC28::miniTn10-*tet*$^{\#77}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH407 NLC28::miniTn10-*tet*$^{\#78}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH408 NLC28::miniTn10-*tet*$^{\#79}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH409 NLC28::miniTn10-*tet*$^{\#80}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH410 NLC28::miniTn10-*tet*$^{\#97}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH411 NLC28::miniTn10-*tet*$^{\#100}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH412 NLC28::miniTn10-*tet*$^{\#102}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH413 NLC28::miniTn10-*tet*$^{\#106}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH414 NLC28::miniTn10-*tet*$^{\#111}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH415 NLC28::miniTn10-*tet*$^{\#118}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH416 NLC28::miniTn10-*tet*$^{\#123}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH417 NLC28::miniTn10-*tet*$^{\#132}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH418 NLC28::miniTn10-*tet*$^{\#134}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH419 NLC28::miniTn10-*tet*$^{\#140}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH421 NLC28::miniTn10-*tet*$^{\#143}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH422 NLC28::miniTn10-*tet*$^{\#144}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH423 NLC28::miniTn10-*tet*$^{\#145}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH424 NLC28::miniTn10-*tet*$^{\#153}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH425 NLC28::miniTn10-*tet*$^{\#156}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH426 NLC28::miniTn10-*tet*$^{\#161}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH427 NLC28::miniTn10-*tet*$^{\#171}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH428 NLC28::miniTn10-*tet*$^{\#172}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH429 NLC28::miniTn10-*tet*$^{\#174}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH430 NLC28::miniTn10-*tet*$^{\#181}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH431 NLC28::miniTn10-*tet*$^{\#182}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH432 NLC28::miniTn10-*tet*$^{\#184}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH433 NLC28::miniTn10-*tet*$^{\#187}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH434 NLC28::miniTn10-*tet*$^{\#190}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

OH435 NLC28::miniTn10-*tet*$^{\#194}$ *att*Tn7::miniTn7-*lac* ϕ80d*lac*::Tn7 pOX38*gen*

Figure 5-4, MiniTn7-*lac* papillation in transduced mutant background:

OH3000 NLC28 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* (wt)
NLC28 *att*Tn7::Tn7 (OH14) mated with CW51 pOX38*gen*::miniTn7-*lac* (OH149)
NLC28 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* transconjugates selected with Tp + Gn

NLC28::miniTn10-*tet*$^{\#x}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
NLC28 *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac* (OH3000) transduced to Tc^R with miniTn10-*tet*$^{\#x}$ from OH501-695

OH3001 NLC28::miniTn10-*tet*$^{\#3}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

OH3002 NLC28::miniTn10-*tet*$^{\#4}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

OH3003 NLC28::miniTn10-*tet*$^{\#6}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

OH3004 NLC28::miniTn10-*tet*$^{\#17}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

OH3005 NLC28::miniTn10-*tet*$^{\#20}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

OH3006 NLC28::miniTn10-*tet*$^{\#43}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

OH3007 NLC28::miniTn10-*tet*$^{\#49}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

OH3008	NLC28::miniTn10-*tet*$^{\#50}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3009	NLC28::miniTn10-*tet*$^{\#53}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3010	NLC28::miniTn10-*tet*$^{\#57}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3011	NLC28::miniTn10-*tet*$^{\#58}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3012	NLC28::miniTn10-*tet*$^{\#61}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3013	NLC28::miniTn10-*tet*$^{\#62}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3014	NLC28::miniTn10-*tet*$^{\#73}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3015	NLC28::miniTn10-*tet*$^{\#75}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3016	NLC28::miniTn10-*tet*$^{\#79}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3017	NLC28::miniTn10-*tet*$^{\#106}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3018	NLC28::miniTn10-*tet*$^{\#111}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3019	NLC28::miniTn10-*tet*$^{\#118}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3020	NLC28::miniTn10-*tet*$^{\#123}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3021	NLC28::miniTn10-*tet*$^{\#132}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3022	NLC28::miniTn10-*tet*$^{\#134}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3023	NLC28::miniTn10-*tet*$^{\#171}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3024	NLC28::miniTn10-*tet*$^{\#174}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3025	NLC28::miniTn10-*tet*$^{\#182}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*
OH3026	NLC28::miniTn10-*tet*$^{\#190}$ *att*Tn7::Tn7 pOX38*gen*::miniTn7-*lac*

