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Engineered Adenovirus for Selective Replication in Tumors and Druggable Control of Virus Progression

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by

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Chapter 2, in part is currently being prepared for submission for publication of the material. Partlo William; O'Shea Clodagh. The dissertation author was the primary investigator and author of this material.

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ABSTRACT OF THE DISSERTATION

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The oncolytic Adenovirus has shown promise as a cancer treatment and is under development in numerous laboratories. Two important requirements of an oncolytic Adenovirus are safety and effectivity. These requirements translate to selectivity against replication in normal cells and potent replication in tumor cells. Often, potency is sacrificed for selectivity, resulting in limited clinical effectivity. The work described in this dissertation attempts to engineer an oncolytic Adenovirus with selectivity based on an arbitrary transcription factor while maintaining wildtype or near-wildtype replication

kinetics in the targeted cell type. The example transcription factor used in this work is p53. A significant percentage of all tumors have been found to be p53-null, while all normal cells are p53-positive. An oncolytic Adenovirus based on the p53 status would thus be a powerful clinical tool.

Development of a robust method for assessing Adenovirus replication kinetics is described. This method employs a genetic modification to the Adenovirus genome such that each infected cell is forced to express a fluorophore. This fluorophore expression is monitored over time to create an exponential growth curve as the virus passes through its initial lifecycle and produces secondary and tertiary infections. With this fluorescence-based viral kinetics assay, the replication kinetics of any Adenovirus type infecting any cell type can be quantified.

A selectively replicating Adenovirus was created by placing a single, critical Adenovirus ORF under control of the Two Step Transcriptional Activation (TSTA) system. For a positively regulated virus, the TSTA system is used with a selective, but weak promoter driving expression of the Tet-On transcriptional activation factor and the Tet-Response Element (TRE) promoter driving expression of the chosen Adenovirus ORF. For a negatively-regulated virus, Tet-On is replaced with the TetR transcriptional repressor while the TRE promoter is replaced with the CMV-Tet-O promoter, subject to repression by TetR.

Negative selectivity based on transcriptional activity of p53 is demonstrated with approximately 100X differential between $p53^{+/+}$ and $p53^{-/-}$ cell lines.

CHAPTER ONE:

Historical Methods for Imparting Selectivity in Oncolytic Adenovirus Constructs

Introduction

As this thesis was being written, the latest cancer statistics for the US were published[1]. This data is summarized in fig. 1.1a for females and fig. 1.1b for males, taken from reference 1. These curves engender many explanations for their trends. For example, lung cancer death rates peaked and began to fall coincident with the rate of smoking in males and females. Breast, colon, and prostate cancer death rates fell as a result of early screening. Even stomach cancer death rates fell early in the 20th century due to improved water and food purity. None of these trends were the result of any dramatic new cancer treatment.

So why the enormous effort currently expended on development of cancer treatments in thousands of laboratories around the world? Because it is expected that these trend lines will not all intersect zero, and even if they did the crossing point would occur more than 50 years from now if the present slopes remained constant. Additionally, there are many cancers shown in these figures that exhibit no decreasing trend at all and few with a troubling rise, however so slight.

Since cancer is a multifaceted disease[2], clinics need a large and varied set of tools to address the full spectrum of cancer types presented by their patients. The O'shea lab has chosen to develop oncolytic adenoviruses as a cancer treatment because the molecular program of the adenovirus has been found to closely match that of a broad spectrum of cancer types[3,4].

Like any other cancer treatment, success of oncolytic adenoviruses can be judged on two major features; safety and efficacy. The safety aspect of oncolytic adenoviruses is

addressed by engineering the adenovirus to be selective for tumor cells vs. normal cells. Efficacy can be addressed in several ways; greater potency via enhanced replication rate, greater cell killing via bystander effect, or improved activation of the immune system. Immune activation by oncolytic adenoviruses has become a hotly pursued goal just in the last few years. At the start of the work on this dissertation in 2013, there was little talk of activating the immune system via oncolytic viruses as a cancer treatment. But, now in 2018, there are few treatments that do not somehow include an immune activation component. This radical shift in approach is due to the success of anti-PD-1/PD-L1[5] and anti-CTLA-4[6] antibodies as well as the FDA approval of T-VEC[7], an oncolytic cancer treatment based on the HSV-1 herpes virus with immune system activation as its proposed mechanism of action.

Oncolytic Adenovirus Selectivity

In the oncolytic adenovirus field, selectivity and safety are often considered the same. If a virus can be made selective for only tumor cells, then an enhanced safety profile is expected. The literature describes two general approaches for engineering selectivity into an oncolytic adenovirus; deletions or mutations of endogenous Ad genes, or replacing one or more endogenous Ad promoters with tumor selective promoters.

Adenovirus Deletions/Mutations to Achieve Tumor Selectivity

The E1B-55k protein, produced by the Ad during the early phase of its lifecycle, is known to mediate degradation of p53[8]. It was thought that deletion of E1B-55k from the

Ad genome would render it selective for replication only in cells lacking a transcriptionally active p53 protein[9,10]. Unfortunately, this predicted dependence was not born out[11,12].

The E1A protein, the earliest protein expressed during the Ad lifecycle, is responsible for binding to and degrading the retinoblastoma (Rb) protein, thus preventing Rb from performing its repressive function on E2F-activated genes[13]. This binding is mediated by two conserved regions, CR1 and CR2. Mutations or deletions to the CR2 region have been used to impart selectivity between actively dividing cells and quiescent cells[14,15]. And combined mutations or deletions to both CR1 and CR2 have been employed[16].

Once Rb repression is eliminated another Ad protein, E4-ORF6/7, performs the function of stabilizing the E2F/DP1 dimer responsible for driving expression of E2F-activated genes[17]. By combining the E1A CR2 deletion with complete deletion of E4-ORF6/7, greater differential between tumor and normal cells has been demonstrated[18].

Rather than engineer mutations *a priori*, some researchers have used directed evolution to produce an oncolytic adenovirus with desired properties. The authors of reference 19 mixed multiple serotypes in a dish of colon cancer cells, allowing cross-serotype genome mixing. After multiple passages on the colon cancer cells, candidate viruses were then screened for differential replication between colon cancer cells and normal epithelial cells. The candidate with highest potency in colon cancer cells and greatest differential for normal epithelial cells was sequenced and found to be a complex combination of Ad3 and Ad11 serotypes. No explanation is given for the reported selectivity.

Replacement of Endogenous Ad Promoters with Tumor-Specific Promoters

One disadvantage of the mutation/deletion method is that selectivity choices are limited to pathways involved in the Ad lifecycle. If a chosen cancer pathway is not part of the Ad lifecycle, then no amount of Ad genome mutations would render the Ad virus selective in replication based on this pathway. To address this limitation, many researchers have replaced endogenous Ad promoters with tumor specific promoters in an effort to impart selectivity between tumor cells and normal cells.

An early example of this type of oncolytic virus construct is described in reference 20. In this construct, the E1A promoter was replaced with the α-feto-protein gene promoter found to be highly expressed in 70-80% of hepatocellular carcinoma cancers, but not in normal adults. Other tumor-specific promoters have been used to replace the E1A promoter, such as the E2F promoter[21, 22] selective for rapidly dividing cells, and the secretory leukoprotease inhibitor (SLPI) promoter[23] found to be highly expressed in a broad range of human carcinomas.

Dissatisfied with the level of selectivity afforded by replacing a single Ad promoter, researchers have also replaced two endogenous Ad promoters with a tumor-specific promoter. Reference 24 describes replacing both the E1A and E4 promoters with the E2F-1 promoter. The E1A and E1B promoters have also been replaced with tumor-specific promoters, as described in reference 25.

Modifications to Enhance Oncolytic Virus Potency

A general problem found in the literature referenced in the proceeding paragraphs is a loss of potency in exchange for selectivity. To address this decrease in virus potency, researchers have made additional changes to the Ad genome either to increase the viral replication rate or to increase the level of cell killing.

Researchers have found mutations that shorten the time between initial cellular entry and progeny virion release. One example mutation is a truncation in the E3-19K protein such that its endoplasmic reticulum retention signal is lost, leading to insertion into the plasma membrane, production of a viroporin-like function, and early virion release[26]. Another example is loss of all E3 genes except the Adenovirus Death Protein (ADP), resulting in increased expression of ADP and early virion release[16].

Viruses in the class of increased cell killing fall into two categories; those that produce a toxin directly and those that express an enzyme that converts a prodrug into a toxin. Examples of toxins directly expressed by an oncolytic Ad are hyaluronidase[27] and onconase[28]. Examples of enzymes that convert a prodrug into a toxin are HSV-1 thymidine kinase[29] and cytosine deaminase[30]. The produg for both of these examples is 5-fluorocytosine.

Immune Activation by Oncolytic Ad

As mentioned in the introduction, immune activation has become a hot topic in oncolytic virus development just in the last few years. The idea is simple, viral infection of a tumor strongly activates the immune system facilitating discovery of tumor neoantigens and expansion of T-cells specific to these neoantigens. By this mechanism

distant metastases are eliminated by activated T-cells rather than the administered oncolytic virus.

Though adenovirus infection is known to be highly immunogenic[31], the region around a tumor is often in a state of immunological anergy[32]. Thus, arming the virus with an immune stimulating gene is thought to be necessary for achieving greatest effect. Examples of immune stimulating payloads described in the literature are; granulocyte macrophage-colony-stimulating factor (GM-CSF)[33-39], IL-12[38], Bispecific T-cell Engager (BiTE)[40], OX40 ligand[41], and anti-PD-1 antibody[42].

The challenge with development of immune stimulating payloads placed in a human oncolytic adenovirus is testing in a non-human model. Human Ad does not replicate in mice[43], so the many murine models of cancer are ill-suited for testing human, replication-competent, oncolytic Ad.

Conclusions

Tremendous progress has been made in the development of oncolytic adenovirus agents. The negative cost in replication kinetics as a result of changes made for selectivity are now better understood and appreciated. Mechanisms for enhancing potency beyond improved replication kinetics have been developed and tested. And a multitude of immune stimulating payloads have been suggested, with some currently in human clinical trials[34,39]. The remaining chapters of this dissertation will describe work toward an oncolytic adenovirus with selectivity based on the presence or absence of an arbitrary transcription

factor. Emphasis is placed on maintaining wildtype or near-wildtype replication kinetics while imparting selective replication control.

Figures

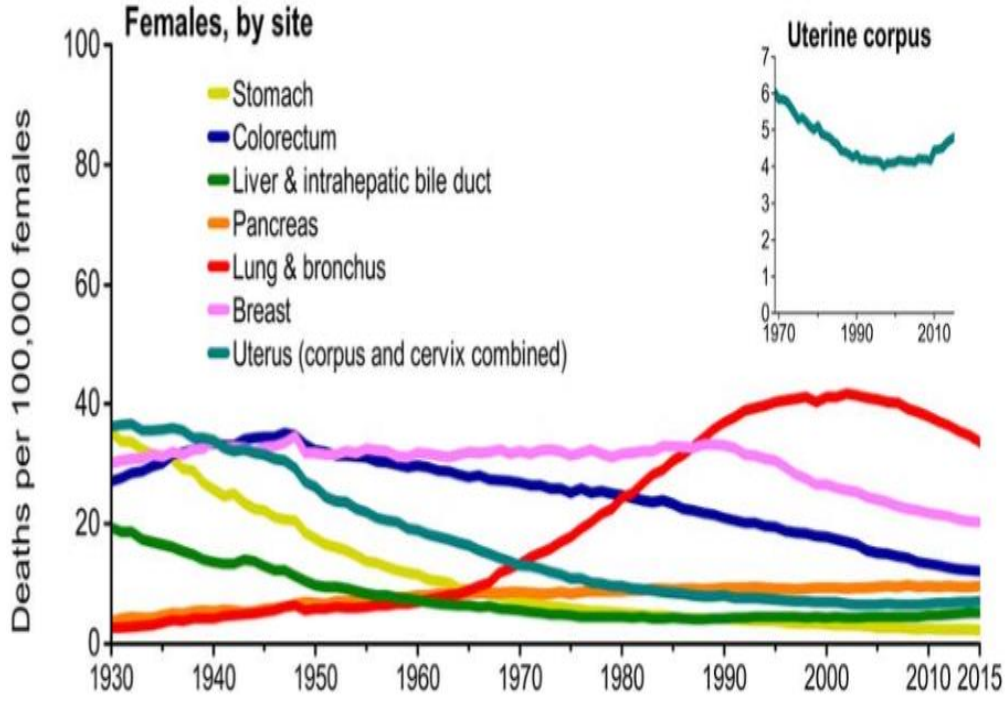

Figure 1.1a. Trends in cancer death rates for females in the US since 1930. Graph taken from reference 1.

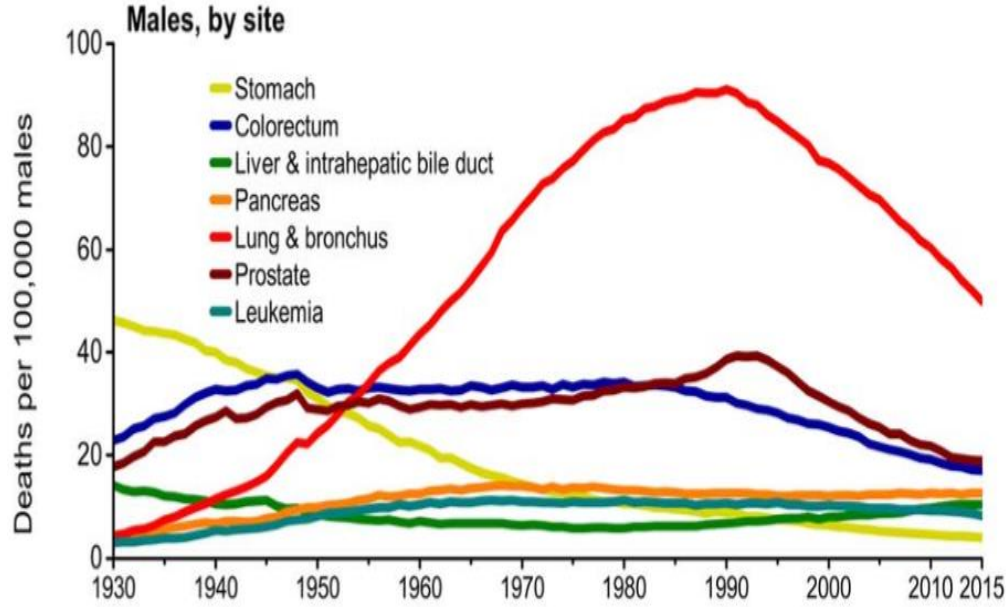

Figure 1.1b. Trends in cancer death rates for males in the US since 1930. Graph taken from reference 1.

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CHAPTER TWO:

Fluorescence-Based Viral Kinetics Assay

Historical Methods for Measuring Adenovirus Replication Kinetics

Since the overarching goal of this thesis project is to engineer an Adenovirus that exhibits rapid replication in tumor cells but slow or no replication in normal cells, it is important to have an accurate assay for assessing virus replication kinetics.

Historically, the single step growth assay has been employed to compare virus kinetics under different conditions or to compare the kinetics of one virus to another. The single step growth assay was first described by Emory and Delbruck in 1939[1]. Their biological model system was a bacteriophage infecting *Escherickia coli*. Though the bacteriophage model is different from that of Adenovirus, the basics of viral lifecycle remains the same: initial particle entry, co-opt host cellular machinery, new particle assembly, and escape via cellular lysis.

In their paper, Emory and Delbruck initially perform a multi-time point measurement of bacteriophage concentration vs. time after initial infection with a low Multiplicity of Infection (MOI). The graph of these results is reproduced in Fig 2.1 (the red text has been added for this dissertation). This dataset was painstaking to produce. Each time point on this graph represents the harvest of infected bacteria followed by a separate plaque assay to determine the concentration of infectious viral particles. Three separate virus lifecycles can be clearly observed, with each successive lifecycle producing approximately the same multiplication in number of virus progeny. After three lifecycles, there is sufficient dephasing such that the clearly observable steps between lifecycles is lost, but the slope of the log-phase growth remains the same.

Based on these results, measurement of a single viral lifecycle was proposed as a method to estimate the log-phase growth of virus under the reasonable assumption that each lifecycle exhibits constant duration and constant multiplication factor.

Single Step Virus Kinetics Assay

In practice, the single step kinetics assay requires that all host cells be infected during initial application of the virus to be tested. This requirement ensures that there are no remaining host cells to take up newly created virus after the first round of the virus life cycle. Upon infecting with a known MOI, and after a period of time equal to the virus life cycle, the host cells and supernatant are harvested and newly-created virions are quantified. The increase in virion count from initial infection to final harvest is considered a measure of virus kinetics.

There are two challenges with using the single step growth curve for assessing virus kinetics. The first challenge is that the full life cycle of the virus is not assayed and the second is the need for an accurate titer for the virus.

With respect to the virus life cycle, cell lysis and secondary infection are not measured in the single step growth assay. Exclusion of cell lysis and secondary infection are known defects in this assay and examples of viruses that perform well in the single step growth assay, but actually have poor kinetics have been described. The most notable example is Ad5 with a deletion of the Adenovirus Death Protein (ADP)[2]. Such ΔADP viruses exhibit no notable defect in their cell entry, co-option, and assembly steps, but are defective in the cell lysis step. Since the single step growth assay requires harvest of host

cells and artificial lysis to release all newly produced virions this defect is not observed, resulting in an incorrect assessment of replication kinetics close to that of wt virus for these ΔADP mutants.

Accurate knowledge of the virus titer is critical for producing usable results with the single step growth assay. The titer of the virus to be tested is essential for calculating the conditions of initial infection while the final titer of the virus produced after one life cycle is also required to calculate the multiple of virus expansion. Techniques have been developed for measuring the titer for wt versions of commonly used Ad serotypes such as Ad5, but for engineered versions of Ad5 or for the less common Ad serotypes there are no available techniques that accurately reflect the true concentration of Plaque Forming Units (PFUs).

Commonly used surrogates to infer the PFU concentration are listed below.

- 260nm/280nm absorbance
- qPCR to quantify viral genomes
- ELISA against viral proteins
- Immuno-staining for viral proteins
- Cell viability assay

Each of these measurements have flaws which can lead to inaccurate assessment of viral PFU concentration. The 260nm/280nm absorbance measurement assumes that the sample is pure and has no non-viral proteins or DNA. It also assumes that all measured DNA (via 260nm absorbance) is part of a complete and packaged viral genome. The qPCR measurement also assumes that every DNA fragment detected by the chosen qPCR primers

is part of a complete and packaged viral genome. Neither of these assumptions is true in practice. Performing ELISA against one of more viral proteins requires a virus of known titer to produce a standard curve, as well as the assumption that the test virus produces the same number and type of vial proteins per PFU. Many viruses used within this dissertation are deleted for expression of one or more viral proteins and thus would be "under counted" against a wt standard by the ELISA method. Immuno-staining for viral proteins upon infection at low MOI accurately measures the number of Infectious Units (IU), but does not reflect the number of PFUs. And finally, the cell viability assay attempts to infer PFU concentration based on the virus ability to kill cells. Any virus engineered to exhibit a bystander effect would greatly exaggerate its PFU concentration.

Criteria for Ideal Viral Kinetics Assay

With these challenges and deficiencies in mind, the following is a set of criteria for an idealized viral kinetics assay.

- Applicable to all Ad serotypes
- Usable with any host cell line
- Non-destructive
- High throughput
- Insensitive to initial virus concentration
- Measure entire viral life cycle: initial entry, co-opt cellular machinery, viral particle assembly, cell lysis, and secondary, and tertiary infections

The reasoning behind most of these criteria should be obvious except possibly for the high throughput requirement. The high throughput requirement is driven by the desire to match the high throughput assembly protocol recently developed in the O'Shea lab[3] and described in the chapter 6. This protocol facilitates the rapid assembly of plasmids containing whole Ad genomes by combining four entry vector plasmids via multi-site Gateway cloning[4], Sequence and Ligation-Independent Cloning (SLIC)[5] or Gibson cloning[6]. Depending on the choice of cloning method, this protocol is called Adsembly or AdSLIC.

The primary reasoning behind the requirement for insensitivity to an accurately known virus titer is the difficulty of determining the titer for all Ad serotypes, as described in previous paragraphs. A second reason behind the desire for a kinetics assay that is insensitive to initial titer is the desire to skip the entire virus production, purification, and titering steps and simply transfect the whole Ad genome plasmid produced by the Adsembly or AdSLIC protocol directly into the cell type of interest. A typical work-flow for constructing, producing, and testing an engineer Ad virus is shown in fig. 2.2. These steps can be both time consuming and expensive. The initial step of producing the whole Ad genome plasmid is amenable to high throughput techniques, but the production, purification, and testing steps do no scale well when the number of different engineered viruses is large. If, instead, one could simply transfect the whole Ad genome plasmid directly into the cell type of interest and measure the virus kinetics, high throughput screens of randomly or systematically modified Ad viruses would be possible and practical.

Fluorescence-Based Virus Kinetics (FBVK) Assay

One method to determine the kinetics of a virus without knowledge of its initial titer would be to infect the cell type of interest with a low MOI and monitor the number of infected cells over a period of several virus lifecycles, similar to the work of Emory and Delbruck. A semi-log plot of the exponential growth in number of infected cells versus time would yield a straight line proportional to the exponential growth rate. This method essentially reduces viral kinetics measurement to that similar to yeast and bacteria where turbidity of the media is used to infer the number of yeast or bacteria in the culture.

How best to infer the number of virally infected cells? We chose to engineer the virus to force expression of a fluorophore upon infection. Under the assumption that each infected cell produces the same number of fluorophores on average, a simple measurement of fluorescence intensity provides a reading proportional to the number of infected cells. One could also image the cells and count those that are positive for the fluorophore, thus providing an infected cell count, but such a method is not high throughput since we need to make multiple cell count measurements per each Ad life cycle of approximately 48 hours and we wish to simultaneously monitor a large number of virus/cell type conditions.

There are many examples of Ad viruses engineered to express a fluorophore[7-11]. A frequently used construct for fluorophore expression within the Ad genome is in fusion with the E1A gene (e.g. E1A-GFP)[12-17]. Based on this body of literature, an Ad5 virus expressing the E1A-GFP fusion was constructed and tested. Fig 2.3 shows the measured fluorescence, both raw and background subtracted, over an 8 day period. These results

were disappointing because the signal-to-background was approximately 1:2 and the signal to noise of about 2. Deriving a reliable exponential growth parameter from this measured data would be problematic.

Though the E1A-GFP fusion has been frequently used in the literature, it has only been used as a marker for individual infected cells during microscopy, not as a means to form an aggregate signal from a mixture of infected and uninfected cells. When imaging, spatial discrimination plays are role in reducing the perceived background level. That is, cells that are GFP-, and the media itself, may still exhibit a low level of auto fluorescence, but since these cells, and media, are spatially removed from the GFP+ cells, so their fluorescence is easily ignored. When using a plate reader to record the total fluorescence from the entire well, no such spatial discrimination is available. Thus, if only a few cells are GFP+ in a large field of GFP- cells, the background fluorescence can exceed that of the signal from the few GFP+ cells. Such is the case for the measurement shown in fig 2.3.

Optimized Conditions for Fluorescence Readout

Faced with the challenge of low signal-to-background when using a plate reader, there are several potential improvements;

- Chose the highest quantum efficiency fluorescent protein
- Optimize the location within the Ad genome for maximum expression
- Modify the media for minimum background fluorescence
- Chose a fluorescent protein with excitation and emission

wavelengths located within a “dark” spectral region of the media

The field of biology has developed a very wide range of fluorescent proteins, covering essentially the full visible spectrum and extending into the UV and IR regions. Table 2.1 lists many of the common fluorescent proteins available along with their spectral properties[18]. The fluorescent protein YPet has two advantages over eGFP. The first advantage is higher brightness compared to eGFP by a factor of 2.4X. The second advantage is a shift in the excitation and emission wavelengths into a region of greatly reduced fluorescence from the media. Fig 2.4 shows the measured fluorescence for DMEM media, with and without phenol red, with emission measured at the peak for eGFP (fig 2.4a) and for YPet (fig 2.4b). Switching from eGFP with phenol red-containing media to YPet with phenol red-free media results in a reduction in background of approximately 10X and a signal-to-background improvement of 24X.

Besides optimizing the choice of fluorescent protein, its expression level and stability are also critical for maximizing measured signal. In the E1A-GFP fusion, the expression level of GFP follows that of the Ad E1A gene. In addition, because it is expressed as a fusion product the GFP experiences the same degradation fate as that of E1A. It has been shown that the major splice-forms of E1A are rapidly degraded[19], and thus so too is GFP when in fusion with E1A. In order to separate the fate of the fluorescent protein from that of the co-expressed endogenous Ad protein, we made use of the self-cleaving peptide sequence, P2A, taken from the porcine teschovirus-1[20,21]. This peptide sequence, when augmented with a leading GSG sequence, can approach a cleavage

efficiency of 90%. Since the cleavage efficiency has been shown to be context dependent, we constructed several viruses to determine the P2A cleavage efficiency at different points in the Ad5 lifecycle. The following virus constructs were made and tested:

CMBT-352: YPet-E1A

CMBT-379: YPet-P2A-E1A

CMBT-456: E1B-55k-P2A-YPet

CMBT-403: YPet-P2A-ADP

CMBT-445: Fiber-P2A-YPet

CMBT-446: Fiber-GS-P2A-YPet

Note that the GS linker in CMBT-446 contains an additional GSGSGSGSGS added to the GSG leading sequence of the P2A sequence.

293-E4 cells were infected with an MOI of 10 and harvested 36 hours post infection. The resulting proteins were probed with antibodies against E1A, E1B-55k, Fiber, and GFP with the results shown in fig 2.5. Fortunately, common antibodies to GFP cross-react to YPet. Little or no E1A is expected at this point in the virus lifecycle, but the E1A-YPet fusion product can be found in both in α-E1A blot and the α-GFP blot. We infer from this result that the E1A-YPet fusion product is more stable than endogenous E1A. A much stronger signal can be found in the α-GFP blot for the YPet-P2A-E1A construct, demonstrating that the P2A cleavage allows the YPet protein to avoid the degradation fate of E1A. Note also the band shift due to the 21 amino acid residue from P2A fused to YPet.