Figure 5-5, MiniTn10-*lac* papillation:

NLC28::miniTn10-*tet*$^{\#x}$ pSIL
NLC28 transduced to Tc^R with miniTn10-*tet*$^{\#x}$ from OH501-695
NLC28::miniTn10-*tet*$^{\#x}$ transformed to Cm^R with pSIL

OH2027	NLC28::miniTn10-*tet*$^{\#3}$ pSIL
OH2028	NLC28::miniTn10-*tet*$^{\#4}$ pSIL
OH2029	NLC28::miniTn10-*tet*$^{\#6}$ pSIL
OH2030	NLC28::miniTn10-*tet*$^{\#17}$ pSIL
OH2031	NLC28::miniTn10-*tet*$^{\#20}$ pSIL
OH2032	NLC28::miniTn10-*tet*$^{\#43}$ pSIL
OH2033	NLC28::miniTn10-*tet*$^{\#49}$ pSIL
OH2034	NLC28::miniTn10-*tet*$^{\#50}$ pSIL
OH2035	NLC28::miniTn10-*tet*$^{\#53}$ pSIL
OH2036	NLC28::miniTn10-*tet*$^{\#57}$ pSIL
OH2037	NLC28::miniTn10-*tet*$^{\#58}$ pSIL
OH2038	NLC28::miniTn10-*tet*$^{\#61}$ pSIL
OH2039	NLC28::miniTn10-*tet*$^{\#62}$ pSIL
OH2040	NLC28::miniTn10-*tet*$^{\#73}$ pSIL
OH2041	NLC28::miniTn10-*tet*$^{\#75}$ pSIL
OH2042	NLC28::miniTn10-*tet*$^{\#79}$ pSIL
OH2043	NLC28::miniTn10-*tet*$^{\#106}$ pSIL
OH2044	NLC28::miniTn10-*tet*$^{\#111}$ pSIL
OH2045	NLC28::miniTn10-*tet*$^{\#118}$ pSIL
OH2046	NLC28::miniTn10-*tet*$^{\#123}$ pSIL
OH2047	NLC28::miniTn10-*tet*$^{\#132}$ pSIL

OH2048 NLC28::miniTn10-*tet*$^{\#134}$ pSIL

OH2049 NLC28::miniTn10-*tet*$^{\#171}$ pSIL

OH2050 NLC28::miniTn10-*tet*$^{\#174}$ pSIL

OH2051 NLC28::miniTn10-*tet*$^{\#182}$ pSIL

OH2052 NLC28::miniTn10-*tet*$^{\#190}$ pSIL

Cloning miniTn10-*tet* insertions:

ID Reference number = Muntant #. Insert Restriction Type. Isolate #

Insert Restriction Types:

C = PstI / XhoI D = PstI / SacI H = PstI I = KpnI

Strain #	Strain	(Cloned Mutant # and ID Reference)	Plasmid Type
OH2507	HB101 pOH55	(miniTn10-*tet*$^{\#4}$ ref - 4.D.1)	pBluescript
OH2549	HB101 pOH96	(miniTn10-*tet*$^{\#6}$ ref - 6.I.8)	pBluescript
OH2582	HB101 pOH128	(miniTn10-*tet*$^{\#6}$ ref - 6.H.1)	pBluescript
OH5240	HB101 pOH88	(miniTn10-*tet*$^{\#17}$ ref - 17.H.6)	pBluescript
OH2511	HB101 pOH59	(miniTn10-*tet*$^{\#20}$ ref - 20.I.50)	pBluescript
OH2513	HB101 pOH61	(miniTn10-*tet*$^{\#20}$ ref - 20.H.35)	pK18
OH2584	HB101 pOH130	(miniTn10-*tet*$^{\#20}$ ref - 20.I.30)	pK19
OH2501	HB101 pOH50	(miniTn10-*tet*$^{\#43}$ ref - 43.H.2)	pBluescript
OH2522	HB101 pOH70	(miniTn10-*tet*$^{\#50}$ ref - 50.H.32)	pK19
OH2560	HB101 pOH106	(miniTn10-*tet*$^{\#53}$ ref - 53.H.38)	pK19
OH2562	HB101 pOH108	(miniTn10-*tet*$^{\#53}$ ref - 53.I.6)	pBluescript