When co-expressed with E1B-55k, an early protein, the cleaved fragment (E1-55K-P2A) and the uncleaved fragment (E1B-P2A-YPet) are found in equal abundance on the α-E1B-55K blot, implying a 50% cleavage efficiency. ADP is expressed at both intermediate and late times with an aggregate cleavage efficiency of approximately 70%. Fiber is the last ORF to be expressed during the late period of the virus lifecycle and shows essentially 100% cleavage efficiency. Note the small band shifts in the α-Fiber blot for the Fiber-P2A-YPet and Fiber-GS-P2A-YPet constructs, due to the presence of the 22AA residue for P2A or the 32AA residue for GSGSGSGSGS-P2A.

Comparisons of Fluorophore Placement within the Ad Genome

With the function of P2A demonstrated in the context of Ad infection, the remaining optimization is the choice of genome location for the YPet insertion. Table 2.2 lists the viruses made and tested during this optimization. Two important criteria were assessed; fluorescent protein expression and virus kinetics. The expression levels were measured for an MOI of 15 and each was measured simultaneously with CMBT-403 (YPet-P2A-ADP) as a reference. The virus kinetics were determined by plotting fluorescence vs. time on a semi-log plot and fitting to the logarithmic growth portion of the curve. Fig 2.6 shows the location of these insertions within the Ad5 genome.

The first notable finding from this optimization is that nearly all constructs produce a higher level of fluorescence than that of the E1A-YPet construct. The second finding is that fusion products always lead to reduced kinetics, if not outright failure. All of the fusion products listed on this table exhibit replication kinetics significantly slower than the

fastest of the cleavage products. A virus is considered failed when it does not produce plaques within 10 days after transfection of its genome into HEK293 cells. The transfection is attempted two additional times to avoid a false declaration of failure.

An interesting failure is the ADP-YPet construct. In this case, even adding the self-cleaving P2A sequence, ADP-P2A-YPet, did not rescue this virus. Yet completely deleting the ADP ORF and replacing it with the YPet ORF yields a successful virus, though with reduced kinetics. The ADP is a single transmembrane protein spanning the nuclear membrane with a 41 amino acid c-terminal tail located in the cytoplasm[22]. The YPet protein contains 239 amino acids, thus we speculate that YPet causes steric interference when fused to the c-terminal of ADP. For the ADP-P2A-YPet construct, a successful cleavage event leaves a 21 amino acid residue on the c-terminal of ADP, yet this much smaller fusion product still causes failure.

Another interesting comparison can be made between viruses of the same design, but assembled in two different ways; Adsembly vs. AdSLIC. As described in reference 3, the Adsembly process constructs a virus from four plasmids using multi-site gateway cloning. This cloning technique is quick, but leaves basepair residues from the recombination sequences at three locations within the Ad genome. The AdSLIC process employs SLIC or Gibson cloning to combine the same four plasmids, and thus does not introduce any extraneous basepairs into the Ad genome. It has been shown that the residues within the Ad genome introduced by the Adsembly process result in reduced virus kinetics[23]. All viruses listed on this table, and described elsewhere in this thesis, with a prefix “CMBT” were constructed using the Adsembly process. All viruses with the

"PCMN" prefix were constructed using the AdSLIC process. The following viruses were built using both methods:

E1A-P2A-YPet (CMBT-432 vs. PCMN950)

DBP-P2A-YPet (CMBT-886 vs. PCMN-968)

YPet-P2A-ADP (CMBT-403 vs. PCMN-421)

E3-14.7k-P2A-YPet (CMBT-890 vs. PCMN-887)

The AdSLIC version of each of these viruses exhibits higher kinetics than its corresponding Adsembly version.

Because of the high fluorescence level and fast kinetics of the YPet-P2A-ADP construct, this readout was chosen for use in the majority of the viruses described in the remaining chapters of this thesis. One exception is the work described in chapter 3. The goal of chapter 3 is to compare the kinetics of several different Ad serotypes and fiber chimeras across a range of different cell types. Because the ADP does not exist in serotypes other than Ad2 and Ad5, we were forced to choose a different readout location.

Plate Reader Instrumentation Optimization

With an optimized virus design chosen, improvements in the instrumentation were then investigated. Three plate readers were characterized, all supplied by TECAN; M200Pro, M200Pro with fat fiber, and M1000Pro. The optical layout of all three models is the same and is shown in Fig 2.7. The difference between these units is mainly in the brightness of the flashlamp and the optical efficiency of the monochrometers and fibers.

The M200Pro and M200Pro with fat fiber, differ only in the diameter of the fiber used to transmit light between the monochrometers and the multiwell plate plate.

To compare the noise performance of these tools, a dilution series of fluorescent beads suspended in 0.25% agarose and plated in a 96 well plate was used to produce a range of fluorescence levels. For each dilution, the fluorescence intensity was measured 200 times and the resulting normalized standard deviation calculated. The raw readings for each plate reader is shown in figs 2.8a through 2.8c. At very low fluorescence levels, it is expected that the plate readers will be shot noise limited and thus the standard deviation will scale as inverse square root of the fluorescence signal. This expectation is born out when plotting instrument noise vs. inverse square root of signal level as shown in Fig 2.9. For all fluorescence levels except for the highest values, the instrument noise increases linearly with inverse of square root of signal level, as expected for a shot noise limited system.

Figs 2.8 and 2.9 show that the noise performance of the M1000Pro is superior to that of the M200s, and the fat fiber version of the M200 is superior to that of the standard M200. All three instruments provide temperature control, but only the M200s provide CO_2 and O_2 control. Since one goal of this thesis project is to show differential replication between tumor cells and normal cells, we need O_2 control to maintain the health of the Small Airway Epithelial Cells (SAECs) used as a model for normal cells. This requirement disqualifies the M1000Pro as a choice, so the M200Pro with fat fiber was chosen as the instrument to be used for all remaining measurements described in this thesis.

With the M200Pro gas control, a plate can be left in the plate reader for an extended period allowing multiple reads and thus greater data density than shown in the original experiment of Fig 2.3. Media evaporation over the period of several days is an issue and was addressed by applying a gas permeable moisture barrier seal. Product number 4ti-0516/96, manufactured by 4titude®, is designed for 96 well plates and is specified to have the following gas permeabilities:

O_2	150 $cm^3/(m^2 \cdot day \cdot bar)$
N_2	38 $cm^3/(m^2 \cdot day \cdot bar)$
CO_2	400 $cm^3/(m^2 \cdot day \cdot bar)$
H_2O	1 $gm/(m^2 \cdot day)$

This low permeability for water vapor results in no measurable fluid loss even after 10 continuous days in the plate reader.

Detailed Assay Description

A typical assay setup is shown in Fig 2.10. A 96 well plate is seeded with the cell type of interest in all wells except for the upper right four wells. The two upper right most wells are filled with fluorescent beads suspended in 0.25% agarose and are used as a normalization constant in case of plater reader sensitivity drift. The next two wells in the same row are left empty to be used for background subtraction in a terminal WST-1 cell viability assay performed at the end of the FBVK assay. The lowest four wells in this last column are seeded with cells but given only a mock infection, and used for background subtraction.

The remaining 11 columns of wells are infected with virus using a dilution series starting with an MOI of 15 and decreasing in steps of 3X. The use of this dilution series has several advantages. To calculate an accurate ln-slope of the virus kinetics, the initial infection of a well must be limited to allow virus exponential expansion. Depending on the virus and cell type combination, different levels of MOI achieve the desired level of initial infection. Several MOIs of this dilution series almost always result in the desired level of initial infection, producing kinetics curves amenable to fitting. A second feature of this dilution series is that the higher MOI values, principally the MOI=15, lead to infection of all cells during the initial infection. Under this condition we can measure the resulting single step kinetics curve described earlier in this chapter. And finally, the lowest MOI values lead to essentially single viral particle infections. Using a cell count of 50,000 cells per well, and assuming Poison statistics for virus uptake per well, the three lowest MOI values lead to the following cells with 0, 1, or 2 virions:

MOI	0 Virions	1 virion	2 virions
0.062	47,090	2,825	85
0.021	49,015	975	10
0.0069	46,666	333	1

Figs 2.11a and 2.11b show the measured results when applying all of the improvements described in the preceding paragraphs. The signal-to-background is now 50:1 and the signal-to-noise is approximately 2000:1.

To demonstrate that these exponential growth values are not specific to the choice of fluorescent protein, PCMN-871 was constructed to express mCherry-P2A-ADP instead

of YPet-P2A-ADP. The measured fluorescence vs. time for this virus is shown in Fig 2.12. The difference in the ln-slope values between PCMN-421 of 2.20 (+0.20/-0.23) and PCMN-871 of 2.10 (+0.08/-0.06) are not considered significant. The major reason for lower signal level for PCMN-871 is the reduced quantum efficiency of mCherry compared to YPet. The quantum efficiency of mCherry is only 20% of that for YPet. A second reason for the reduced signal level is lower responsivity of the plate reader's photocathode to the red-shifted fluorescence of mCherry.

Example Results

An excellent example of a potential false result produced by the single step viral kinetics assay is a comparison of viruses with deleted or mutated ADP. Fig 2.13 shows the measured fluorescence curves for the following viruses: YPet-P2A-ADP, ΔADP[YPet], and YPet-GS-ADP. Two additional viruses were constructed but could not be produced: ADP-GS-YPet and ADP-P2A-YPet. The kinetic slopes for these two failures is declared as zero.

Based on the curves shown in Fig 2.13, if a 48 hour single step kinetics assay were used to compare YPet-P2A-ADP against ΔADP[YPet], one would conclude that the ΔADP virus exhibits equal or better replication kinetics compared to that of wt virus. This conclusion is based on the higher late protein expression levels for ΔADP at 48 hours post infection compared to that of wt virus. But, a fit to the log-phase growth portion of the curves clearly shows a dramatic defect in virus kinetics for the ΔADP virus. These results fit well with what is known about the biology of the ADP protein[24]. The ADP protein is

expressed during the late stage of viral infection and is required for efficient cell lysis and release of viral progeny. A defect in this protein, or its absence would not impact the expression levels of late proteins, but would negatively impact the ability of the virus to produce secondary and tertiary infections, thus slowing its kinetics.

A clinical example of kinetics comparison is shown in Fig 2.14, wt Ad5 virus vs. ONYX-015. ONYX-015[25] is an Ad5 virus with a deletion in E1B-55k expression. Deletion of E1B-55k is an attempt to render this virus selective for tumor cells lacking p53 transcriptional activity based on the knowledge that E1B-55k was responsible for degrading p53. This virus is an approved treatment for head and neck cancers in China, but shows limited effectivity. The dramatically reduced kinetics of this virus, as compared to wt, could explain the poor clinical results obtained with this virus

As a final example, Fig 2.15 shows a comparison between wt Ad5 and an Ad5 virus deleted for expression of all of the E3 genes except ADP (ΔE3-12.5k, ΔE3-6.7k, ΔE3-19k, ΔE3-RIDα, ΔE3-RIDβ, ΔE3-14.7k). The explanation for the significantly faster kinetics of the E3-deleted virus is unknown at this time. This result is included as an example of a virus engineered to be faster than wildtype and may have clinical applications as a more potent oncolytic.

Conclusions

The development of the FBVK assay allows us to compare the kinetics between different viruses and across a variety of conditions and cell types. For much of the work described in following chapters, the ln-slope produced by the FBVK assay will be used to

determine if an engineered virus exhibits its intended design characteristic. Where it be differential replication between normal and tumor cells, improved replication within a targeted cell type, or replication controlled by the application of a drug, the FBVK can give insight into the successes and failures of these virus designs.

Chapter 2, in part is currently being prepared for submission for publication of the material. Partlo, William; O'Shea Clodagh. The dissertation author was the primary investigator and author of this material.

Figures and Tables

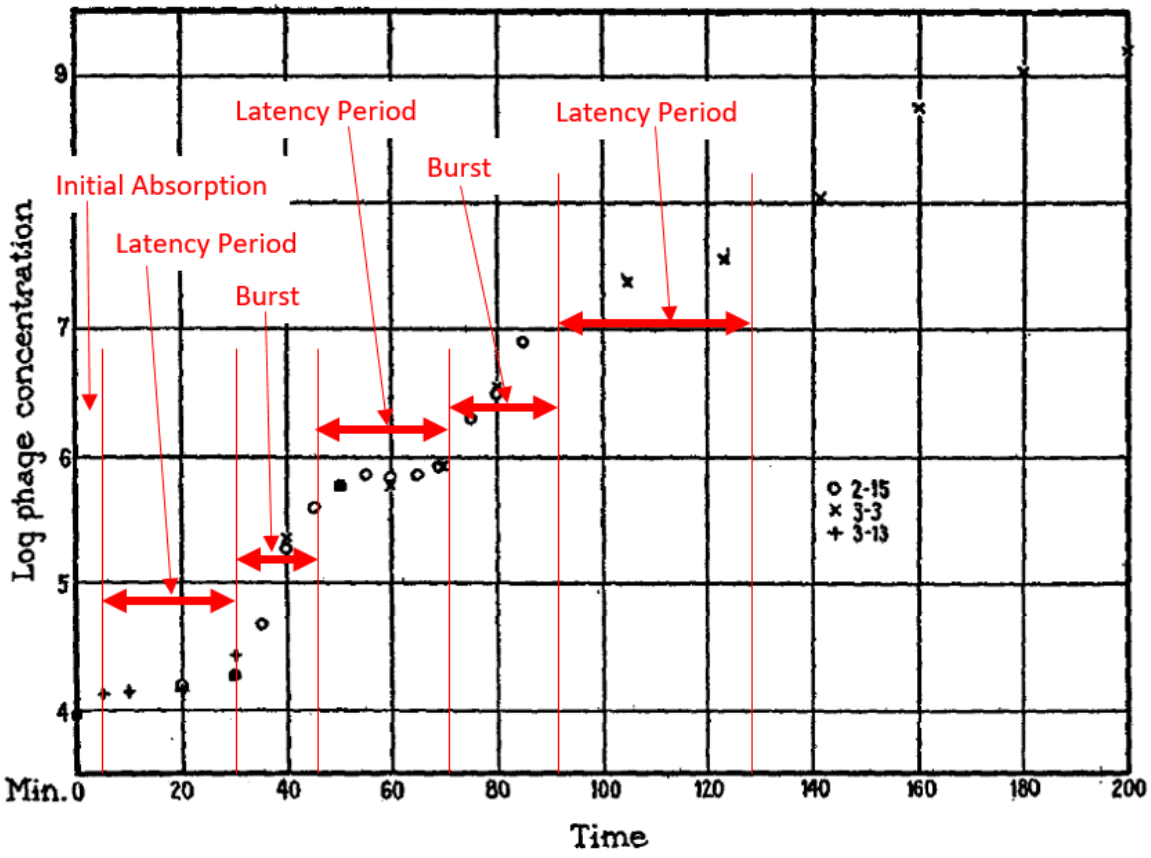

FIG. 2. Growth of phage in the presence of growing bacteria at 37°C.

A diluted phage preparation was mixed with a suspension of bacteria containing 2×10^8 organisms per cc., and diluted after 3 minutes 1 to 50 in broth. At this time about 70 per cent of the phage had become attached to bacteria. The total number of infective centers was determined at intervals on samples of this growth mixture. Three such experiments, done on different days, are plotted in this figure. The same curve was easily reproducible with all phage preparations stored under proper conditions.

Figure 2.1. Plot of bacteriophage concentration vs. time at initial infection with low MOI. Plot reproduced from reference 1.

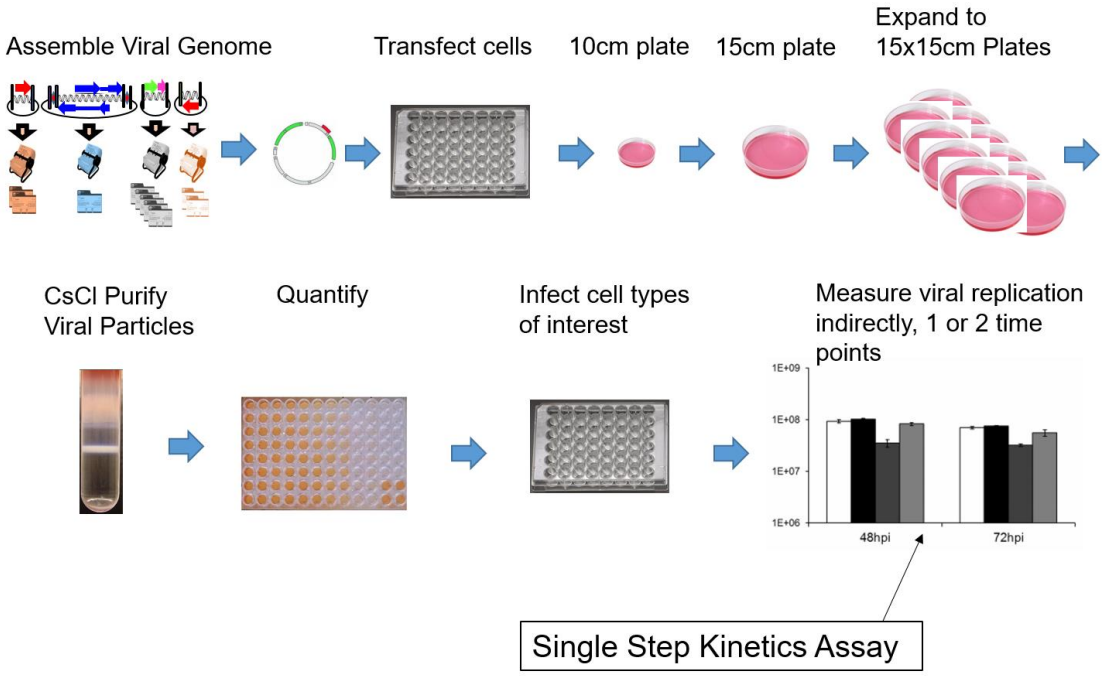

Figure 2.2. Typical workflow for construction, production, and testing of an engineered Adenovirus

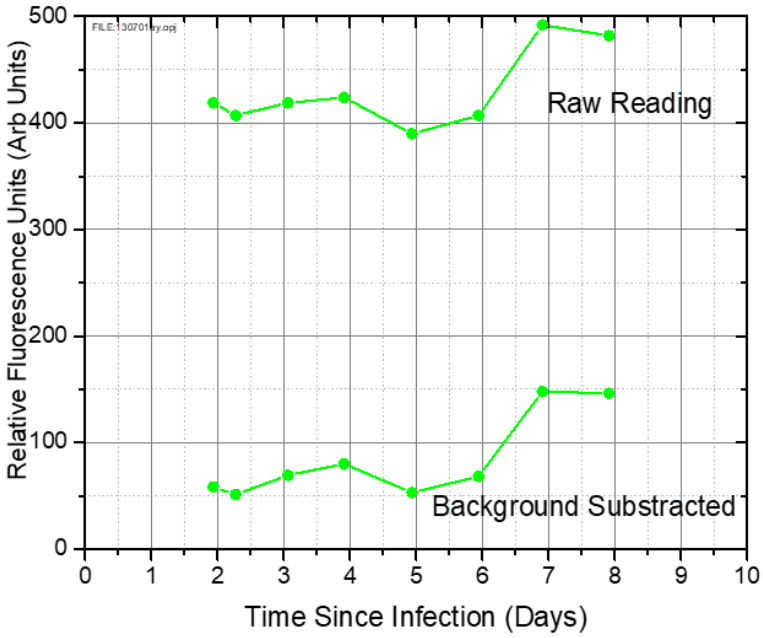

Figure 2.3. Measured Fluorescence vs. time from Ad5 with E1A-GFP fusion.

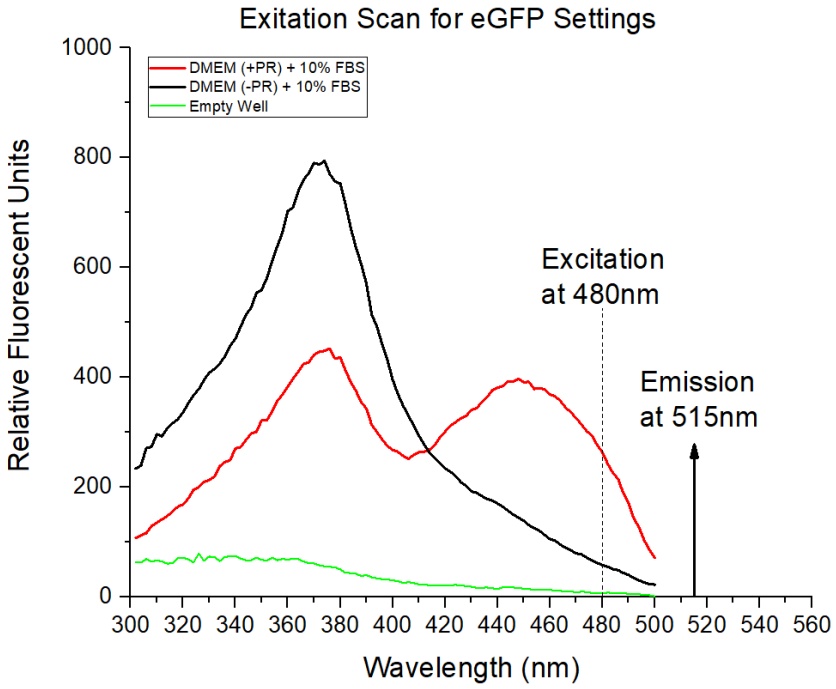

Figure 2.4a. Measured fluorescence spectrum for DMEM media at eGFP emission peak.

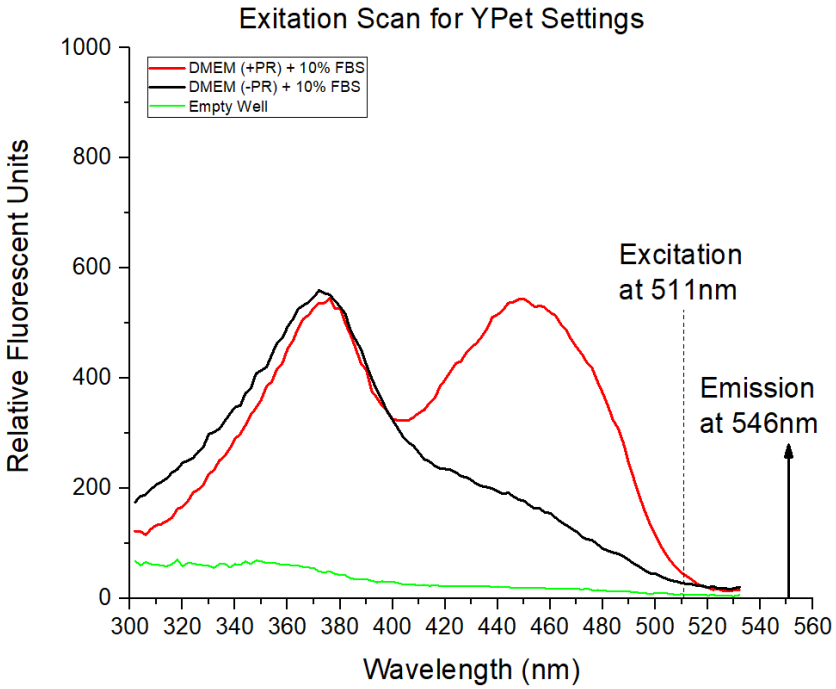

Figure 2.4b. Measured fluorescence spectrum for DMEM media at YPet emission peak.

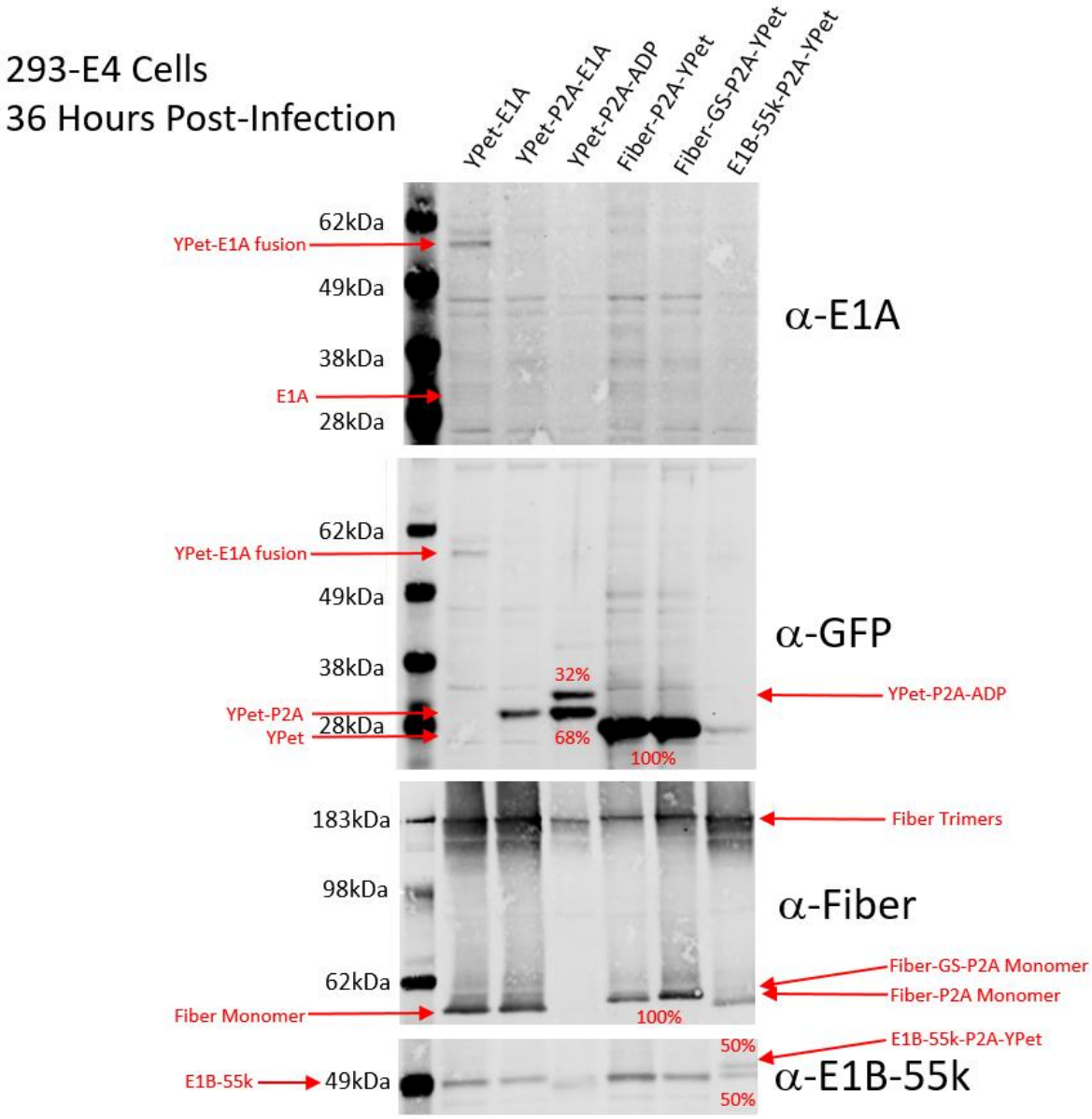

Figure 2.5. The cleavage efficiency of the self-cleaving peptide sequence, P2A, is context dependent. The cleavage efficiency is approximately 50% early in the virus lifecycle (E1B-55k-P2A-YPet), but is essentially 100% for mRNAs expressed late in the virus lifecycle (Fiber-P2A-YPet).

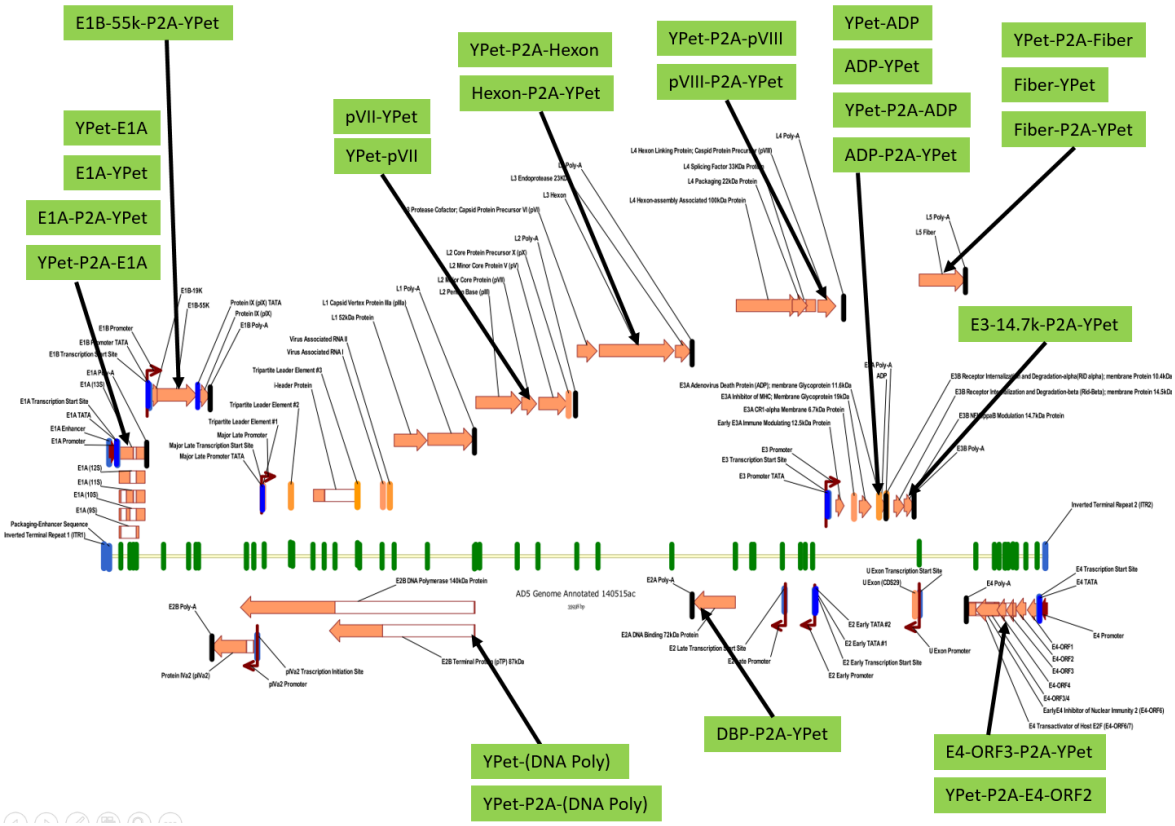

Figure 2.6. Graphical representation of the fluorophore insertion locations within the Ad5 genome.

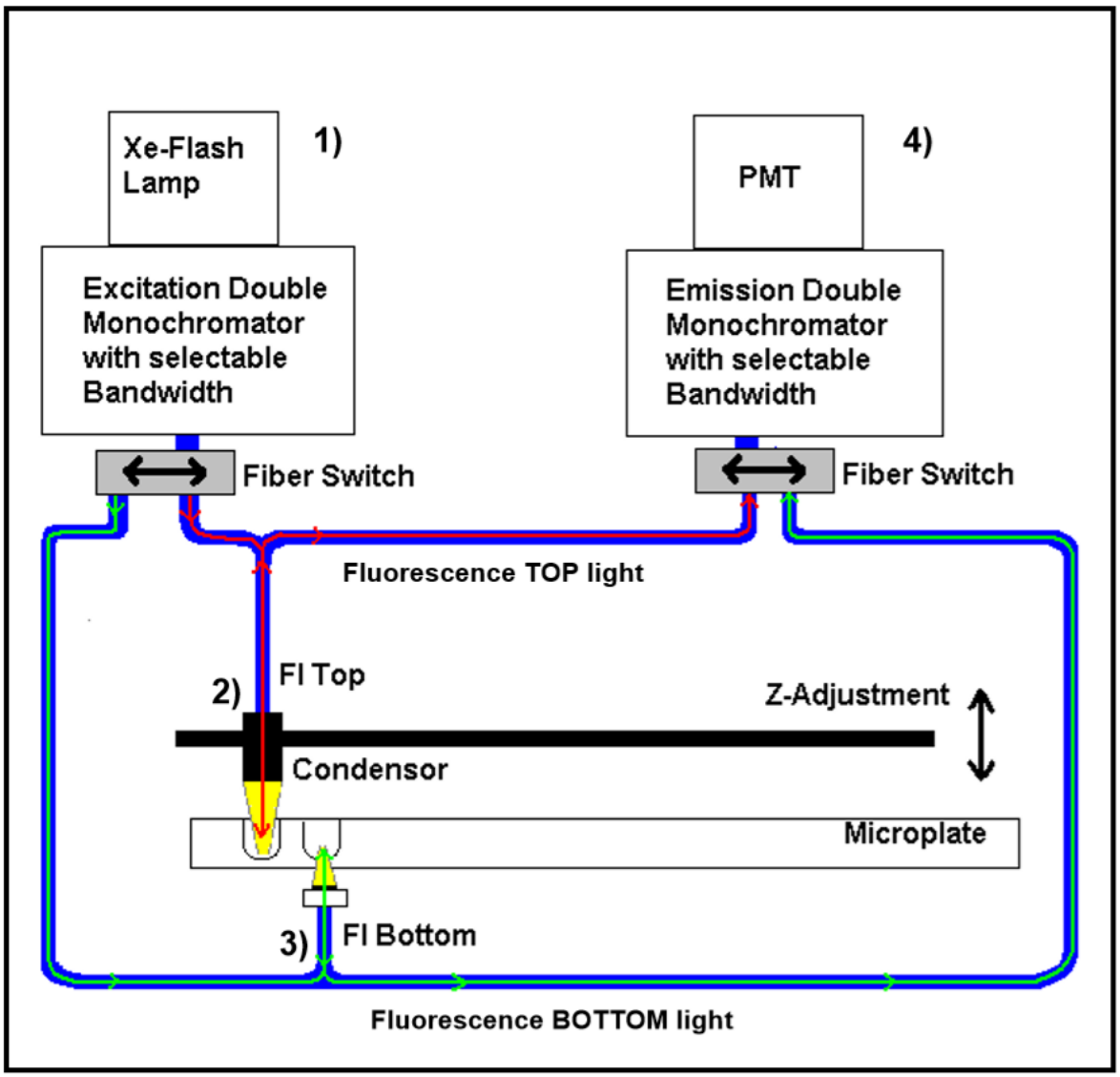

Figure 2.7. Optical layout of TECAN plate readers (figure taken from Infinite M1000Pro users manual).

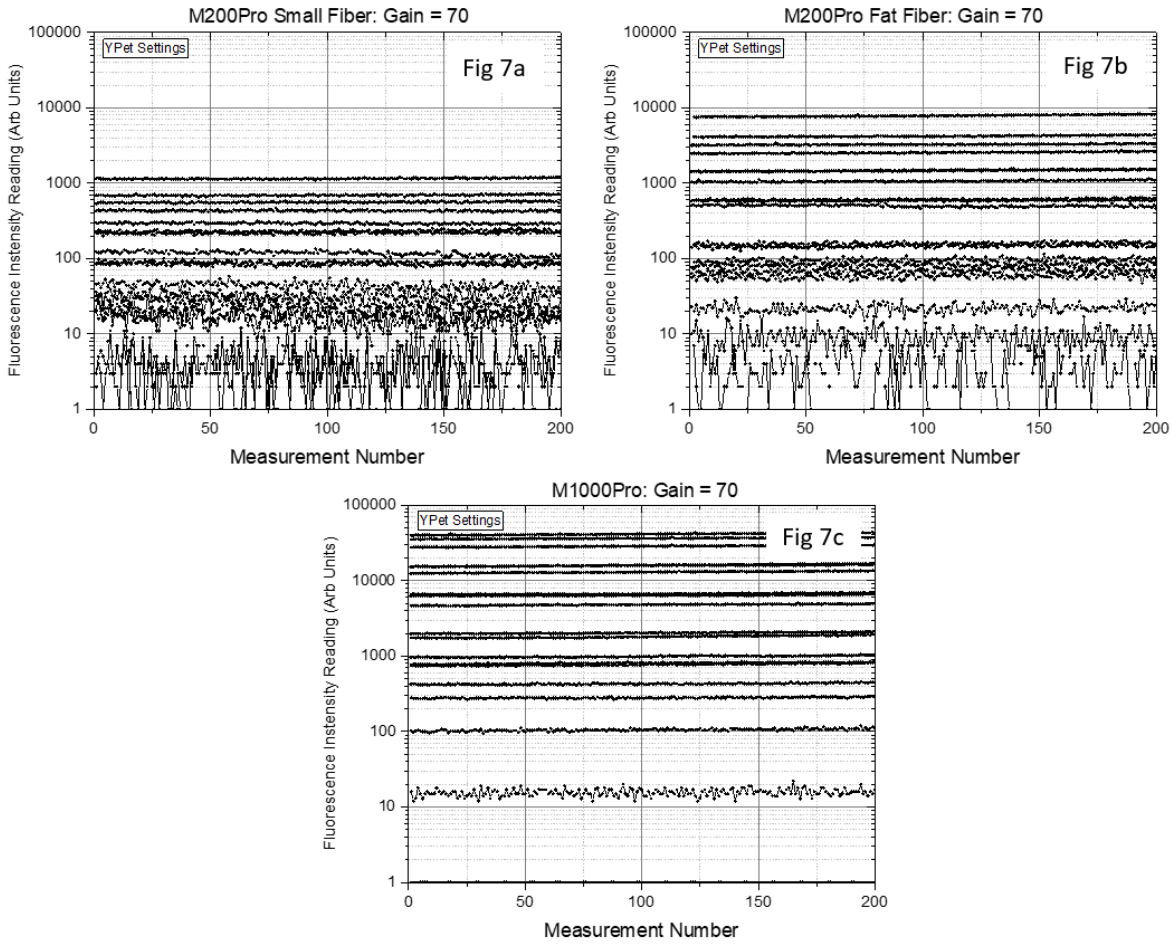

Figure 2.8. Measured fluorescence intensity for a dilution series of fluorescent beads suspended in agarose. 200 measurements made for each fluorescent bead dilution. Plate reader type: a) M200Pro, b) M200Pro with fat fiber, C) M1000Pro.

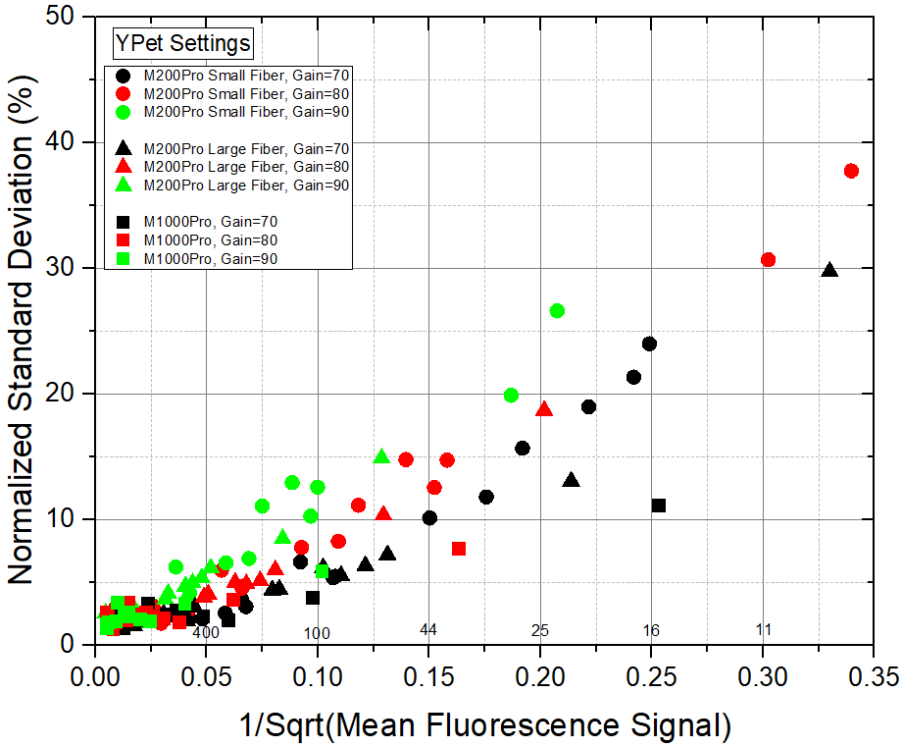

Figure 2.9. Normalized standard deviation of measured fluorescence intensity vs. square root of measured fluorescence for each instrument.

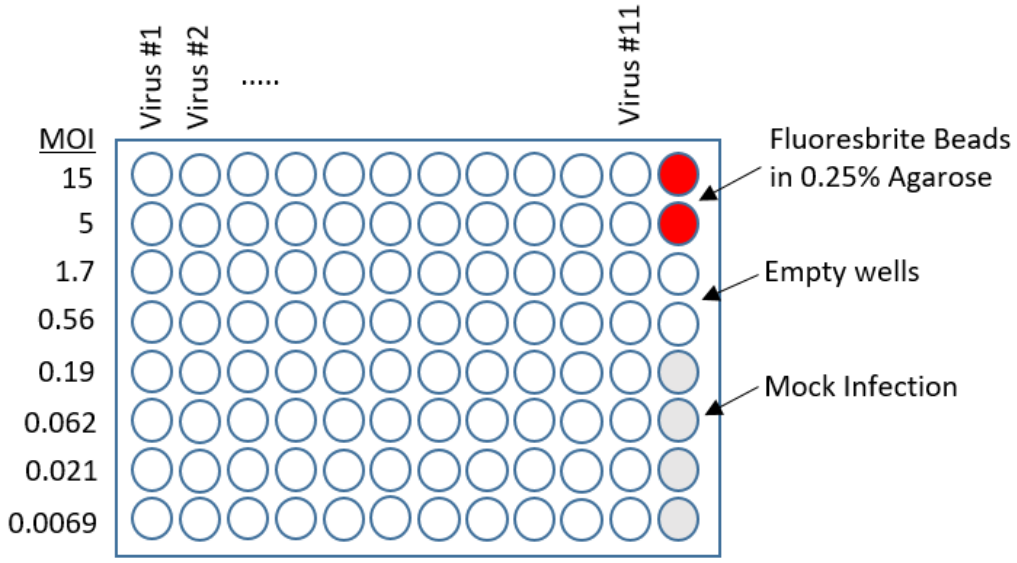

Figure 2.10. Layout for FVBK assay. 11 different viruses can be monitored on a single 96 well plate. The 12th column of wells is used for normalization constant, empty wells for later WST-1 cell viability assay, and Mock infected wells for background subtraction.

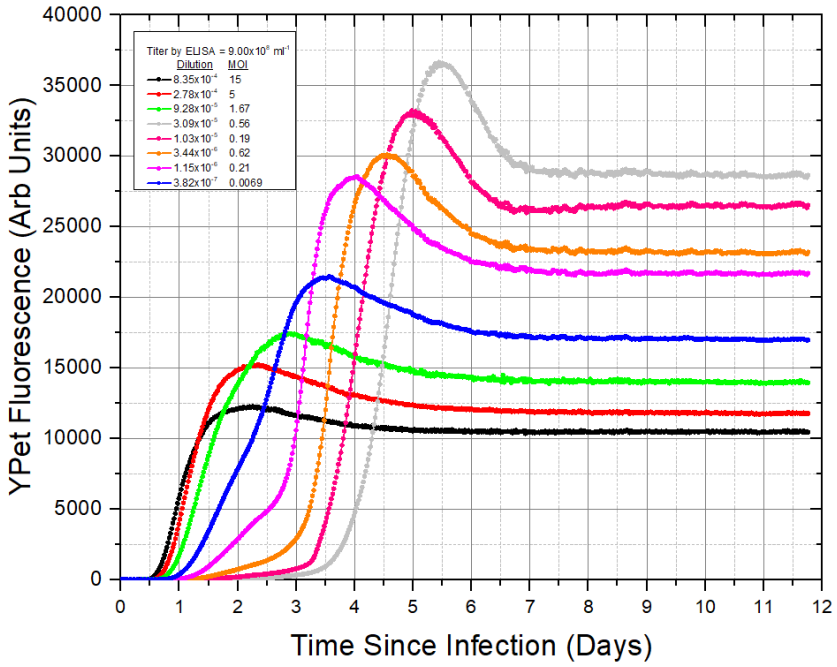

Figure 2.11a. Measured fluorescence signal vs. time for PMCN-421 (YPet-P2A-ADP) infecting A549 cells, plotted on a linear scale.

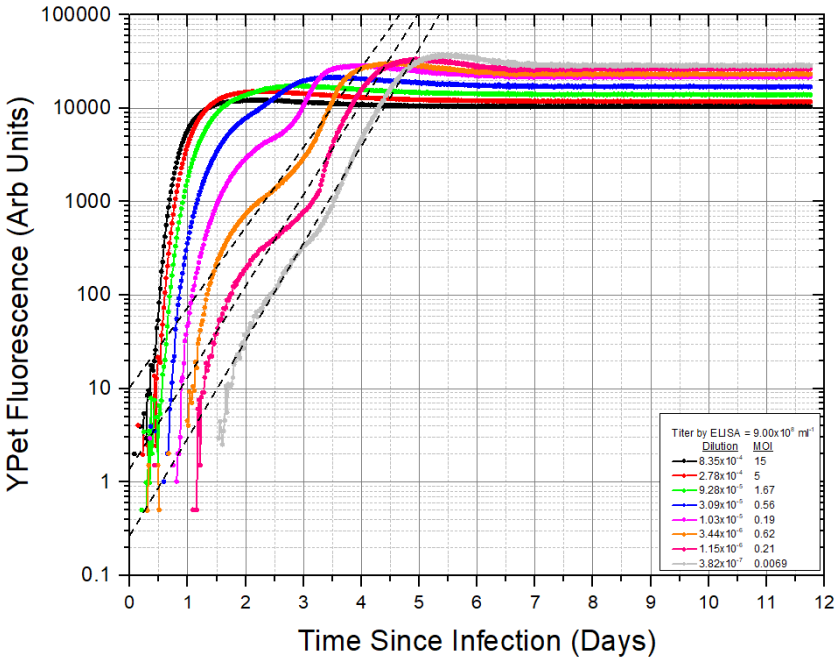

Figure 2.11b. Same data as shown in Fig 2.10a, but plotted on a semi-log graph for PCMNA-421 (YPet-P2A-ADP). The dashed lines show the fits to the logarithmic growth portion of the curves with a resulting ln-slope of 2.20 days^{-1} (+0.20/-0.23)

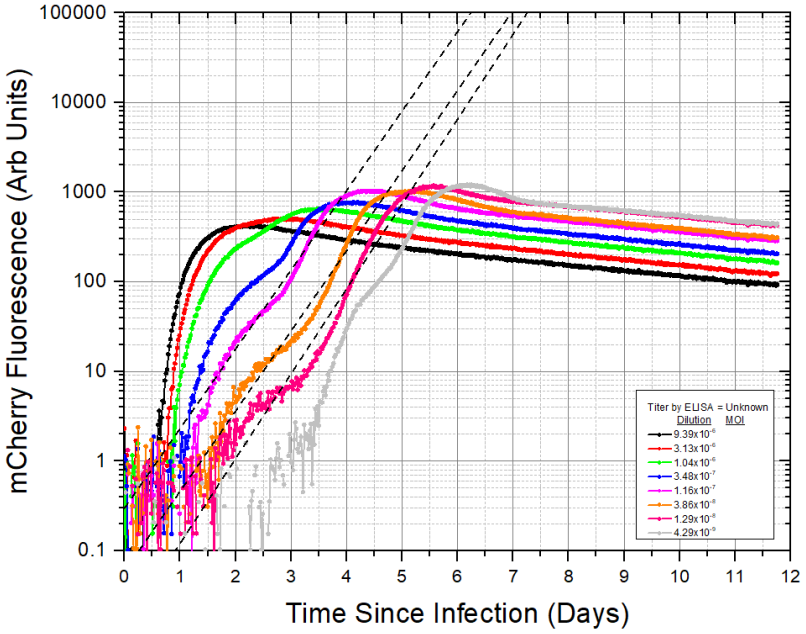

Figure 2.12. Measured fluorescence signal vs. time for PMCN-871 (mCherry-P2A-ADP) infecting A549 cells. The lower signal levels and higher noise values are a result of the lower quantum efficiency of mCherry compared to YPet. The resulting ln-slope is 2.10 $days^{-1}$ (+0.08/-0.06).

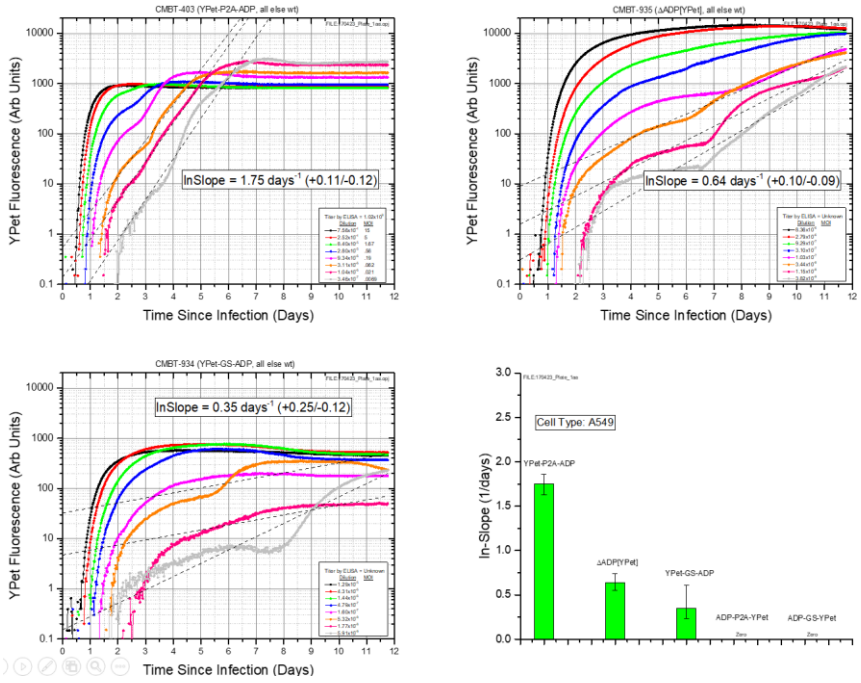

Figure 2.13. Kinetics comparison of viruses with fluorophores expressed coincident with ADP.

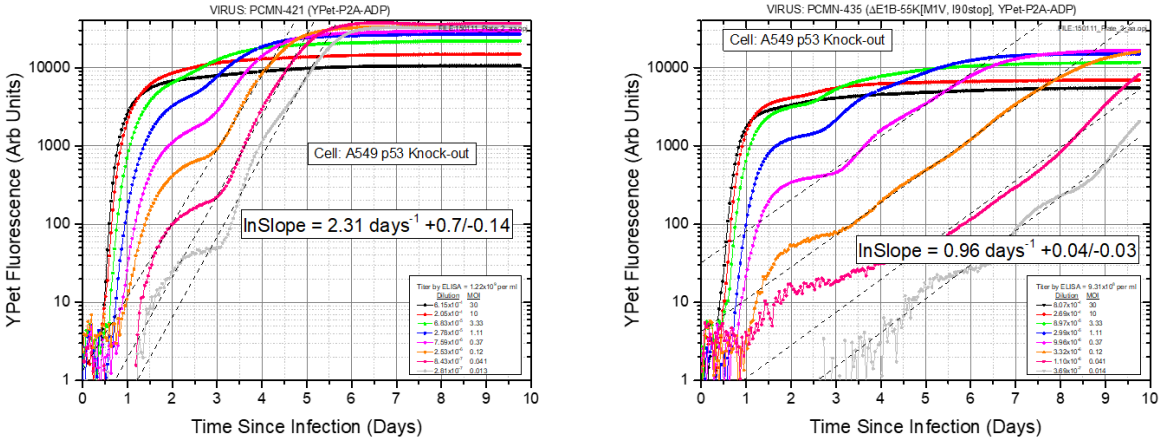

Figure 2.14. Kinetics comparison between wt Ad5 and ONYX-015, an Ad5 virus deleted for expression of E1B-55.

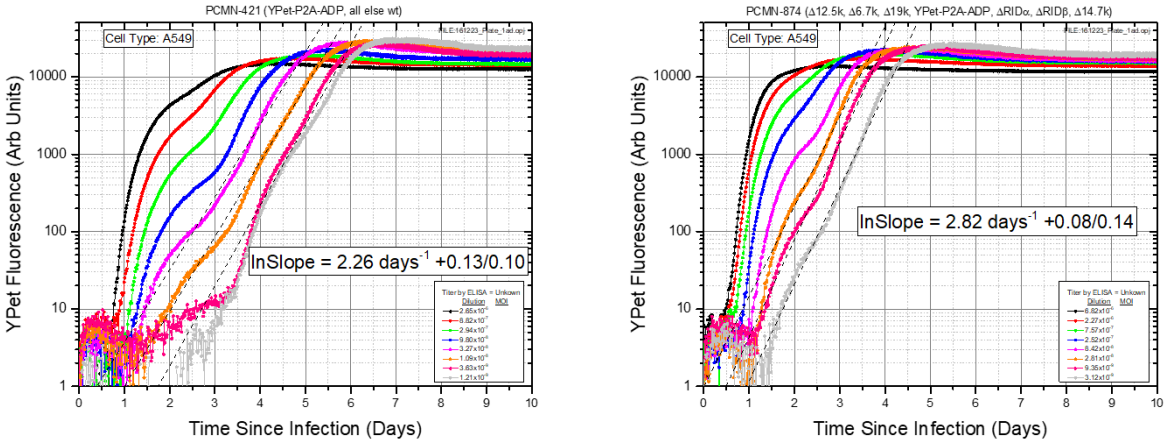

Figure 2.15 Kinetics comparison between wt Ad5 and Ad5 deleted for all E3 genes except ADP.