Strain #	Strain	(Cloned Mutant # and ID Reference)	Plasmid Type
OH2508	HB101 pOH56	(miniTn10-*tet*#57 ref - 57.D.2)	pBluescript
OH2563	HB101 pOH109	(miniTn10-*tet*#61 ref - 61.I.2)	pK18
OH2564	HB101 pOH110	(miniTn10-*tet*#61 ref - 61.I.2Q)	pK18
OH2565	HB101 pOH111	(miniTn10-*tet*#61 ref - 61.I.3Q)	pK18
OH2567	HB101 pOH113	(miniTn10-*tet*#61 ref - 61.H.33)	pK18
OH2568	HB101 pOH114	(miniTn10-*tet*#62 ref - 62.I.3)	pK19
OH2569	HB101 pOH115	(miniTn10-*tet*#62 ref - 62.I.2)	pK19
OH2572	HB101 pOH118	(miniTn10-*tet*#62 ref - 62.I.9)	pK18
OH2544	HB101 pOH92	(miniTn10-*tet*#73 ref - 73.I.76)	pBluescript
OH2519	HB101 pOH67	(miniTn10-*tet*#75 ref - 75.H.10)	pBluescript
OH2552	HB101 pOH99	(miniTn10-*tet*#79 ref - 79.I.27)	pBluescript
OH2553	HB101 pOH100	(miniTn10-*tet*#79 ref - 79.I.29Q)	pBluescript
OH2554	HB101 pOH101	(miniTn10-*tet*#79 ref - 79.I.29)	pBluescript
OH2556	HB101 pOH102	(miniTn10-*tet*#79 ref - 79.I.30)	pBluescript
OH2526	HB101 pOH74	(miniTn10-*tet*#106 ref - 106.H.14)	pBluescript
OH2527	HB101 pOH75	(miniTn10-*tet*#106 ref - 106.H.15)	pBluescript
OH2528	HB101 pOH76	(miniTn10-*tet*#106 ref - 106.H.40)	pK19
OH2502	HB101 pOH51	(miniTn10-*tet*#111 ref - 111.H.2)	pBluescript
OH2577	HB101 pOH123	(miniTn10-*tet*#118 ref - 118.H.34)	pK18
OH2557	HB101 pOH103	(miniTn10-*tet*#123 ref - 123.I.21Q)	pBluescript
OH2558	HB101 pOH104	(miniTn10-*tet*#123 ref - 123.I.69)	pK18
OH2585	HB101 pOH131	(miniTn10-*tet*#123 ref - 123.I.47)	pK19
OH2523	HB101 pOH71	(miniTn10-*tet*#132 ref - 132.H.12)	pBluescript
OH2579	HB101 pOH125	(miniTn10-*tet*#171 ref - 171.H.59Q)	pK19

Strain #	Strain	(Cloned Mutant # and ID Reference)	Plasmid Type
OH2580	HB101 pOH126	(miniTn10-*tet*#171 ref - 171.I.60)	pK19
OH2530	HB101 pOH78	(miniTn10-*tet*#174 ref - 174.H.2)	pBluescript
OH2532	HB101 pOH80	(miniTn10-*tet*#174 ref - 174.H.11)	pBluescript
OH2533	HB101 pOH81	(miniTn10-*tet*#174 ref - 174.H.12)	pBluescript
OH2534	HB101 pOH82	(miniTn10-*tet*#174 ref - 174.I.22)	pBluescript
OH2535	HB101 pOH83	(miniTn10-*tet*#174 ref - 174.I.23)	pBluescript
OH2536	HB101 pOH84	(miniTn10-*tet*#174 ref - 174.I.24)	pBluescript
OH2537	HB101 pOH85	(miniTn10-*tet*#174 ref - 174.I.42)	pK19
OH2583	HB101 pOH129	(miniTn10-*tet*#174 ref - 174.H.41)	pK19
OH2504	HB101 pOH53	(miniTn10-*tet*#182 ref - 182.I.37)	pBluescript
OH2541	HB101 pOH89	(miniTn10-*tet*#190 ref - 190.H.24.1)	pBluescript
OH2543	HB101 pOH91	(miniTn10-*tet*#190 ref - 190.H.8)	pBluescript

Chapter 6: Summary and Perspective.....338

No Strains

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