Table 2.1. Common fluorophores and their properties (taken from ref. 18)

Protein (Acronym)	Ex (nm)	Em (nm)	EC x 10^{-3}	QY	*in vivo* Structure	Relative Brightness (% of EGFP)	References
Blue Fluorescent Proteins							
EBFP	383	445	29.0	0.31	Monomer*	27	Patterson, *et al*., 1997
Sapphire	399	511	29.0	0.64	Monomer*	55	Zapata-Hommer, *et al*., 2003
T-Sapphire	399	511	44.0	0.60	Monomer*	79	Zapata-Hommer, *et al*., 2003
Cyan Fluorescent Proteins							
ECFP	439	476	32.5	0.40	Monomer*	39	Cubitt, *et al*., 1995
mCFP	433	475	32.5	0.40	Monomer	39	Zacharias, *et al*., 2002
Cerulean	433	475	43.0	0.62	Monomer*	79	Rizzo, *et al*., 2004
CyPet	435	477	35.0	0.51	Monomer*	53	Nguyen and Daugherty, 2005
AmCyan1	458	489	44.0	0.24	Tetramer	31	Matz, *et al*., 1999
Midoriishi Cyan	472	495	27.3	0.90	Dimer	73	Karasawa, *et al*., 2004
Green Fluorescent Proteins							
EGFP	484	507	56.0	0.60	Monomer*	100	Heim, *et al*., 1995
aceGFP	480	505	50.0	0.55	Monomer*	82	Gurskaya, *et al*., 2003
TurboGFP	482	502	70.0	0.53	Monomer*	110	Shagin, *et al*., 2004
Emerald	487	509	57.5	0.68	Monomer*	116	Cubitt, *et al*., 1999
Azami Green	492	505	55.0	0.74	Monomer	121	Karasawa, *et al*., 2003
ZsGreen	493	505	43.0	0.91	Tetramer	117	Matz, *et al*, 1999
Yellow Fluorescent Proteins							
EYFP	514	527	83.4	0.61	Monomer*	151	Miyawaki, *et al*., 1999
Topaz	514	527	94.5	0.60	Monomer*	169	Tsien, 1998
Venus	515	528	92.2	0.57	Monomer*	156	Nagai, *et al*., 2002
mCitrine	516	529	77.0	0.76	Monomer	174	Griesbeck, *et al*., 2001
YPet	517	530	104	0.77	Monomer*	238	Nguyen and Daugherty, 2005
PhiYFP	525	537	124	0.39	Monomer*	144	Shagin, *et al*., 2004
ZsYellow1	529	539	20.2	0.42	Tetramer	25	Matz, *et al*., 1999
mBanana	540	553	6.00	0.70	Monomer	13	Shaner, *et al*., 2004
Orange and Red Fluorescent Proteins							
Kusabira Orange	548	559	51.6	0.60	Monomer	92	Karasawa, *et al*., 2004
mOrange	548	562	71.0	0.69	Monomer	146	Shaner, *et al*., 2004
dTomato	554	581	69.0	0.69	Dimer	142	Shaner, *et al*., 2004
dTomato-Tandem	554	581	138	0.69	Monomer	283	Shaner, *et al*., 2004
DsRed	558	583	75.0	0.79	Tetramer	176	Matz, *et al*., 1999
DsRed2	563	582	43.8	0.55	Tetramer	72	Bevis and Glick, 2002
DsRed-Express (T1)	555	584	38.0	0.51	Tetramer	58	Bevis and Glick, 2002
DsRed-Monomer	556	586	35.0	0.10	Monomer	10	Clontech, 2005
tangerine	568	585	38.0	0.30	Monomer	34	Shaner, *et al*., 2004
mStrawberry	574	596	90.0	0.29	Monomer	78	Shaner, *et al*., 2004
AsRed2	576	592	56.2	0.05	Tetramer	8	Matz, *et al*., 1999
mRFP1	584	607	50.0	0.25	Monomer	37	Campbell, *et al*., 2002
JRed	584	610	44.0	0.20	Dimer	26	Shagin, *et al*., 2004
mCherry	587	610	72.0	0.22	Monomer	47	Shaner, *et al*., 2004
HcRed1	588	618	20.0	0.015	Dimer	1	Gurskaya, *et al*., 2001
mRaspberry	598	625	86.0	0.15	Monomer	38	Wang, *et al*., 2004
HcRed-Tandem	590	637	160	0.04	Monomer	19	Fradkov, *et al*., 2002
mPlum	590	649	41.0	0.10	Monomer	12	Wang, *et al*., 2004
AQ143	595	655	90.0	0.04	Tetramer	11	Shkrob, *et al*., 2005

Table 2.2. Viruses with various fluorophore inserts infecting A549 cells. Expression levels are relative to CMBT-403 set to 10,000. The ln-slope value for virus kinetics is the exponential growth factor determined by fitting to a semi-log plot of fluorescence vs. time. Viruses labeled as "failed" could not be produced in 293 cells.

Virus Number	**Insertion**	**Expression Level**	**Ln-slope (days^{-1})**
CMBT-352	YPet-E1A	100	0.67 (+0.13/-0.13)
CMBT-379	YPet-P2A-E1A	1000	1.30 (+0.24/-0.23)
CMBT-1058	E1A-YPet	100	1.46 (+0.23/-0.30)
CMBT-432	E1A-P2A-YPet	600	1.82 (+0.18/-0.18)
PCMN-950	E1A-P2A-YPet	500	2.12 (+0.12/-0.15)
CMBT-456	E1B-55k-P2A-YPet	400	1.72 (+0.22/-0.11)
CMBT-590	YPet-DNA Polymerase	Failed	
CMBT-530	YPet-P2A-DNA Polymerase	350	1.82 (+0.39/-0.37)
CMBT-886	DBP-P2A-YPet	4,000	1.54 (+0.09/-0.10)
PCMN-968	DBP-P2A-YPet	4,000	1.68 (+0.30/-0.21)
CMBT-934	YPet-ADP	7,000	0.36 (+0.25/-0.13)
CMBT-403	YPet-P2A-ADP	10,000	1.75 (+0.11/-0.12)
PCMN-421	YPet-P2A-ADP	10,000	2.20 (+0.20/-0.25)
CMBT-1190	ADP-YPet	Failed	
CMBT-429	ADP-P2A-YPet	Failed	
CMBT-930	ΔADP[YPet]	90,000	0.64 (+0.10/-0.09)
CMBT-890	E3-14.7k-P2A-YPet	150,000	0.97 (+0.08/-0.08)
PCMN-887	E3-14.7k-P2A-YPet	150,000	1.27 (+0.12/-0.06)
CMBT-457	YPet-P2A-E4-ORF2	Failed	
CMBT-900	E4-ORF3-P2A-YPet	1,200	0.63 (+-.09/-0.05)
CMBT-899	YPet-pVII	Failed	
CMBT-893	pVII-YPet	100	No Replication in A549
CMBT-847	Hexon-P2A-YPet	Failed	
CMBT-863	YPet-P2A-Hexon	Failed	
CMBT-534	pVIII-P2A-YPet	800	2.00 (+0.40/-0.32)
CMBT-873	YPet-P2A-pVIII	Failed	
CMBT-407	YPet-P2A-Fiber	Failed	
CMBT-938	Fiber-YPet	Failed	
CMBT-445	Fiber-P2A-YPet	50,000	0.34 (+0.05/-0.04)

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CHAPTER THREE:

Kinetics Comparison Between Ad3, Ad5, Ad9, and Ad34 Serotypes and their Fiber and Shaft Chimeras

Adenovirus Tropism

An important condition for a potent oncolytic Ad is to match the tropism of the virus to that of the cell type or tissue of interest. For example, an oncolytic based on the human Ad9 serotype, with ocular tropism, would not be expected to exhibit fast replication in a prostate tumor. Each serotype for the human adenovirus has evolved molecular characteristics that make it best suited for replication in a subset of tissue types. These characteristics include initial binding affinity, cell entry, and coopting host cellular machinery. There is an extensive body of literature describing genetic modifications to the Ad fiber in order to change or broaden the range of cell types with which the fiber knob binds with high initial binding affinity[1-24]. Much modifications include replacement of the fiber knob, and/or shaft and knob, with one from an alternate serotype, insertion of a heterologous peptide sequence with a known binding affinity into the fiber knob, or fusion of a heterologous peptide sequence to a capsid protein. All of these genetic modifications lead to a fully assembled virion that presents a binding surface with different, and hopefully, higher affinity for a targeted cellular receptor.

The motivations behind the extensive pervious work on modifying Ad tropism are two-fold. The first is to engineer a virus with highly specific tropism for a selected tissue type or tumor type. Such selectivity should increase the safety profile of any treatment using this virus since off-target viral entry would be minimized. The second motivation for modifying Ad tropism is to achieve pan-tropic properties of an Ad virus. Such a virus could then be used to treat a variety of tumor or tissue types without the need for genetic

re-engineering. It is the search for pan-tropic performance that leads us to investigate Ad serotypes beyond just Ad5.

The 57 known serotypes of Adenovirus provide us with a rich and diverse set of components from which to choose. Table 3.1 shows these serotypes arranged into 8 subgroups, along with the proposed tropism for each subgroup[25].

Replication Competent Virus vs. Vector

The majority of the work cited in references 1-24 involve Ad vectors rather than replication competent viruses. Due to the lack of replication in the target cell for a vector, there is no method, nor any need, to measure the replication kinetics of such modified viruses. One can imagine a modification that leads to highly efficient target cell entry, but causes very slow new virion assembly. Such a virus would work fine as a vector, but not as a replicating oncolytic virus. Because this thesis project is focused on replicating viruses, rather than on vectors, impacts to the full lifecycle of the virus must be assessed when judging the performance of a modified virus inside a target cell type. The Fluorescence-Based Viral Kinetics (FBVK) assay described in chapter 2 was developed to address the need for comparing kinetics across a range of Ad serotypes and within a variety of cell types.

Kinetics Testing Across Serotypes and Chimeras

Table 3.1 lists the human Ads collected by subgroup, showing their known receptors and tropism. We made the assumption that Ad serotypes within a subgroup have

similar characteristics and thus we chose a sample from four major subgroups as representative examples for study.

Subgroup	Serotype
B1	Ad3
B2	Ad34
C	Ad5
D	Ad9

We also constructed fiber and fiber/shaft chimeric viruses as listed in tables 3.2 and 3.3. The nomenclature used to describe these serotypes and chimeras is detailed in Fig 3.2. For example, Ad5 5/5/5 represents wildtype Ad5 and Ad5 5/5/34 represents Ad5 with an Ad34 knob.

With these serotypes and chimeras in mind, the challenge is to choose a location for the fluorophore that can be common to all constructs. As shown in chapter 2, the YPet-P2A-ADP construct exhibits high signal level and fast virus replication when inserted into the Ad5 genome. Unfortunately, serotypes other than Ad2 and Ad5 do not possess an equivalent ORF to ADP. It has been shown that the E3 genes of all serotypes are dispensable in tissue culture[26], so we restricted our choices of fluorophore location to the E3 ORFs. Fig 3.1 shows maps of the ORFs for each Ad subgroup[27]. Homology analysis shows that only one ORF is common to all of the subgroups, E3-14.7k. This ORF is highlighted in red. Besides its existence in all subgroups, E3-14.7k has the advantage that it is located at the very end of the E3 region and nearly at the end of the Major Late Transcript. Fusing a fluorophore to the c-terminus of this ORF is expected to be least

likely to impact the splicing program for all ORFs upstream. All of the serotypes and chimeras listed in tables 3.2 and 3.3 include an E3-14.7k-P2A-YPet readout (or equivalent for Ad3, Ad9, and Ad34 cores).

Each of these constructs was initially tested in A549 cells (lung adenocarcinoma), with the kinetics curves shown in Figs 3.3a-3.3f. Dashed lines on each graph show the best fits to the ln-phase growth portion of each curve. The resulting ln-slope values are summarized in Fig 3.4. It was surprising to find that wt Ad34 exhibits equivalent, or slightly better, kinetics compared to wt Ad5. Ad5 is thought to have natural tropism for lung tissue, while renal tissue is the proposed natural host for Ad34. But, the even greater surprise was the significantly faster kinetics for the Ad34 34/5/5 chimera, but not the Ad34 34/34/5 chimera. The difference between these two chimeric viruses is the choice of shaft. Use of the Ad5 shaft in this chimeric virus leads to a significant enhancement in replication kinetics.

To help understand possible reasons for this shaft-dependent improvement, Fig 3.5 schematically shows the fibers for each of the four serotypes used in this study. Though the tail and knob portions of each fiber are nearly identical in size, based on amino acid count, each shaft differs considerably in length. The Ad5 shaft is approximately 4 times longer than that of the Ad34 shaft. Our results corroborate findings already in the literature. For example, Wu et. al.[28] studied the transduction efficiency of chimeric viruses based on Ad5 and Ad37 in A549 cells. Their results show that the transduction efficiency with (Ad5 shaft + Ad5 knob) was almost 7 times higher than that of (Ad37 shaft + Ad5

knob). The Ad37 shaft consists of 138 AAs, and thus is slightly longer than the Ad34 shaft with 88 AAs, but still significantly shorter than the Ad5 shaft with 354 AAs.

Virus Replication Kinetics in PC3 Cells

As noted in the introduction of this chapter, these serotypes and chimeras where constructed in order to test their kinetics in cell types to be used in the development of the positively-regulated virus described in chapter 4. Because the chosen target organ for the positively-regulated virus is the prostate, we measured the kinetics of all serotypes and chimeras when infecting PC3 cells. The PC3 cell line is a canonical prostrate cell line used in prostate cancer research. PC3 cells do not require androgen signaling to initiate cell growth and multiplication. We will use this cell line as a negative control for viruses engineered to replicate in the presence of prostate-specific antigen promoter activation.

Upon infecting with the matrix of serotypes and chimeras, we found no evidence of replication for any of the viruses. Fig 3.6 shows an example of the kinetics curves for ad5 wt (Ad5 5/5/5, PCMN-887) using the same conditions as those for the A549 infections. Each dilution reached a maximum fluorescence signal after 2-3 days and remained constant for rest for the assay. These curves look just like those for a replication-defective virus. These results do not match those from the literature showing replication of Ad5 wt, and its chimeras, in PC3 cells[29-31]. In previous work we found that periodic media exchange was required to maintain the host cells in a state able to support virus replication. To test if periodic media exchange was required for PC3 cells, we repeated the infections but this time performed a 50% volume media exchange every 24 hours, with the results

shown in Fig 3.7. We found that with this rate of media exchange, the fluorescence growth stalled near the end of each 24 hour period and rapidly increased upon media exchange. Under these media exchange conditions, there is some evidence of virus replication, but at a very slow rate.

The stall in fluorescence growth near the end of each 24 period gave us a hint that something within the media was becoming exhausted or saturated, and each media exchange returned conditions back to those supportive of virus replication. Based on these curves, we hypothesized that a more frequent media exchange rate would ameliorate the media exhaustion. To that end, we repeated the infections again, but this time with 50% volume media exchange every 12 hours, with the results shown in Fig 3.8. The curves now show no stall in fluorescence signal growth and there is clear evidence of virus replication.

With the media exchange conditions addressed, a comparison between all the serotypes and chimeras was made, as shown in Fig 3.9. The chimeric virus, Ad5 5/5/34, shows significantly better kinetics than wt Ad5 and would be the logical choice for a starting baseline for a prostate-specific positively-controlled oncolytic virus.

Virus Replication Kinetics in a Variety of Tissue Types

We can apply this matrix of serotypes and chimeras to any cell time amendable to standard tissue culture techniques. Figs 3.10a-3.10f show the measured ln-slops for all the serotypes and chimeras when infecting cell lines representing seven different tumor types. Among the wt viruses, Ad5 shows the greatest consistency in replication kinetics across

this varied list of cell types, with Ad34 a close second. We were surprised to find that the wt versions of Ad3 and Ad9 do not exhibit notably high kinetics in any of the cell types tested. The tropism for Ad3 listed in table 3.1 includes respiratory, yet Ad3 performs poorly in A549 cells (lung adenocarcinoma). Ocular tropism is listed for both Ad3 and Ad9, so testing a cell type taken from the eye might lead to better performance.

When the Ad5 core is used in a knob-only chimera (Fig 3.10b), it can be made essentially pan-tropic when paired with the Ad34 knob. The Ad34 knob is thought to bind to the CD46 receptor and CD46 is expressed on all nucleated cells[32]. Besides its near-ubiquitous expression, the CD46 receptor has been found to be upregulated in certain cancers as a means to avoid the complement system[33]. These aspects of CD46 and the Ad34 knob, when combined with the Ad5 core, make it a promising choice as an oncolytic virus.

Except for the anomalously fast kinetics of Ad34 34/5/5 in A549 cells (Fig 3.10f), none of the other serotype cores, alone, or when combined with the Ad5 knob or Ad5 shaft and knob show improvement over wt Ad5 in any of the seven cell lines tested. This high effectivity of the Ad5 core may explain its dominant seroprevalence (table 3.1).

Conclusion

As a starting point for development of a pan-tropic oncolytic virus, the Ad5 5/5/34 chimera exhibits the desired properties. This demonstrates highly efficient entry in a broad range of tumor cell types and replicates well after initial entry.

It is interesting that several of the serotypes tested never show best kinetics in any of the example cell lines tested. One would have thought that superior performance of each serotype would follow each of their published tropism. The fallacy with this assumption is that the published tropisms for many of the Ad serotypes are simply the first organ from which an isolate is obtained. First discovery of a serotype in an organ, possibly under unusual circumstances, does not necessarily dictate that the discovered serotype is optimized for said organ and is superior to all other known serotypes.

Figures and Tables

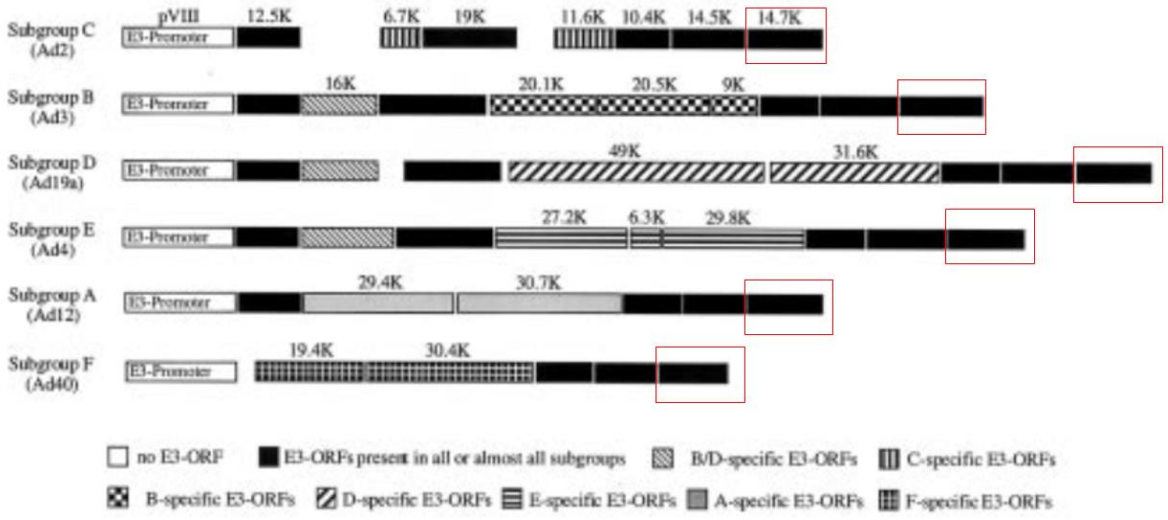

Figure 3.1. Maps of the ORFs for the E3 regions of the subgroups of human Ad, taken from reference 27. The ORF homologous to E3-14.7k is highlighted for each subgroup.

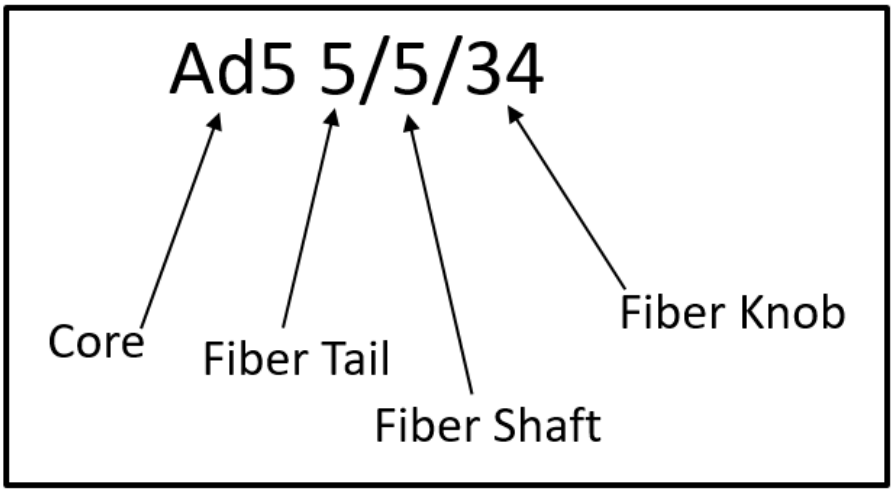

Figure 3.2. Nomenclature used to describe Ad serotypes and chimeras. This example represents an Ad5 virus with an Ad34 knob.

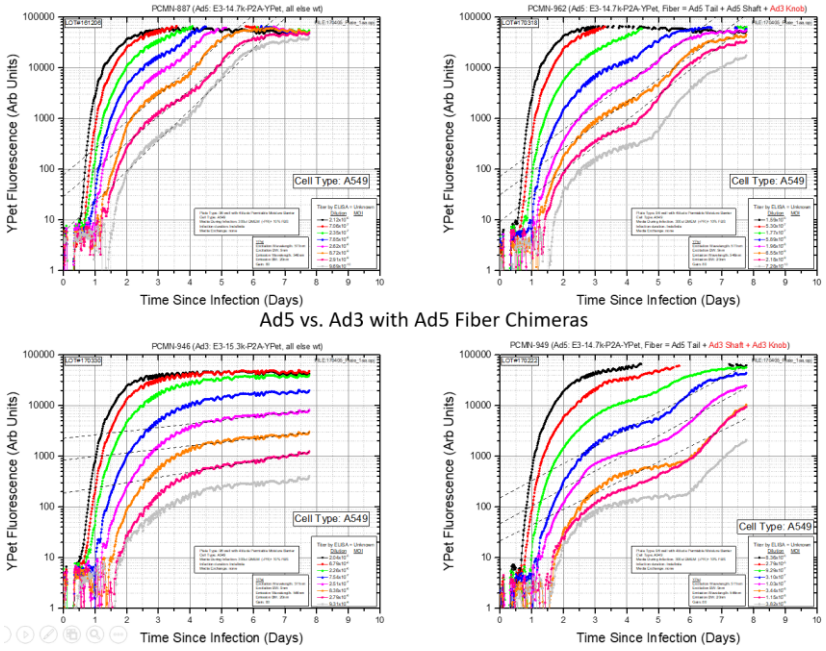

Figure 3.3a. Kinetics curves for Ad5 5/5/5 (wt), Ad3 3/3/3 (wt), Ad5 5/5/3, and Ad5 5/3/3 in A549 cells.

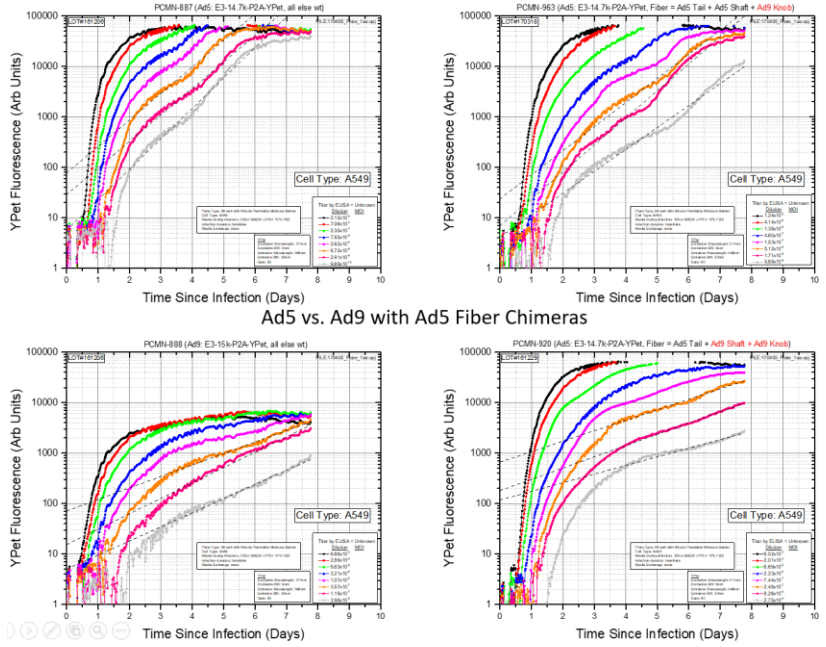

Figure 3.3b. Kinetics curves for Ad5 5/5/5 (wt), Ad9 9/9/9 (wt), Ad5 5/5/9, and Ad5 5/9/9 in A549 cells.

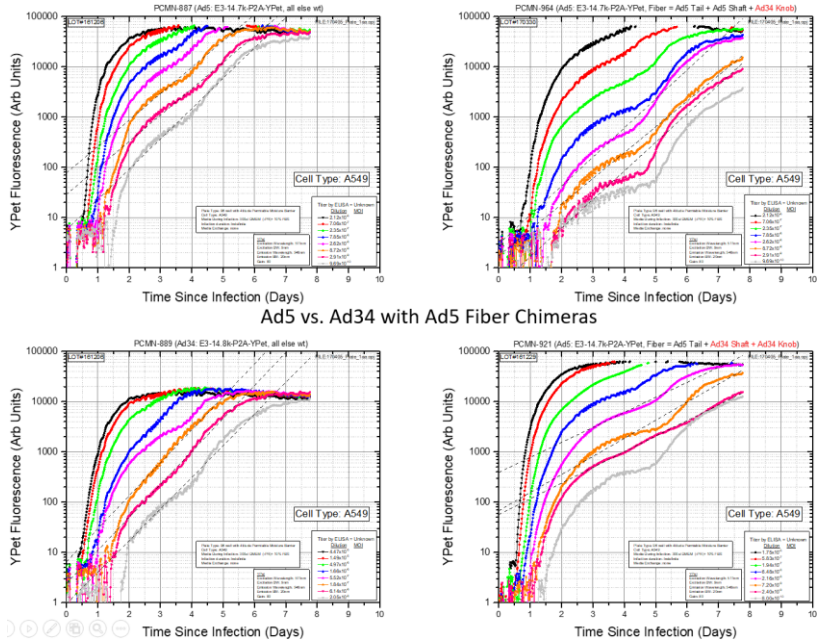

Figure 3.3c. Kinetics curves for Ad5 5/5/5 (wt), Ad34 34/34/34 (wt), Ad5/5/34, and Ad5 5/34/34 in A549 cells.

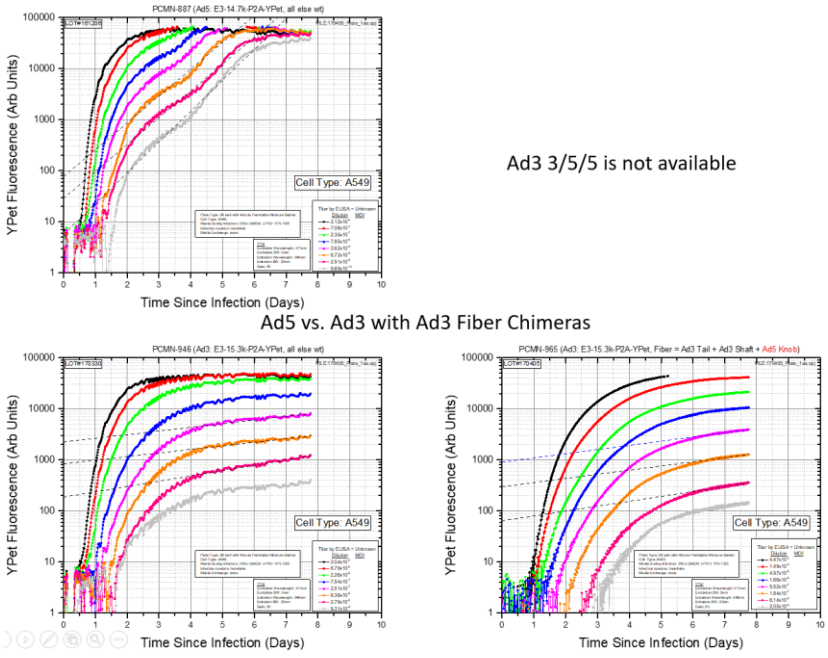

Figure 3.3d. Kinetics curves for Ad5 5/5/5 (wt), Ad3 3/3/3 (wt), Ad3 3/3/5, and Ad3 3/5/5 (data not available) in A549 cells.

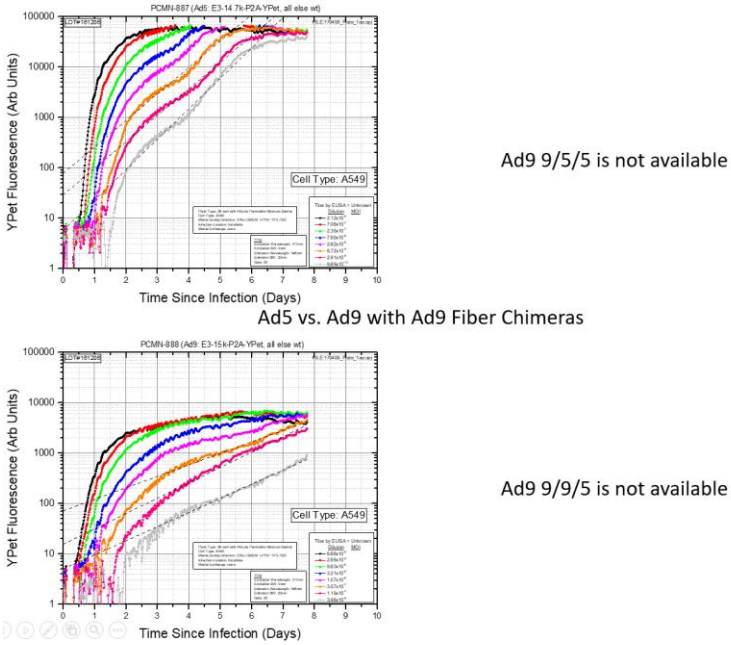

Figure 3.3e. Kinetics curves for Ad5 5/5/5 (wt), Ad9 9/9/9 (wt), Ad9 9/9/5 (data not available), and Ad9 9/5/5 (data not available) in A549 cells.

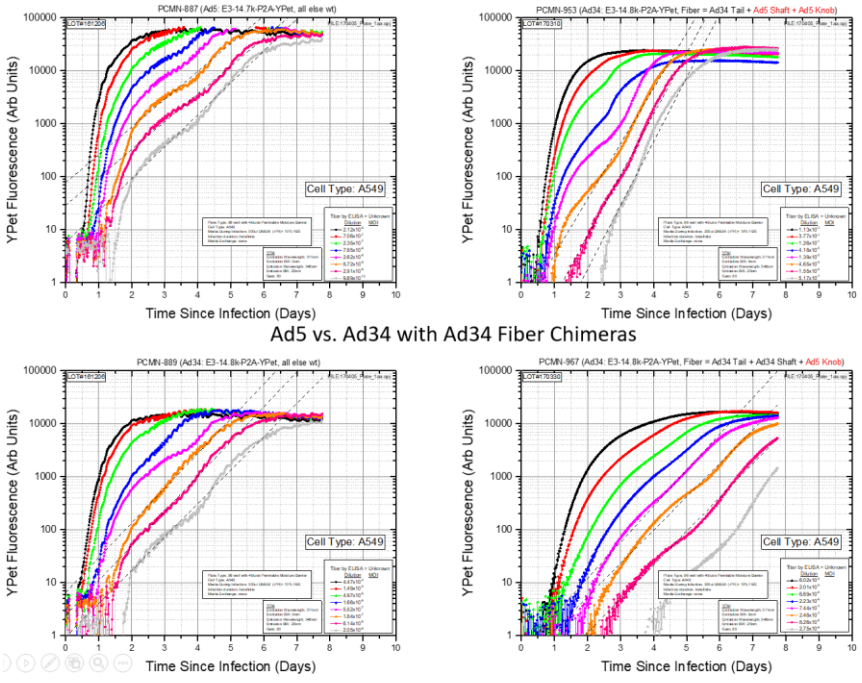

Figure 3.3f. Kinetics curves for Ad5 5/5/5 (wt), Ad34 34/34/34 (wt), Ad34 34/34/5, and Ad34 34/34/5 in A549 cells. Note the significantly faster kinetics for Ad34 34/5/5 compared to all others.

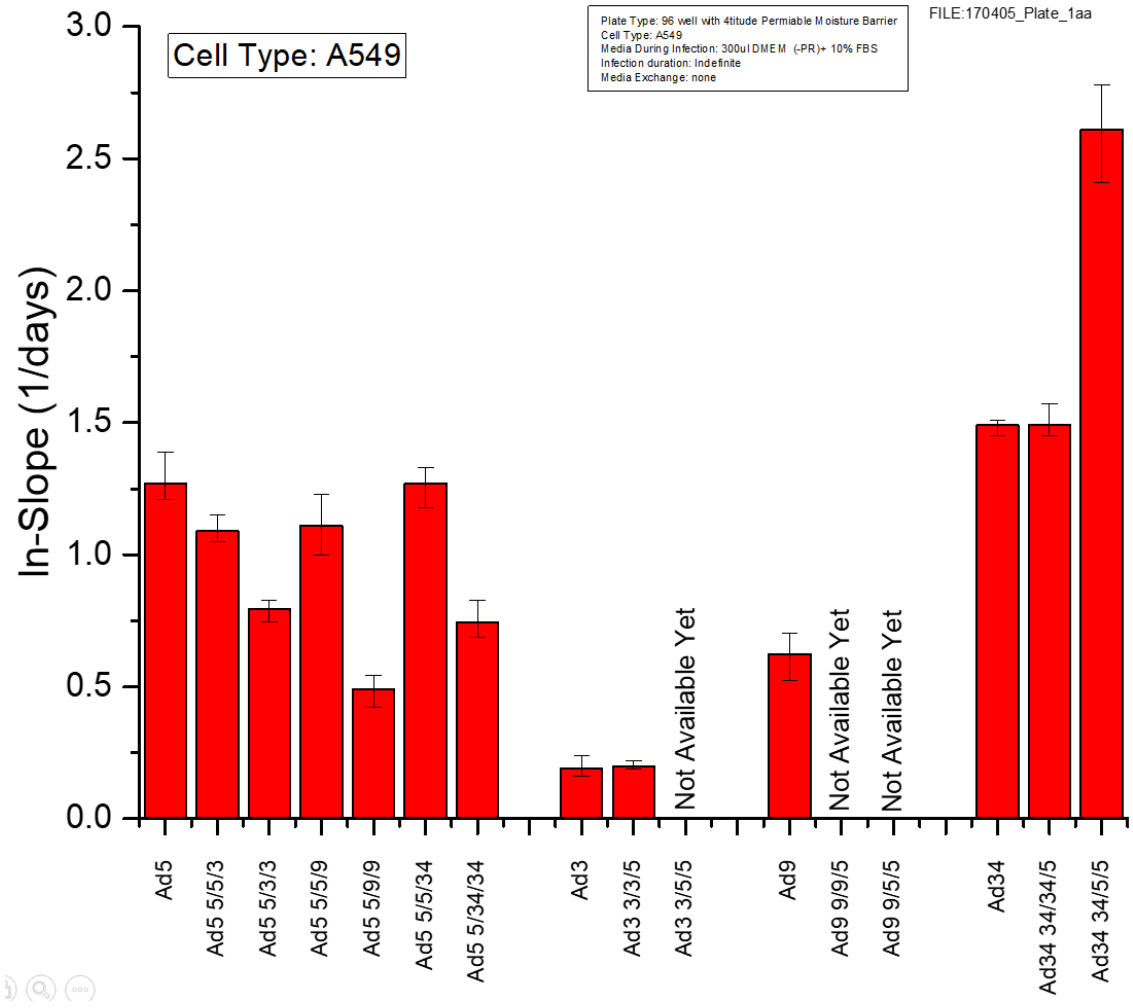

Figure 3.4. The calculated ln-slopes for each serotype and chimera in A549 cells. Note the surprising result that wt Ad34 is slightly faster than wt Ad5 and the Ad34 34/5/5 chimera is significantly faster than wt Ad5, even though Ad5 has natural tropism for lung tissue and Ad34 has tropism for renal cells.

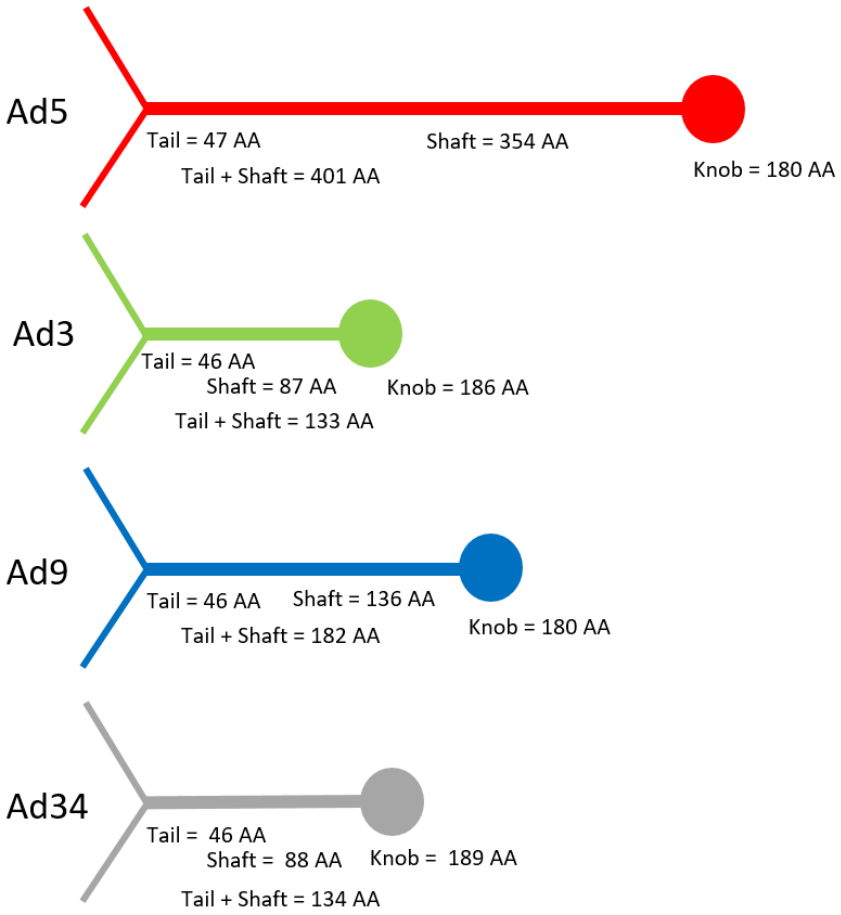

Figure 3.5. The fibers for each of the Ad3, Ad5, Ad9, and Ad34 serotypes. Listed are the amino acid count for the tail, shaft, and knob portions of each fiber. The tails and knobs are all nearly identical in size, but the length of each shaft, as inferred by AA count, is significantly different. The shaft length ratios are as follows: Ad5/Ad3 = 4.1X, Ad5/Ad9 = 2.6X, and Ad5/Ad34 = 4.0X.

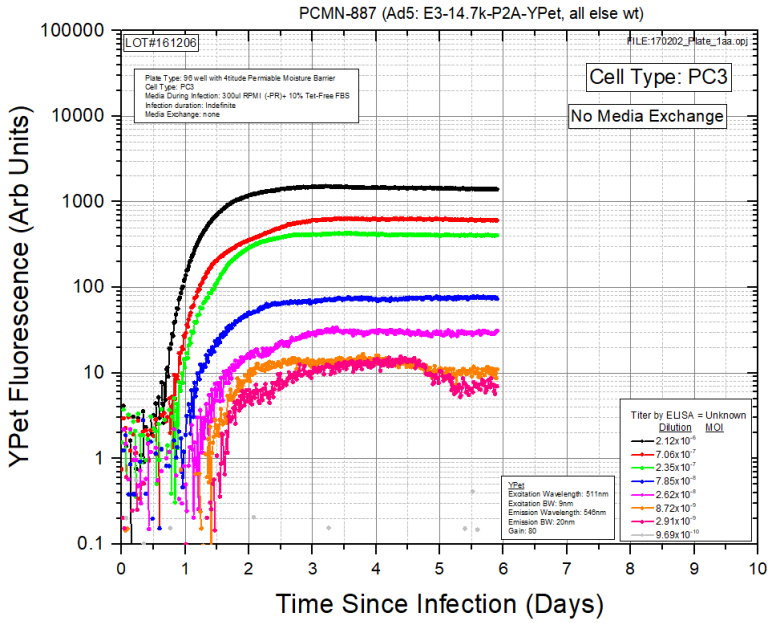

Figure 3.6. Kinetics curves for Ad5 wt (Ad5 5/5/5, PCMN-887) when infecting PC3 cells without any media exchange.

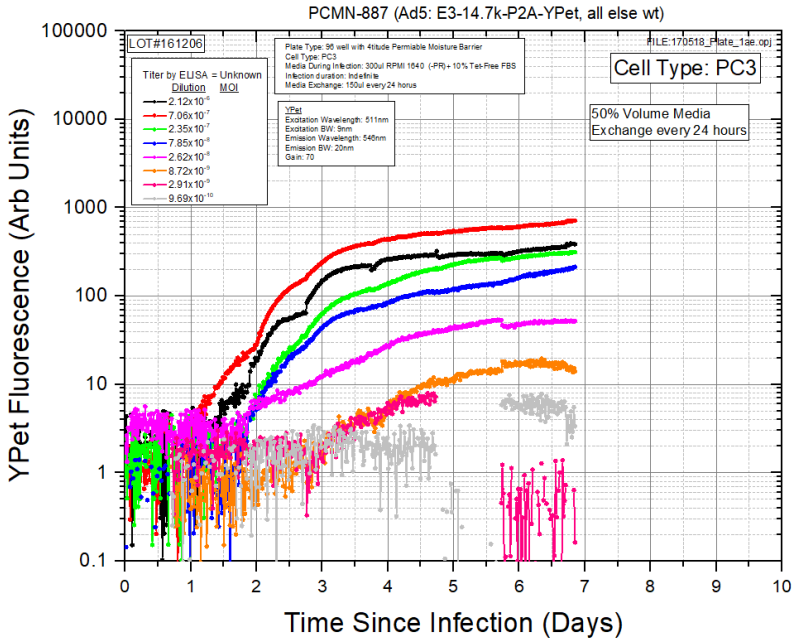

Figure 3.7. Kinetics curves for Ad5 wt (Ad5 5/5/5, PCMN-887) when infecting PC3 cells with 50% volume media exchange every 24 hours. Note the stall in fluorescence growth near the end of each 24 hour period with rapid increase following media exchange (red and black curves).

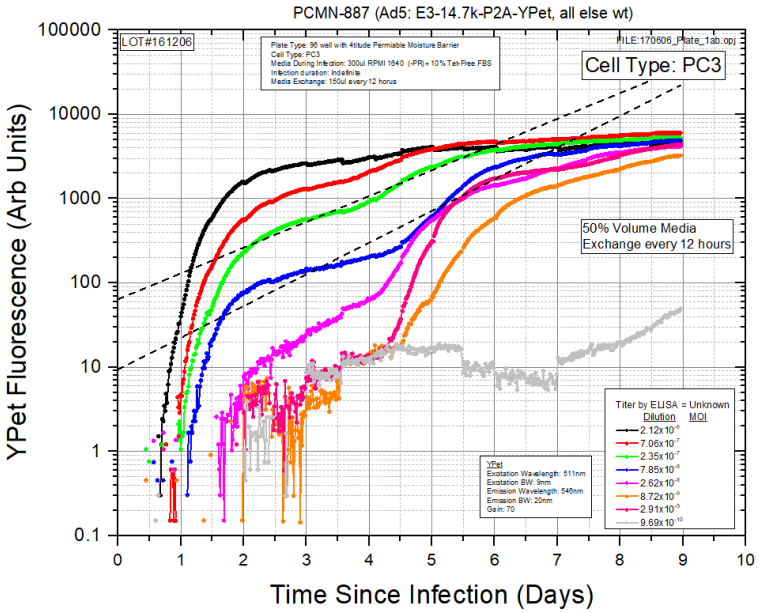

Figure 3.8. Kinetics curves for Ad5 wt (Ad5 5/5/5, PCMN-887) when infecting PC3 cells with 50% volume media exchange every 12 hours. Note the lack of stall in fluorescence signal growth, as well as clear evidence of virus replication.

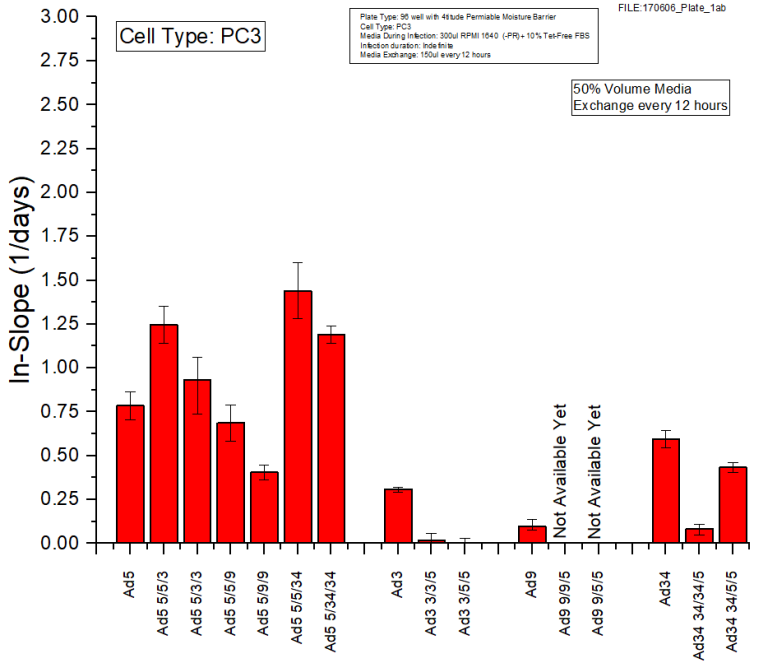

Figure 3.9. Measured ln-slope values for all serotypes and chimeras when infecting PC3 cells with 50% volume media exchange every 12 hours. Note the significant improvement in replication kinetics when the Ad5 core and shaft is paired with the Ad34 knob (Ad5 5/5/34).

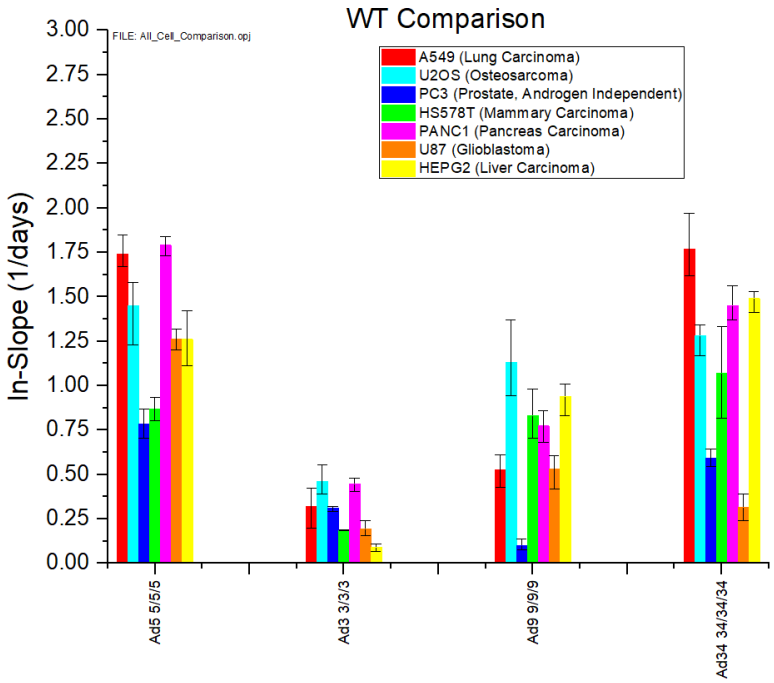

Figure 3.10a. Comparison of kinetics for wt Ad3, Ad5, Ad9, and Ad34.

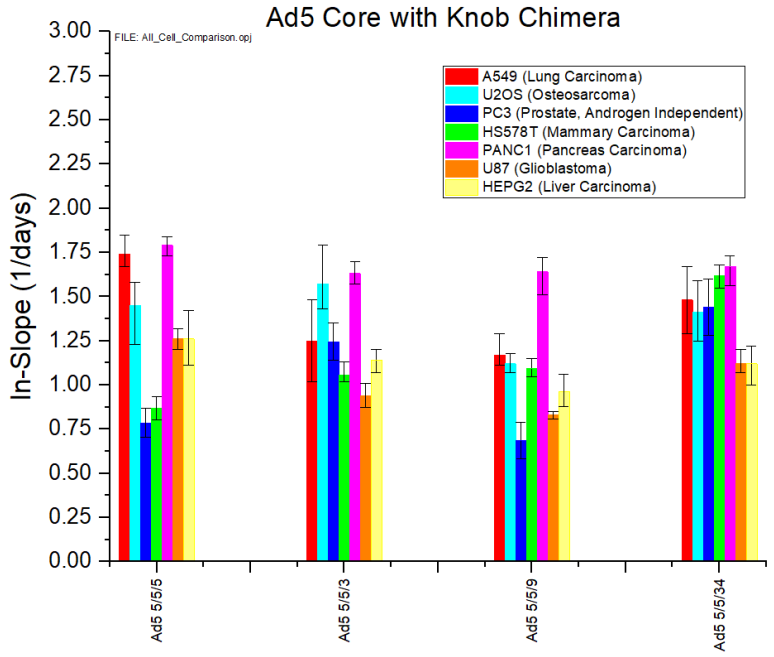

Figure 3.10b. Comparison of kinetics for wt Ad5 and the knob-only chimeras with Ad5 core.

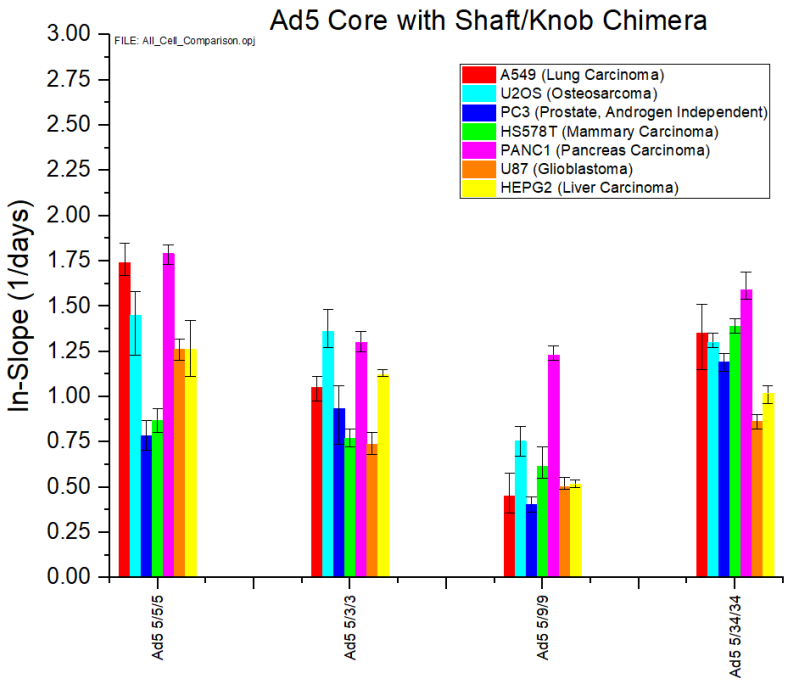

Figure 3.10c. Comparison of kinetics for wt Ad5 and the knob-and-shaft chimeras with Ad5 core.

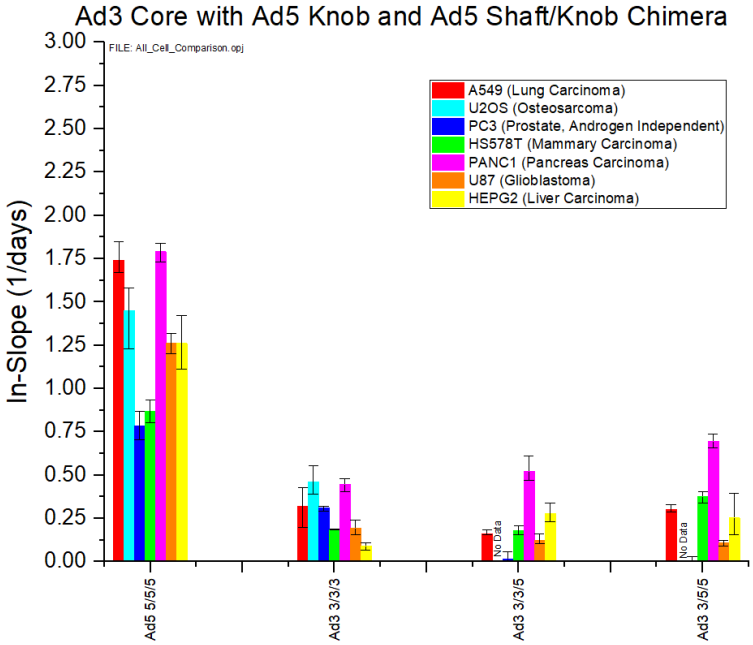

Figure 3.10d. Comparison of kinetics for wt Ad5, wt Ad3, knob-only, and knob-and-shaft chimeras with Ad3 core.

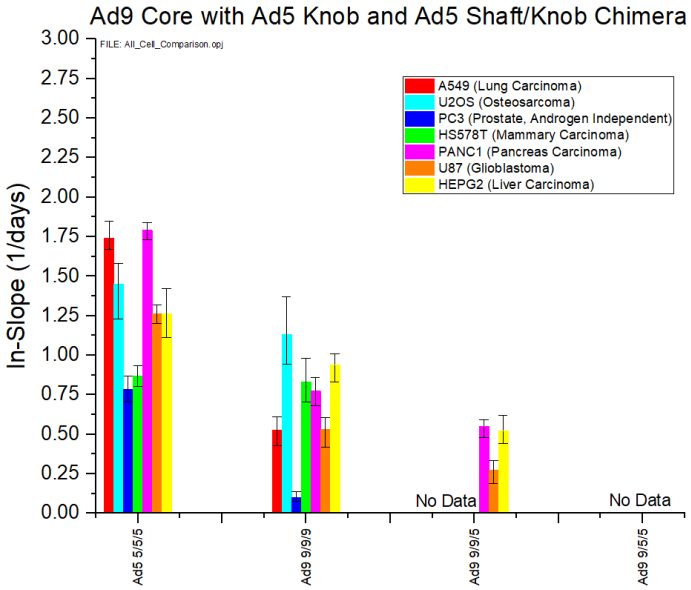

Figure 3.10e. Comparison of kinetics for wt Ad5, wt Ad9, knob-only, and knob-and-shaft chimeras with Ad9 core.

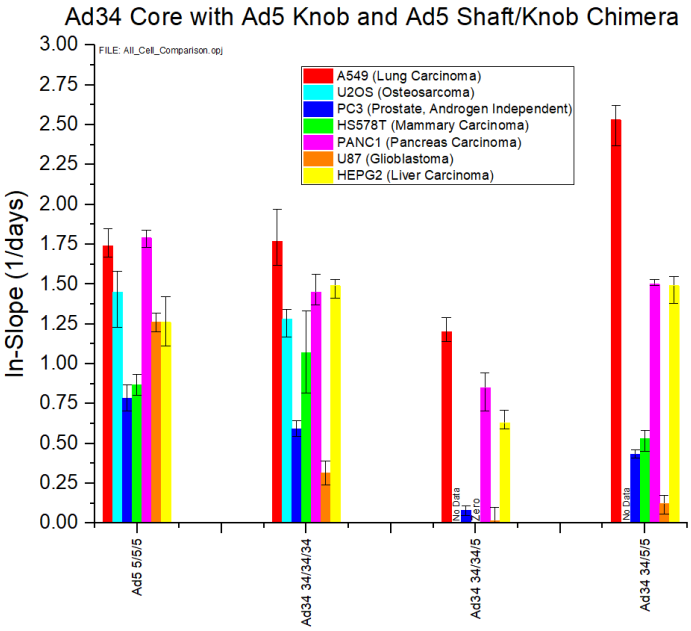

Figure 3.10f. Comparison of kinetics for wt Ad5, wt Ad34, knob-only, and knob-and-shaft chimeras with Ad34 core.

Table 3.1. Human Ad serotypes ordered by subgroup A through G, listing known receptors and tropism. Reproduced from reference 25.

Species	Serotype	Receptor(s)	Tropism:	Seroprevalence (%)	Fibre shaft repeats
A	12, 18, 31	CAR, fIX, fX	Cryptic (enteric, respiratory)	35–70	23
B1	3, 7, 16, 21, 50	CD46, 'X', fX CD80, CD86	Respiratory, ocular	2–15 (Ad16, 21, 50) 35–70 (Ad3, 7)	6
B2	11, 14, 34, 35	CD46, 'X', fX CD80, CD86	Renal, ocular, respiratory	1–3 (Ad11, 34, 35) 18 (Ad14)	6
C	1, 2, 5, 6	CAR, fIX, fX, Lf, DPPC, VCAM-1, HS, MHC1-α2	Respiratory, ocular lymphoid	40–80	22
D	8–10, 13, 15, 17 19, 20, 22–30, 32, 33, 36–39, 42–49, 51	SA, CD46, CAR fx	Ocular (enteric)	3–44	8
E	4	CAR	Ocular, respiratory	45	12
F	40, 41	CAR	Enteric	41 (together)*	12 (short fibre) and 21/22 (long fibre)
G	52	ND	Enteric	ND	9 or 17

ND: not determined.
*Serotypes 40 and 41 are very closely related antigenically.

Table 3.2. Knob-only chimeras

		Knob Donor			
		Ad5	Ad3	Ad9	Ad34
Core Donor	**Ad5**	PCMN-887 Core: Ad5 Fiber Tail: Ad5 Fiber Shaft: Ad5 Fiber Knob: Ad5 Ad5 5/5/5	PCMN-962 Core: Ad5 Fiber Tail: Ad5 Fiber Shaft: Ad5 Fiber Knob: Ad3 Ad5 5/5/3	PCMN-963 Core: Ad5 Fiber Tail: Ad5 Fiber Shaft: Ad5 Fiber Knob: Ad9 Ad5 5/5/9	PCMN-964 Core: Ad5 Fiber Tail: Ad5 Fiber Shaft: Ad5 Fiber Knob: Ad34 Ad5 5/5/34
	Ad3	PCMN-965 Core: Ad3 Fiber Tail: Ad3 Fiber Shaft: Ad3 Fiber Knob: Ad5 Ad3 3/3/5	PCMN-946 Core: Ad3 Fiber Tail: Ad3 Fiber Shaft: Ad3 Fiber Knob: Ad3 Ad3 3/3/3		
	Ad9	PCMN-966 Core: Ad9 Fiber Tail: Ad9 Fiber Shaft: Ad9 Fiber Knob: Ad5 Ad9 9/9/5		PCMN-888 Core: Ad9 Fiber Tail: Ad9 Fiber Shaft: Ad9 Fiber Knob: Ad9 Ad9 9/9/9	
	Ad34	PCMN-967 Core: Ad34 Fiber Tail: Ad34 Fiber Shaft: Ad34 Fiber Knob: Ad5 Ad34 34/34/5			PCMN-889 Core: Ad34 Fiber Tail: Ad34 Fiber Shaft: Ad34 Fiber Knob: Ad34 Ad34 34/34/34

Table 3.3. Shaft/Knob chimeras.

		Shaft/Knob Donor			
		Ad5	Ad3	Ad9	Ad34
Core Donor	**Ad5**	PCMN-887 Core: Ad5 Fiber Tail: Ad5 Fiber Shaft: Ad5 Fiber Knob: Ad5 Ad5 5/5/5	PCMN-949 Core: Ad5 Fiber Tail: Ad5 Fiber Shaft: Ad3 Fiber Knob: Ad3 Ad5 5/3/3	PCMN-920 Core: Ad5 Fiber Tail: Ad5 Fiber Shaft: Ad9 Fiber Knob: Ad9 Ad5 5/9/9	PCMN-921 Core: Ad5 Fiber Tail: Ad5 Fiber Shaft: Ad34 Fiber Knob: Ad34 Ad5 5/34/34
	Ad3	PCMN-951 Core: Ad3 Fiber Tail: Ad3 Fiber Shaft: Ad5 Fiber Knob: Ad5 Ad3 3/5/5	PCMN-946 Core: Ad3 Fiber Tail: Ad3 Fiber Shaft: Ad3 Fiber Knob: Ad3 Ad3 3/3/3		
	Ad9	PCMN-952 Core: Ad9 Fiber Tail: Ad9 Fiber Shaft: Ad5 Fiber Knob: Ad5 Ad9 9/5/5		PCMN-888 Core: Ad9 Fiber Tail: Ad9 Fiber Shaft: Ad9 Fiber Knob: Ad9 Ad9 9/9/9	
	Ad34	PCMN-953 Core: Ad34 Fiber Tail: Ad34 Fiber Shaft: Ad5 Fiber Knob: Ad5 Ad34 34/5/5			PCMN-889 Core: Ad34 Fiber Tail: Ad34 Fiber Shaft: Ad34 Fiber Knob: Ad34 Ad34 34/34/34

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CHAPTER FOUR:

Selective Oncolytic Virus

Introduction

As an example case for demonstrating a positively regulated selective oncolytic virus, we have chosen to engineer selectivity for prostate cells. This choice was based on several factors. First, there is an existing body of literature describing efforts to engineer Ad for selective replication exclusive to prostate cells. Second, the prostate is an organ that can be targeted for complete removal, both cancerous and noncancerous cells, and maintain patent survival. Such is not the case for most other organs, such as pancreas, liver, brain, etc. And third, the present standard of care is a prostatectomy, which necessitates the cutting of nerve fibers responsible for erectile function. Eradicating the prostate via selective Ad infection has the potential for leaving these nerves intact and functional.

Ad-based treatment of prostate cancer falls into two general categories, non-replicating Ad vectors with selective expression of a toxin gene and replication-competent Ad with selective replication in prostate cells. Multiple clinical trials are underway or have been completed employing both non-replicating Ad vectors and replication-selective Ad[1].

Prostate-Specific Expression from Ad Vector

Non-replicating Ad vectors have been used for two purposes in the treatment of prostate cancer; force prostate cells to selectively express a toxin or force prostate cells to selectively express an imaging molecule. Table 4.1 provides a list of examples taken from the literature. Of particular note is the payload HSV-tk. This is the Herpes Simplex Virus-1 Thymidine Kinase and can be used both as a suicide gene or an imaging gene[2]. With

HSV-tk selectively expressed in the target cells, administration of a purine analog (acyclovir, ganciclovir, buciclovir, or penciclovir) leads to phosphorylation and retention in target cells. These analogs are competitive inhibitors of deoxyguanosine triphosphate (dGTP) leading to cell death. For imaging, administration of a radioactive analog 9-(4-^{18}F-fluoro-3-[hydroxymethyl]butyl)guanine ([^{18}F]FHBG) leads to phosphorylation and accumulation in target cells, creating a localized positron emission source for use in Positron Emission Tomography (PET) imaging.

Though selective expression is demonstrated in each of the references listed in table 4.1, most authors comment on the need for higher expression levels than those achieved using the selective promoters chosen to drive prostate-specific expression.

Two-Step Transcriptional Activation (TSTA)

In response to the need for higher expression levels while maintaining selectivity, the TSTA system was developed[3]. This system is shown in Fig 4.1. In the TSTA system a weak promoter drives a strong transcription factor and the strong transcription factor in turn drives expression of the target gene to high levels. In this example, the strong transcription factor is the GAL4-VP16 fusion and the target gene is driven by a promoter consisting of 5 copies of the GAL4 binding sequence combined with a minimal promoter. A weak, but selective, promoter may produce only a low level of the GAL4-V16 transcription factor, but this low level is sufficient to drive high level expression of the target gene.

The TSTA system has been used by multiple authors in the prostate cancer space, with table 4.2 showing a list of examples. These authors report increased expression levels of 100-fold over that produced by the initial, weak promoter. All of the examples found in the literature using the TSTA system apply to non-replicating Ad.

Replacing Endogenous Ad Promoter with Prostate-Specific Promoter

Considerable work has been done to develop a replicating Ad virus that is selective to the prostate. A survey of the literature shows a common theme among these efforts. All examples found in the literature replace one or more of the endogenous Ad promoters with a prostate-specific promoter. Table 4.3 provides a list of prostate-specific promoters and Ad promoter replacements that have been described in the literature. Though each of these examples demonstrated some level of selectivity for prostate cells, none showed the same replication kinetics as wt Ad when infecting prostate cells. Researchers working with Ad selectivity in tissues or cancer types other than prostate have faced the same problem; selectivity comes at a cost of potency.

TSTA Applied to a Replicating Ad

The disappointing kinetics obtained with direct replacement of one or more Ad promoters with a prostate-specific promoter is not surprising when comparing the promoters of table 4.1 and table 4.3. The promoters that provided insufficient prostate-specific expression inside a non-replicating Ad vector (table 4.1) are the same as those used for direct replacement of one or more Ad promoters in the replication-selective Ad

viruses of table 4.3. Viral promoters in general, and Ad promoters specifically, are known to produce high expression levels[28]. Thus, if an Ad promoter is to be replaced while maintaining fast replication kinetics, the replacement promoter must also produce high expression levels.

The the dual requirements for this replacement promoter of tight specificity and high expression might be met with the TSTA system. Rather than use the TSTA system to produce high expression of a target gene, as described in the references of table 4.2, instead we propose use of the TSTA system to replace an Ad promoter. For example, in the TSTA system shown in fig 4.1, instead of driving a target gene with the 5XGAL4 promoter, replace an Ad promoter with the 5XGAL4 promoter. In this example, the weak, prostate-specific promoter forces some low level expression of the GAL4-VP16 fusion transcription factor and this transcription factor goes on to produce a high level of expression of the gene or genes normally activated by the replaced Ad promoter.

For the work described in this dissertation, we chose to use the Tet-On system[29] rather than the GAL4-VP16 system. Our choice was based on the fact that the Tet-On system allows an additional level of control due to its requirement for doxycycline to generate the proper conformational change in the Tet-On protein leading to high affinity binding to the target DNA binding site. The Tet-On, Tet-Off, and TetR systems are shown schematically in fig 4.2. For highest on-state expression and lowest off-state leakage, the 3rd generation Tet-On system[30] with the so called Tet-Response Element 3G (TRE3G) has been employed.

Safe Location in Ad Genome for Exogenous Gene Placement

Use of the TSTA system to control Ad replication faces a challenge in virus design that does not exist with the non-replicating vectors of tables 4.1 and 4.2 nor with the direct promoter replacement viruses of table 4.3. This new challenge is where in the genome to place the genes associated with the TSTA system without negatively impacting the replication kinetics of the virus. For the non-replicating vectors that employ the TSTA system, the choice of location is clear since all of these vectors are E1-region deleted. It is standard practice to place exogenous genes immediately after the left hand Inverted Terminal Repeat Sequence (ITRS) located in the now-vacant E1 region. Since these are non-replicating viruses, the only concern with regard to replication kinetics is during virus production and not during its application in the patient. It is the experience in the O'Shea lab that non-replicating viruses built in this way can be produced to high titer using HEK293 cells[31] to rescue the E1 region deletion, as is standard practice in the literature.

There are many examples of adding exogenous genes to a replication-competent Ad genome. Because of the limited genome capacity of the Ad virion[32], most often endogenous Ad genes are deleted in order to free up genome space. The immunomodulatory E3 genes are dispensable in tissue culture, so these are most often the genes removed[33]. Consequently, the E3 region is often the location for the added exogenous genes[34-38]. Following this historic precedent, we deleted the E3B ORFs, RIDα, RIDβ, and 14.7k, and placed the Tet-On gene in the location of these deletions. We retained the E3B poly-A for use with the Tet-On gene. We cloned three different promoters to drive expression of the Tet-On ORF; E2F1, CMV, and EF1α. We chose

these three promoters because they are considered constitutive and represent three different levels of promoter strength with EF1α > CMV > E2F1. A schematic of these changes to the Ad5 genome is shown in fig 4.3. In addition to these E3 deletions and the insertion of the Tet-On gene, we included the YPet-P2A-ADP modification as a kinetics readout.

The kinetics of these three constructs are shown in fig 4.4 along with a wildtype background for comparison. The construct with the EF1α promoter has a declared ln-slope of zero because it could not be produced. This data suggests that as the promoter strength is increased, the kinetics of the virus is negatively impacted. Also note the slight increase in ln-slope for the virus with E2F1 promoter relative to the wildtype background. This increase has been repeatedly observed and has been attributed to an increase in kinetics caused by the E3B ORF deletion, as shown in fig 4.5.

To better understand the cause of this kinetic defect as the Tet-On gene promoter strength is increased, figs 4.6a through 4.6c show the measured YPet fluorescence for each of the viruses of fig 4.4. Since the ADP is essentially a late protein[39], we can use the YPet fluorescence level produced by the YPet-P2A-ADP as a surrogate for late protein expression. One can see that the fluorescence level for the CMV::Tet-On construct shown in fig 4.6c is significantly lower than the wildtype and the EF1::Tet-On construct. Based on these results, we surmised that placement of the Tet-On gene in the E3B region leads to reduced late protein expression and thus slower viral kinetics. The cause for the lower late protein expression is thought to be transcriptional interference between the Tet-On gene and the Major Late Transcript (MLT). The MLT encodes all of the structural proteins and runs nearly the full length of the upper strand of the Ad5 genome as shown by the blue

arrow in fig 4.7. Our finding of reduced kinetics due to location of an exogenous gene within the Ad5 genome has recently been corroborated by other researchers[40]. Suzuki et. al tested placement of an exogenous gene in various locations of a non-replicating Ad5 vector. They used virus titer yield from HEK293-E4 cells as a measure of optimum placement. They found that an exogenous gene, employing the EF1α promoter, placed in the E3 region led to greatly reduced virus particle yield. Their results and ours lead to the following design rule: Do not place a gene within a gene. That is, do not place an exogenous promoter-ORF-poly-A sequence inside any of the Ad5 endogenous transcripts.

Because the work by Suzuki et. al. was done in a non-replicating Ad5 vector with the E1 and E4 regions deleted, they had the option of placing an exogenous gene in the vacated E1 or E4 regions. For our case, we are attempting to retain the kinetics of a replication competent Ad5 and thus both the E1 and E4 regions are still part of the genome. This gene-within-a-gene design rule is highly constraining, with only 3 possible locations available, as shown in fig 4.8. The three locations are; between the E1A and E1B transcripts, between the E1B transcript and the U gene transcript, and between the MLT and the E4 transcript. For ease of cloning, we chose to work in the region between the MLT and E4 transcript.

A closer look at the sequence data of this region reveals that the full length L5 poly-A of the MLT and the full length E4 poly-A overlap, as shown in fig 4.9. Also shown in this figure is the canonical poly-A sequence as described in reference 41. It is unknown if this overlap has some particular function or is just a clever way to save genome space by using the AATAA sequence of one poly-A as the G/T rich region of another. It is

noteworthy that the E1B poly-A and the U gene poly-A located on the left hand side of the genome also overlap in a similar way.

Given this overlap in poly-A sequences, inserting an exogenous gene between the AATAAA signals of the L5 poly-A and the E4 poly-A would destroy the full length poly-A sequences of both. A solution to this problem is to add a new poly-A sequence to the right or left of the overlapping L5 and E4 poly-As. Use of the minimal SV40 poly-A sequence has a genome cost of only 45 base pairs so we cloned this poly-A to the left of the overlapping poly-As and inserted the Tet-On gene into the space between. This arrangement is shown schematically in fig 4.10 and the resulting measured replication kinetics are shown in fig 4.11. There is no significant loss of kinetics when using the CMV promoter relative to the E2F1 promoter and even the virus using the EF1α promoter could be produced, though it does exhibit a small kinetics defect.

TSTA Control of an Ad Promoter

With a "safe" place in the Ad5 genome to insert the Tet-On gene now available, we can move on to applying the actuator function of the Tet-On system. That is, use the TRE3G activated by the Tet-On transcription factor to impact the kinetics of the virus. Since the TSTA system is meant to allow use of a weak, but selective, promoter as if it were a strong promoter, we chose to replace various Ad promoters with the TRE3G promoter in a fashion similar to the work described in the references of table 4.3. Our expectation was to achieve similar selectivity as described by these researchers, but with better virus kinetics in the selected cell type.

There are 9 known promoters within the Ad5 genome, as shown in fig 4.12. Which to replace with the TRE3G promoter can be down-selected based on several criteria. The first criteria is that the genes driven by the promoter must not be dispensable in tissue culture. This criteria is based on the fact that we wish to show selectivity *in vitro*. Viruses with the E3 and UXP genes deleted can still replicate *in vitro*, so their promoters are eliminated by this first criteria. The second criteria is that the basepairs of the chosen promoter can not also be used on the opposite strand. If we replace such a promoter with the TRE3G promoter, we will also disrupt the base pairs used by another gene running along the opposite strand. This criteria eliminates the E2 early, pIVa2, and Major Late promoters. Applying these two criteria, there are 4 remaining promoters amenable to replacement by the TRE3SG promoter; E1A, E1B, E2 Early, and E4. Constructs replacing three of these four promoters were constructed and tested.

Controlling E1A expression with the TRE3G promoter is appealing because in the off-state there would be no expression of E1A and no initiation of the remainder of the Ad5 lifecycle. Unfortunately, replacing the E1A promoter with TRE3G led to no significant control over virus replication, as shown in fig 4.13. This failure in control is likely due to the numerous transcription factor binding sites located in the ITRS and packing regions located just to the left of the E1A promoter (see appendix A for details). These ITRS and packaging features can not be eliminated while maintaining replication competence.

Though there are no literature examples of replacing the E2 early promoter with a prostate-specific promoter listed in table 4.3, there is president from work in other tissue

types[42]. Controlling E2 early expression is not as appealing as controlling E1A since an infected cell will likely die upon infection due to initial E1A activation. But, because the E2 early promoter controls expression of the Ad5 DNA polymerase, the off-state would not exhibit DNA replication and thus the Ad5 lifecycle would not progress to late gene expression[43]. Fig 4.14 shows the measured replication kinetics for viruses with the E2 early promoter replaced with TRE3G in the presence and absence of doxycycline. To our complete surprise, the control authority is opposite to that of our expectations. The virus kinetics are reduced in the +Dox case and increased in the –Dox case, opposite to what we expected. And this suppression effect in the +Dox condition is exaggerated as the promoter strength is increased; E2F1 < CMV < EF1α. We have no solid explanation for these results. One possibility is based on the fact that the Ad5 virus has to E2 promoters, the E2 early and the E2 late. The E2 early promoter is only activated during the early phase of the Ad5 lifecycle, and the E2 late promoter is only activated during the Ad5 late lifecycle[28]. It is possible that continued activation from the E2 early promoter position by the TRE3G promoter during the late lifecycle causes a kinetic defect.

Replacing the E4 promoter with the TRE3G promoter is the least appealing choice because an infected cell will likely die due to E1A activation and large numbers of copies of the Ad5 genome will be produced due to E2 activation. The measured replication kinetics for constructs with the E4 promoter replaced with the TRE3G promoter are shown in fig 4.15. There is some control authority and the increase/decrease in kinetics vs. +/- Doxycycline is as expected, but there are several problems with these results. First, the on-state using the weak promoter, E2F1, is relatively slow. And second, the off-state for all

three promoters is not as low as desired. And finally, only the CMV promoter case exhibits nearly wildtype kinetics. It seems that this virus design is exquisitely sensitive to the choice of promoter strength. Such a sensitivity makes this design of little practical use since the goal is a virus that is regulated by an arbitrary tissue-specific promoter.

TSTA Control of a Single Ad Gene

Replacement of an Ad promoter with the TRE3G promoter led to mixed and disappointing results. We took a step back and thought again about how one might control the replication kinetics of the Ad5 using an arbitrary promoter. The intent of directly replacing an Ad promoter with the TRE3G promoter was to control the expression of one or more Ad protein(s) and thus control replication by the presence or absence of these proteins. This same effect could be achieved by deleting a single chosen ORF from the Ad genome and placing it under direct control of the TRE3G promoter.

There are 37 known proteins expressed by the Ad5 virus during various stages of its lifecycle (see Appendix A for details). A simple selection criteria was used to down-select to a more manageable number.

Criteria #1: Viral replication must be critically dependent on this protein. If we are to control virus replication through this single protein, it must be critical to the virus life-cycle. This criteria eliminates all of the E3 proteins, E4 proteins, E1B-19k and E1B-55k.

Criteria #2: The ORF for this protein must not interfere with base pairs of an ORF on the opposite strand. We will need to delete the base pairs associated with this protein's endogenous ORF in order to free up genome space and thus if these base pairs are used on

both top and bottom strands we would disrupt other functions when deleting these base pairs.

Criteria #3: This protein must not be a structural protein. The expression levels of the structural proteins during the late time portion of the Ad lifecycle is extremely high. We have little confidence that TRE3G-driven expression of these proteins will produce the appropriate timing and levels required for good virus kinetics.

Criteria #4: Avoid the E1A protein. Since the E1A protein is the first to be expressed, any delay in its expression would lead to a reduction in virus kinetics. There is a time lag associated with the Tet-On system due to the need for initial accumulation of the Tet-On transcription factor prior to high expression from the TRE3G promoter and this time delay if applied to E1A expression would be detrimental to virus kinetics.

Applying these criteria reduces the list of possibilities from 37 to just 3 proteins: L1 52kDa protein, L3 Endoprotease, and DNA Binding Protein (DBP). We chose to clone viruses with the L3 Endoprotease and DBP placed under direct control of the TRE3G promoter. The results for these constructs are shown in figs 4.16 and 4.17. There is limited control when using L3 Endoprotease (fig. 4.16), but excellent control when using DBP (fig. 4.17). Besides the wide control authority found when using DBP, an additional attractive feature of using DBP as the control protein is that its absence prevents efficient genome replication since this protein is responsible for protecting the single-stranded Ad genomes generated by the Ad DNA polymerase[44] during the genome replication cycle.

Prostate-Specific Promoter Testing

With the Ad replication control actuator in hand, we began testing a prostate-specific promoter to be used in combination with TSTA to impart prostate-specific Ad replication. The PSES promoter, first described in reference 7, showed the best performance based on a reading of the literature. This promoter was reconstituted from descriptions in the journal and patent literature[45] and placed in the Ad5 genome located between the separated L5 and E4 poly-A's (see Fig 4.10). The PSES promoter was cloned to drive expression of YPet so that we can compare the expression levels when infecting various cell types. In addition to the PSES promoter, the CMV promoter, and a p53-depedent promoter called PrMinRGC (see chapter 5), were also cloned in the the same location, but in separate Ad5 viruses.

The androgen receptor positive, androgen dependent prostate cell line LNCaP was used as the positive control for the PSES promoter. This cell line is described in the literature as providing the highest activation of the PSES promoter. The androgen receptor negative, androgen independent prostate cell line PC3 was used as a negative control. Additionally, the A549 ($TP53^{+/+}$) cell line and the A549p53KO ($TP53^{-/-}$) cell lines were also included. The resulting expression levels for the three promoters in these four cell lines are shown in Fig 4.18.

The first observation is that the YPet expression levels, when driven by the CMV promoter are all approximately equal between the four cell lines. Equal expression for the CMV construct is taken as evidence that entry and activation by this virus in these four cells lines is approximately equivalent, allowing us to make direct comparisons between the PSES promoter and PrMinRGC promoter results.

To my great disappointment, the PSES promoter shows only a 3.4X differential between LNCap and PCs cells (55 units for LNCaP vs. 16 units for PC3). As a further disappointment, the differential between LNCaP and A549 cell lines is only 2.3X (55 units for LNCaP vs. 24 units for A549). And if this disappointing differential wasn't enough, the strength of the PSES promoter is 73X less than that of the CMV promoter when infecting LNCaP cells (4000 units for CMV vs. 55 units for PSES).

In contrast to the PSES promoter, the PrMinRGC promoter shows both a promising level of differential (100X between A549 and A549p53KO cells) and excellent promoter strength, essentially equal to that of CMV.

Based on these results, I did not pursue the positively-regulated virus based on the PSES promoter. Instead, development of a negatively-regulated virus based on the PrMinRGC promoter was pursued and described in detail in chapter 5.

Conclusions and Future Work

Even though the viruses described in this chapter have not been integrated with a tissue-specific promoter to produce a positively-regulated virus, these viruses with a constitutive promoter have potential in clinical applications. For example, the Dox control of the virus shown in Fig 4.17 could be used as a "safety switch" when treating a patient. This virus has limited replication kinetics in the absence of Dox, thus removal of Dox administration should greatly attenuated the replication of this virus in a patient. Such a safety switch might allow more aggressive treatment, either with administration of higher initial particle count, or by arming the virus with a potent anti-tumor payload or immune-

stimulatory payload. In the event that adverse effects are detected in a patient, Dox administration can be terminated and virus replication brought to a halt.

A further improvement in the safety of this class of virus is to create an even better "off" state. Since the E4-promoter replacement and the DBP replacement viruses both make use of the Tet-On protein driven by the CMV promoter, one can imagine combining the two control circuits as follows. The CMV promoter drives constitutive expression of Tet-On. Expression of the DBP ORF is driven by the TRE3G promoter and the E4 promoter is also replaced by the TRE3G promoter. Administration of Dox would then control both DBP expression and E4 gene expression. In the absence of Dox, the very slow virus of Fig 4.17 would be further handicapped by the slow kinetics of the virus shown in Fig 4.15. The multiplicative negative effects on kinetics from both of these "off states" is expected to result in zero replication.

Figures and Tables

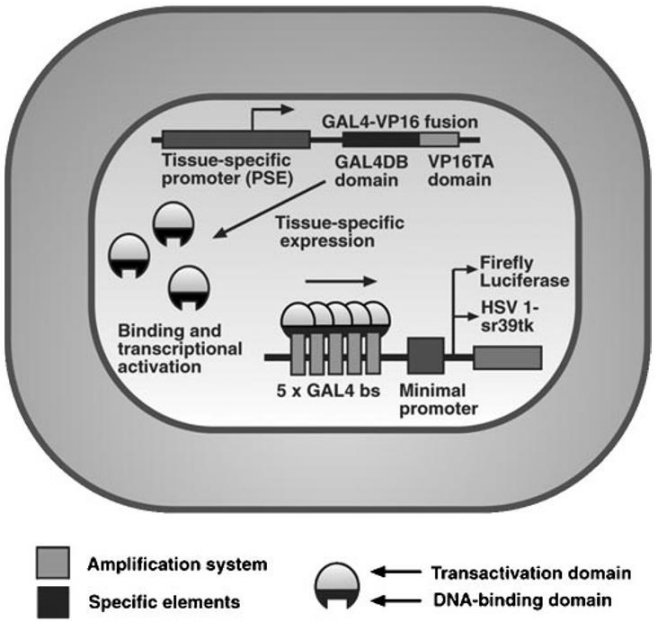


Figure 4.1. Schematic representation of the TSTA system (taken from reference 3).

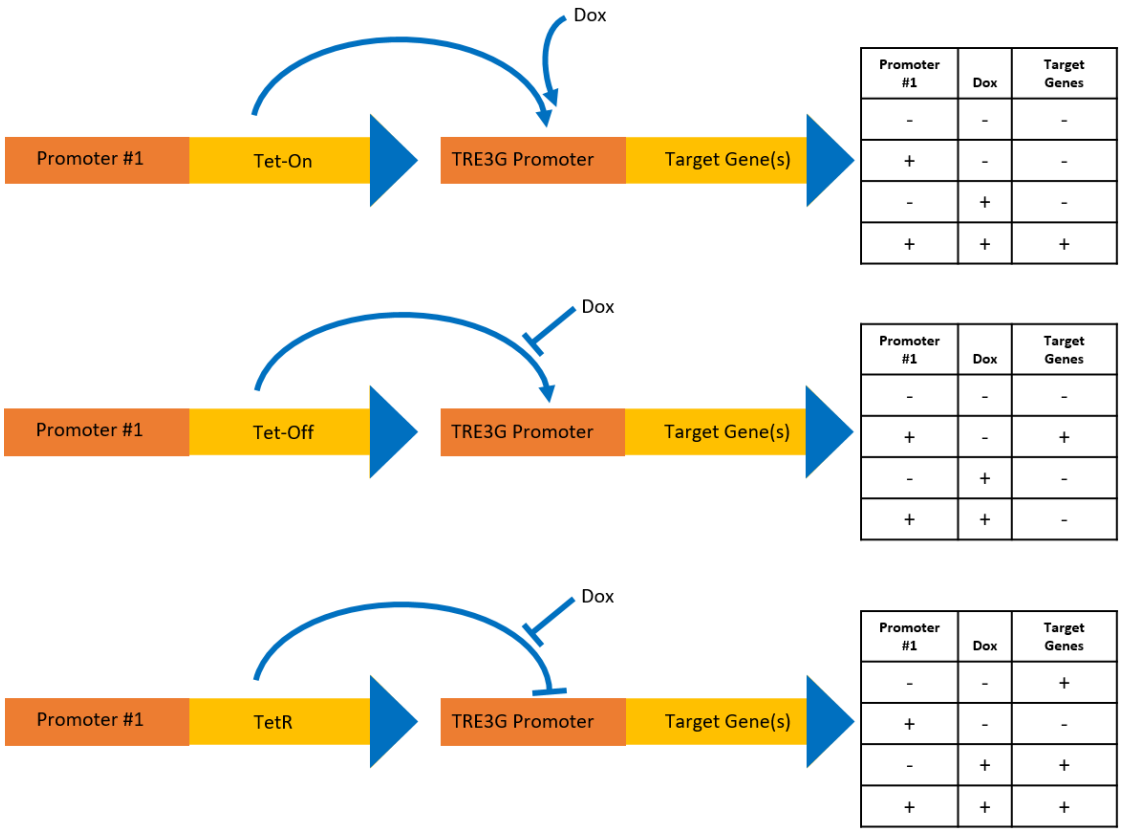


Figure 4.2. Schematic representation of the Tet-On, Tet-Off, and TetR systems.

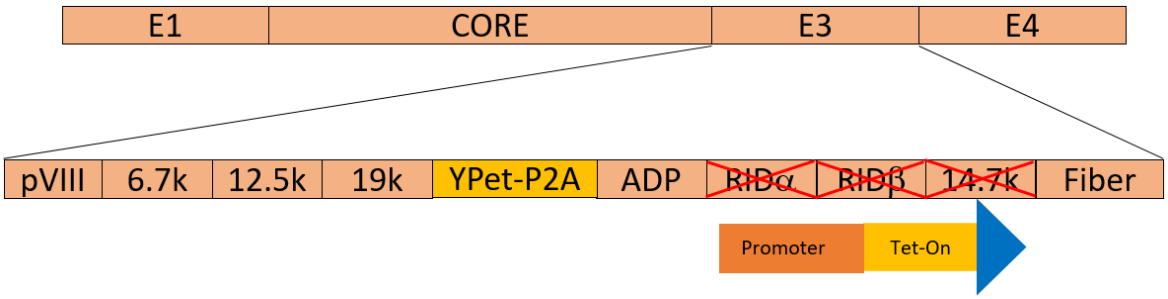


Figure 4.3. Schematic representation of Tet-On gene placed in the E3 region. The E3B poly-A normally used for the deleted RIDα, RIDβ, and 14.7k ORFs is retained and used for the Tet-On gene. The promoter driving the Tet-On ORF is either E2F1, CMV, or EF1α.

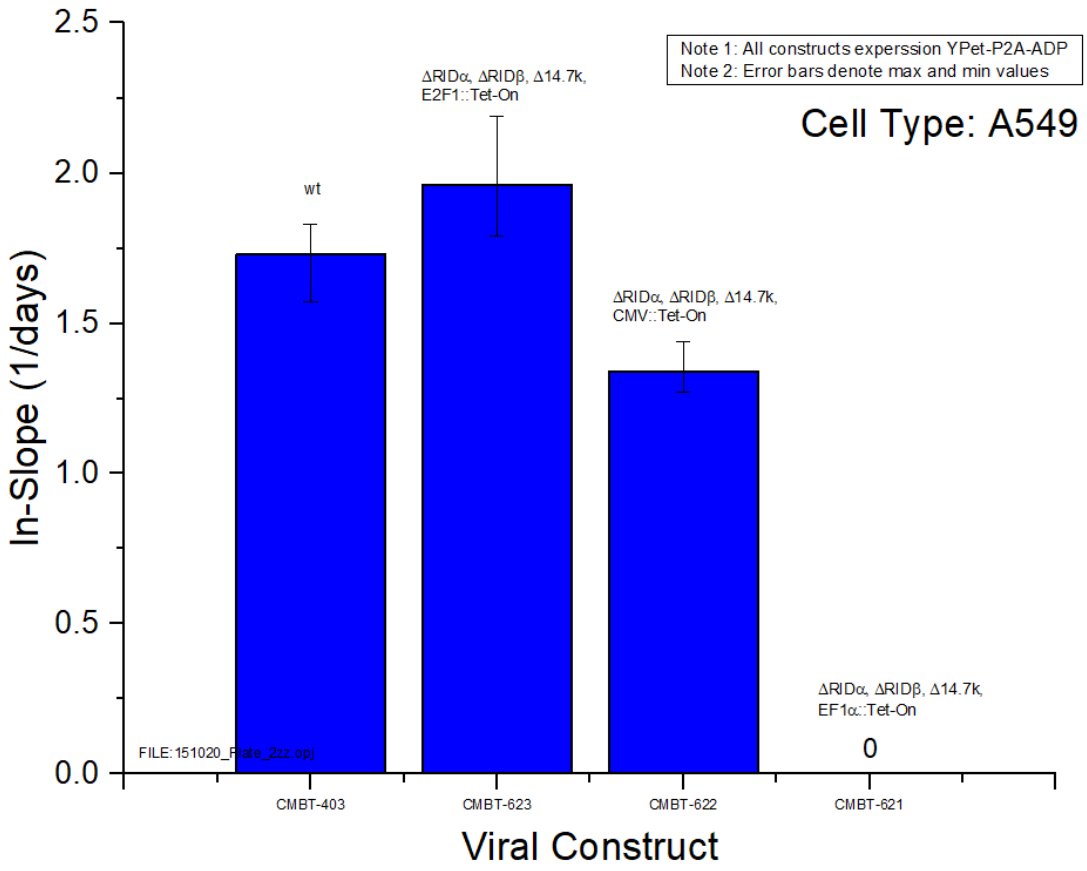


Figure 4.4. Measured replication kinetics of virus constructs with Tet-On gene placed in the E3B region of Ad5 genome.

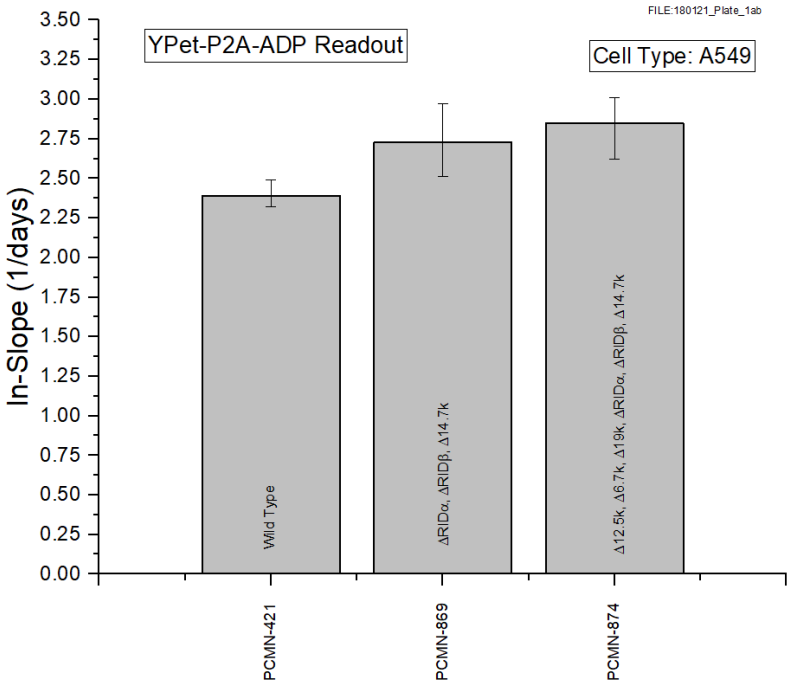


Figure 4.5. Measured replication kinetics comparison between wildtype, E3B deletion, and E3A and E3B deletions.

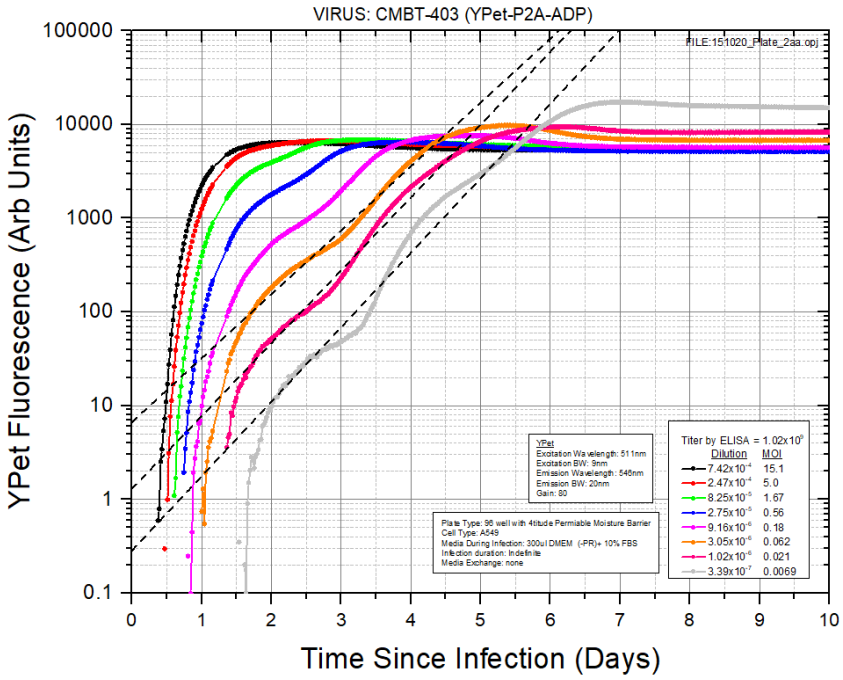


Figure 4.6a. Measured YPet fluorescence versus time for wildtype background.

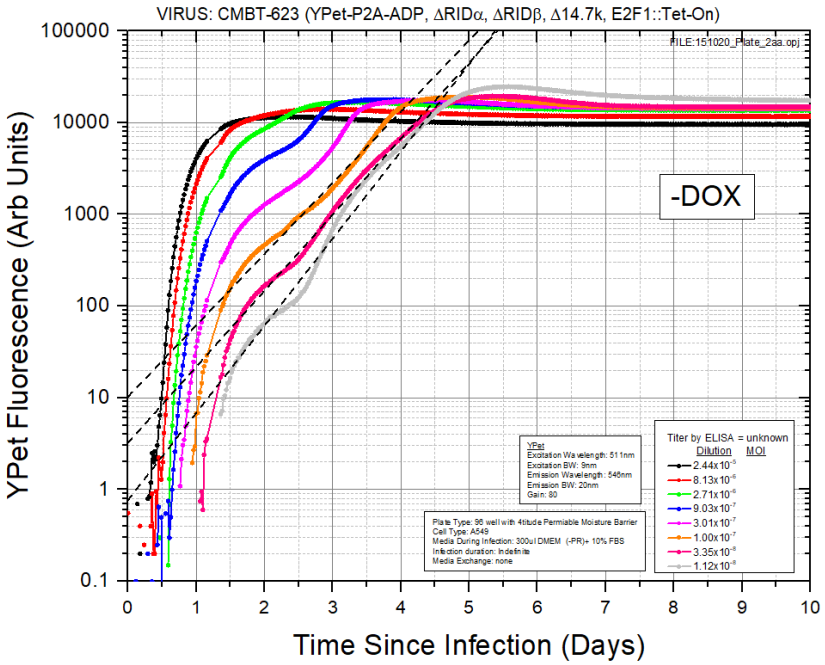


Figure 4.6b. Measured YPet fluorescence versus time for construct with E2F1 promoter driving Tet-On ORF located in the E3B region.

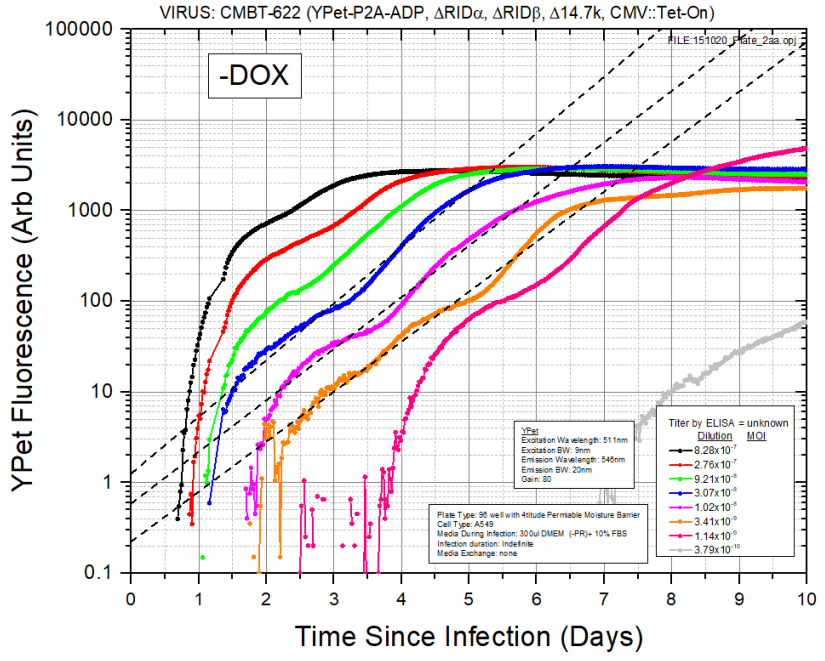


Figure 4.6c. Measured YPet fluorescence versus time for construct with CMV promoter driving Tet-On ORF located in the E3B region.

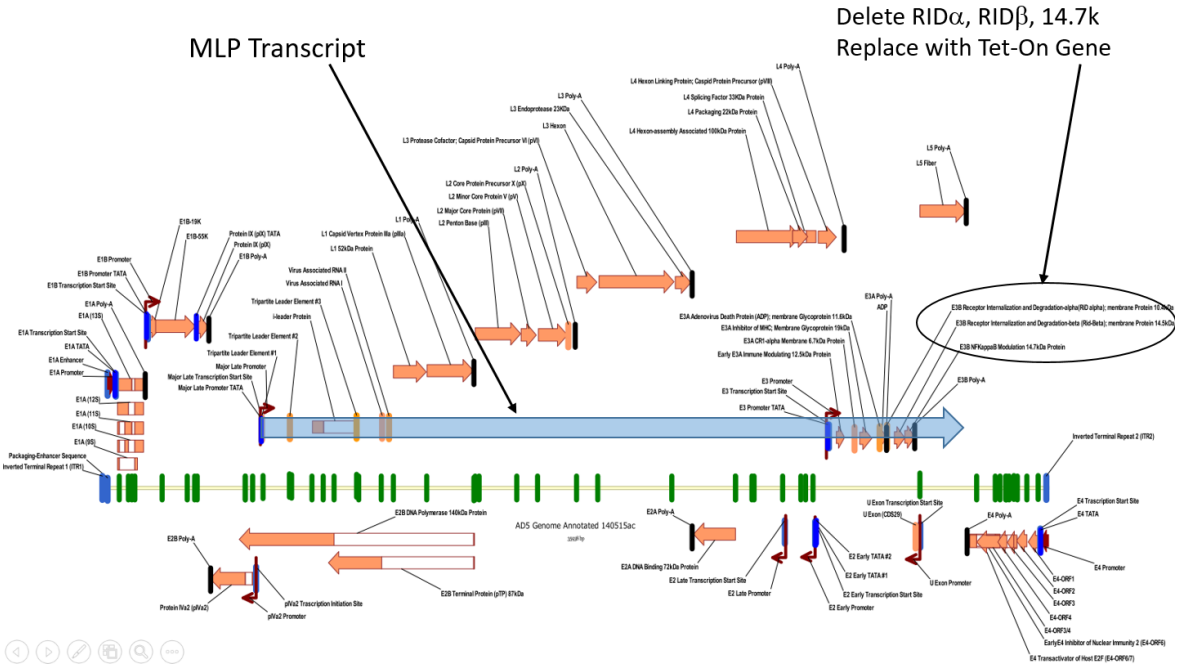


Figure 4.7. Ad5 genome with MLT highlighted with a blue arrow. The E3A region falls within this transcript.

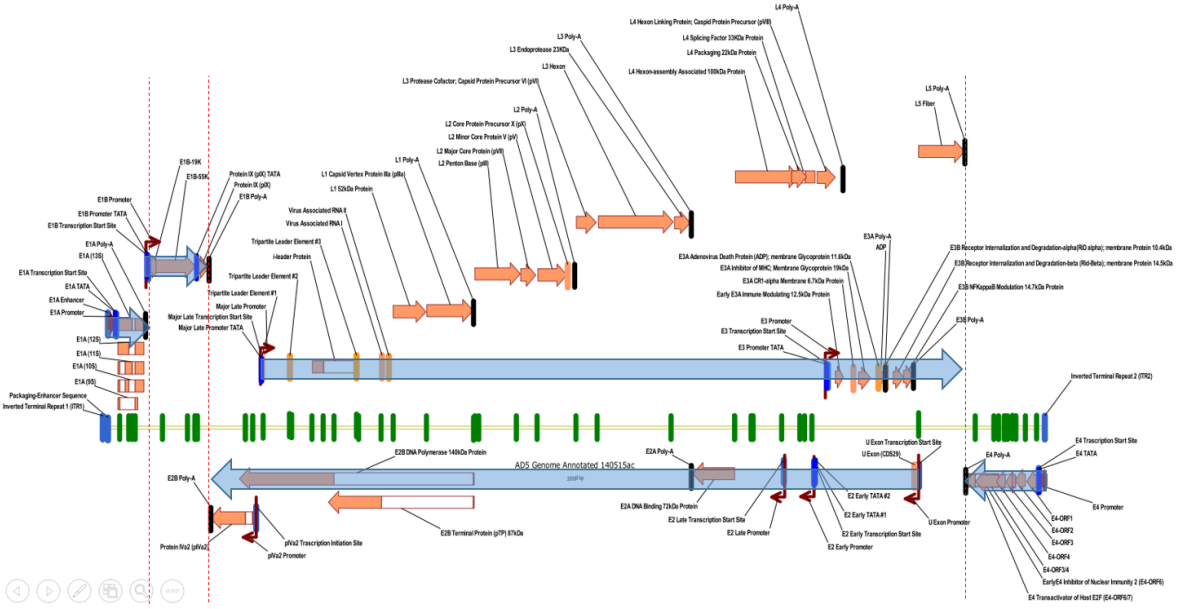
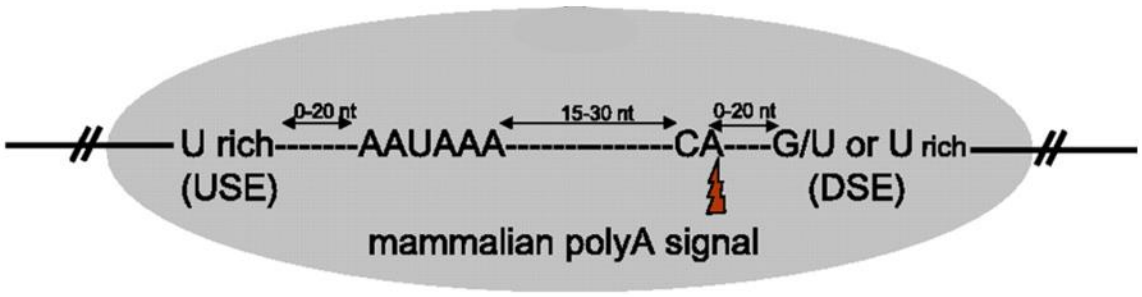


Figure 4.8. The Ad5 transcripts shown with blue arrows and the three possible locations for placing an exogenous gene shown with red dash lines.



Nick J. Proudfoot Genes Dev. 2011;25:1770-1782

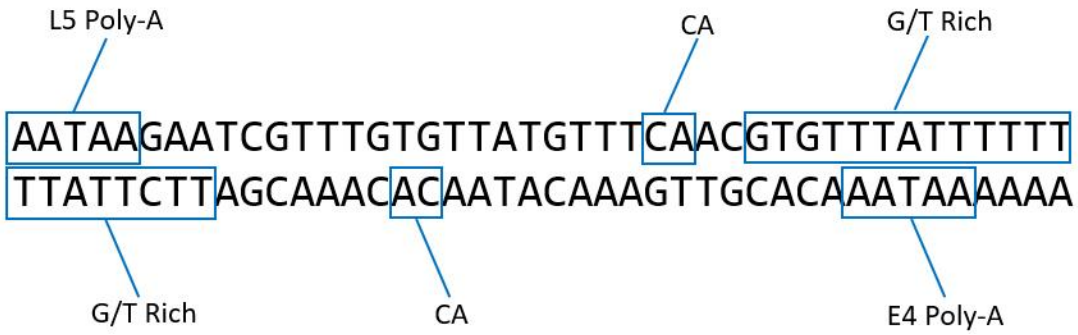


Figure 4.9. Canonical poly-A sequence as described by reference 40 and the overlapping L5 poly-A and E4 poly-A sequences of wildtype Ad5.

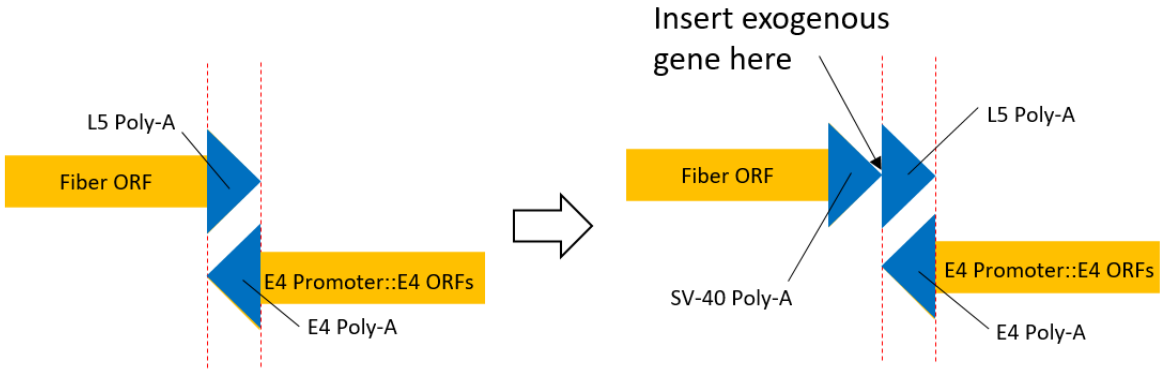


Figure 4.10. Schematic of additional SV40 poly-A sequence, creating location for addition of exogenous gene.

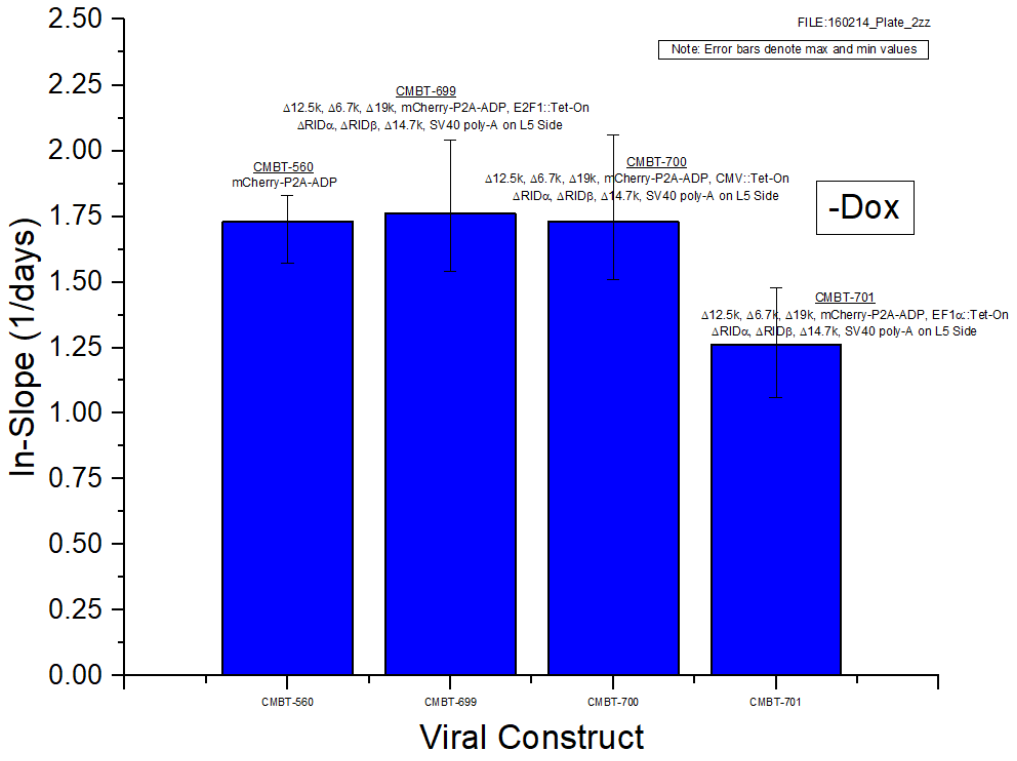


Figure 4.11. Measured replication kinetics of virus constructs with Tet-On gene placed between the L5 and E4 regions.

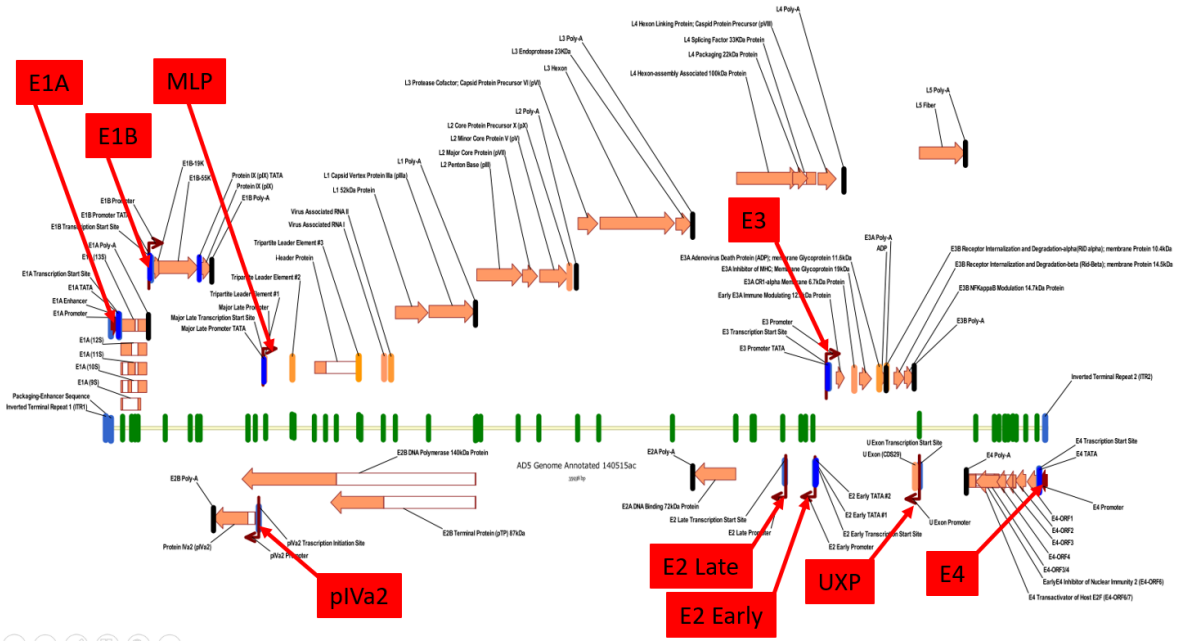


Figure 4.12. The Ad5 genome with all 9 known promoters highlighted in red.

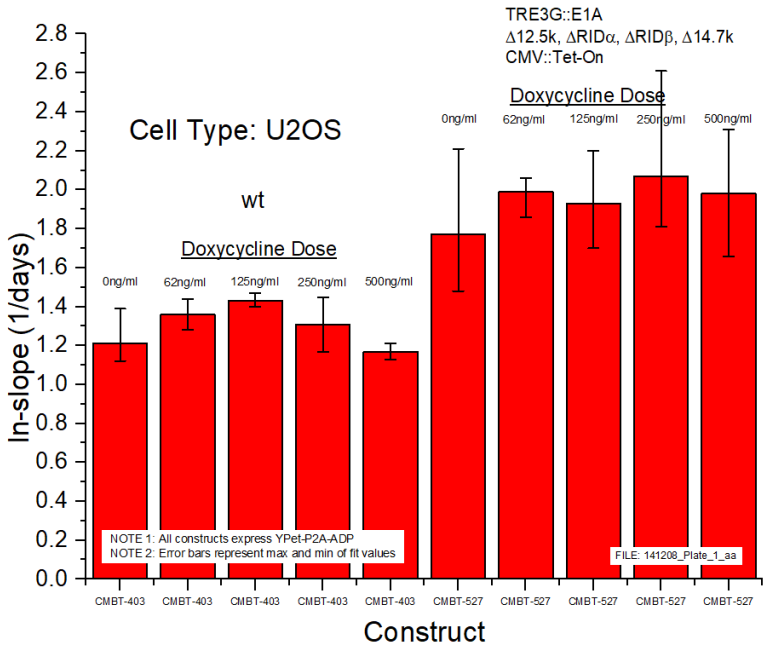


Figure 4.13. Measured virus replication kinetics for wt Ad5 vs. a Ad5 construct with the E1A promoter replaced by the TRE3G promoter (Tet-On is driven by CMV).

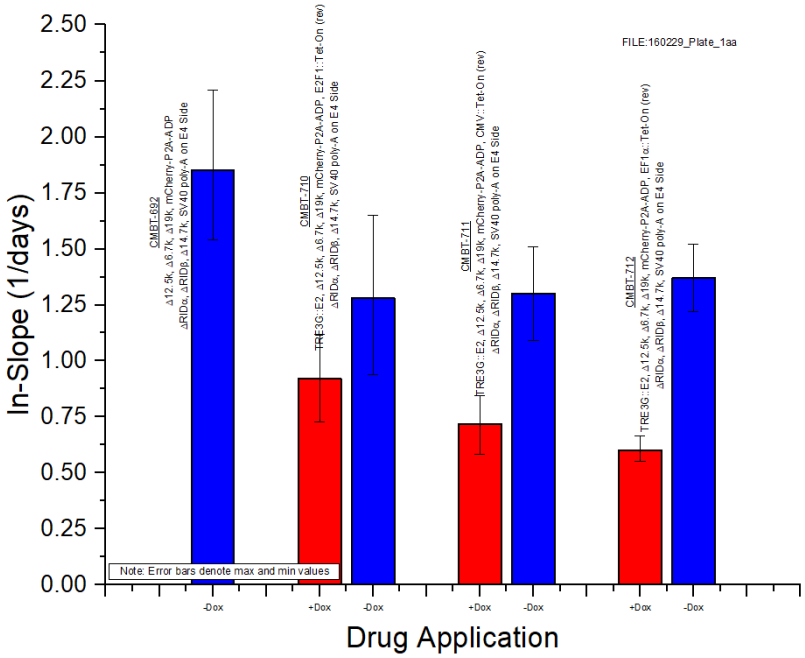


Figure 4.14. Measured replication kinetics for constructs with the E2 early promoter replaced by TRE3G. The Tet-On transcription factor is driven by E2F1, CMV, or EF1α.

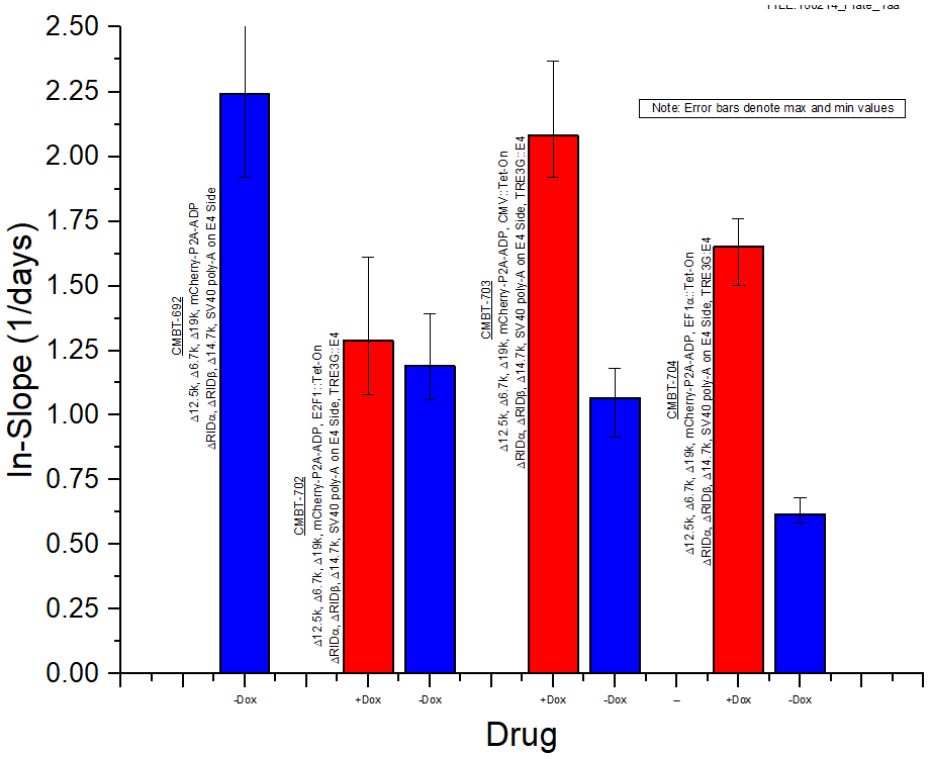


Figure 4.15. Measured replication kinetics for constructs with the E4 promoter replaced by TRE3G. The Tet-On transcription factor is driven by E2F1, CMV, or EF1α.

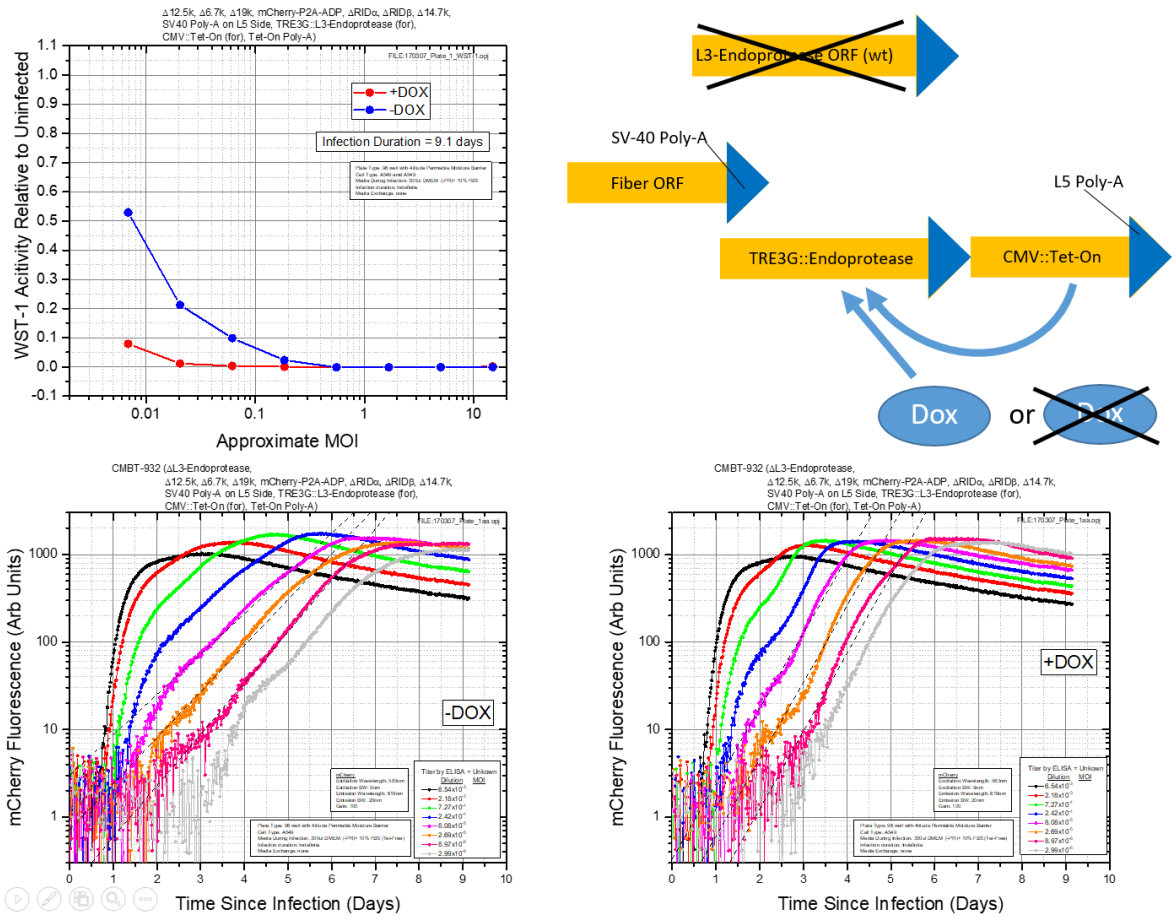


Figure 4.16. Cell viability assay results (upper left), schematic diagram (upper right), and kinetics curves +/-Dox (lower left and right) for virus with Endoprotease placed under direct control of the TRE3G promoter.

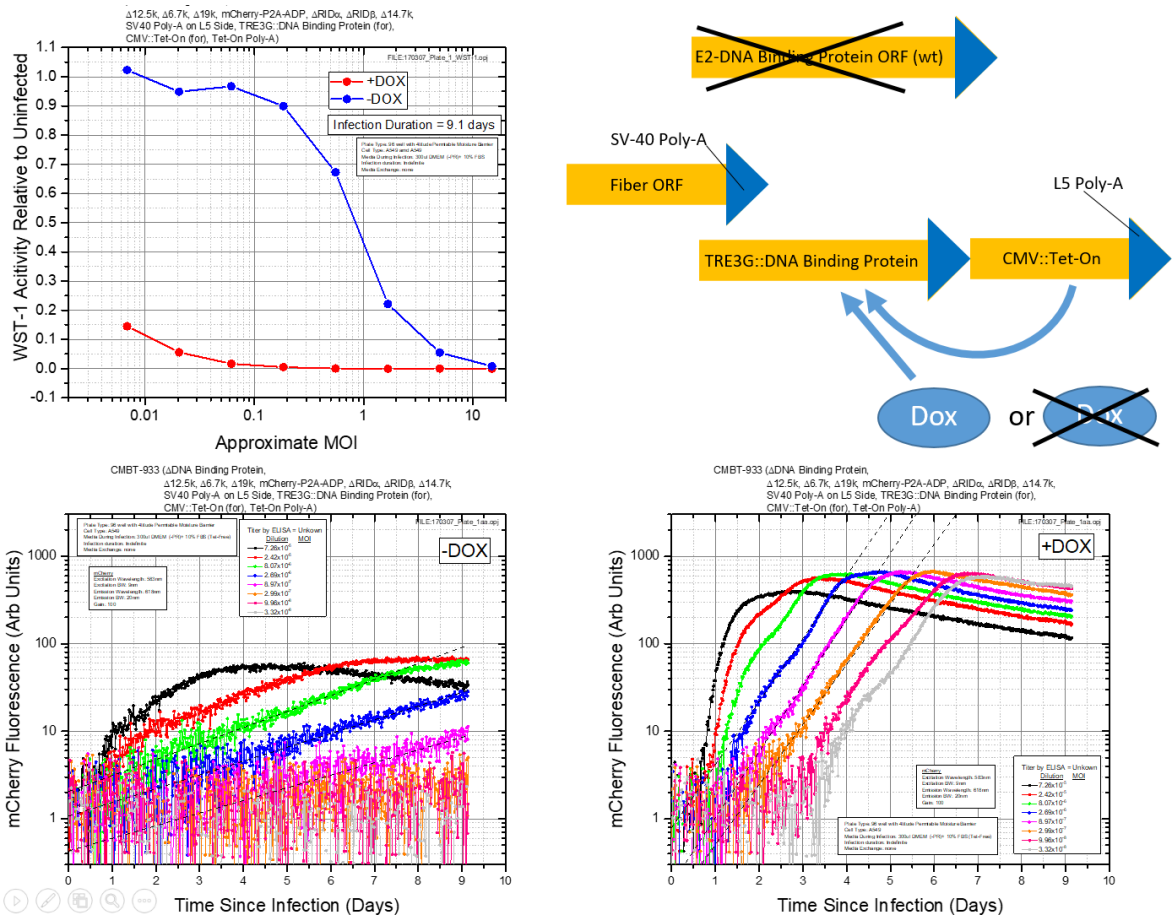


Figure 4.17. Cell viability assay results (upper left), schematic diagram (upper right), and kinetics curves +/-Dox (lower left and right) for virus with DBP placed under direct control of the TRE3G promoter.

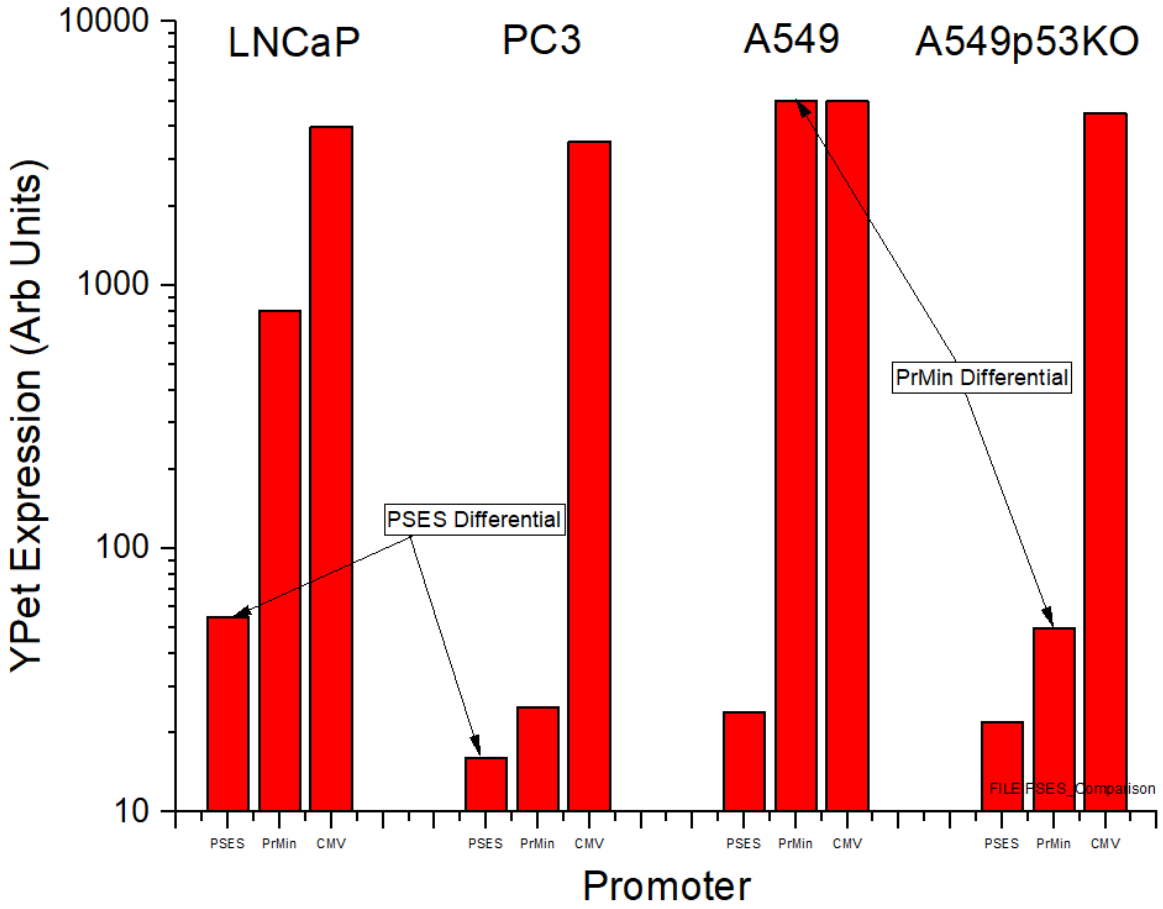


Figure 4.18 Measured YPet expression driven by PSES, PrMin, or CMV promoter upon infection with MOI=10 for cell lines LNCaP, PC3, A549, and A549p53KO. LNCaP and A549 are $p53^{+/+}$. PC3 and A539p53KO are $p53^{-/-}$.

Table 4.1. Prostate-specific promoters and payloads for non-replicating Ad vectors. PSAe = Prostate-Specific Antigen enhancer, PSAp = Prostate-Specific Antigen promoter, PSE-BA = Prostate-Specific Enhancer + androgen receptor binding site, PSES = Prostate-Specific Antigen enhancer + Prostate-Specific Membrane Antigen enhancer, rPB = rat Probasin promoter, PSAe = Prostate-Specific Antigen enhancer, PSMAe = Prostate-Specific Membrane Antigen enhancer, TARPp = T-cell receptor γ-chain Alternate Reading frame Protein promoter, CMV = Cytomegalovirus promoter

Prostate-Specific Promoter	Payload	Reference
PSAe/PSAp	nitroreductase	4
PSE-BA	Luciferase	5
PSAp	Diphtheria Toxin	6
PSES	Luciferase	7
rPB	HSV-tk	8
PSAe/PSMAe/TARPp	Luciferase	9
CMV	mda-7/IL-24	10

Table 4.2. Examples of the TSTA system used for prostate-selective expression of a target gene. PSE = Prostate-Specific Antigen Promoter, PSMA = Protate-Specific membrain Antigen Promoter, PSMAe = Prostate-Specific Membrane Antigen enhancer, TARPp = T-cell receptor γ-chain Alternate Reading frame Protein promoter, PSE-BC = Enhanced Prostate-Specific Antigen Promoter, PSES = Prostate-Specific Antigen enhancer + Prostate-Specific Membrane Antigen enhancer

Prostate-Specific Promoter	Payload	Reference
PSE	Expanded Polyglutamine	3
PSE	Luciferase-HSV1-sr39tk	11
PSE	Luciferase	12
PSE	HSV-tk	13
PSE	Luciferase	14
PSMA	HSV-tk	15
PSMAe/TARPp	Luciferase	16
PSE-BC	Luciferase-HSV1-sr39tk	17
PSES	HSV-tk	18

Table 4.3. Prostate-specific promoters and Ad promoter replaced to impart prostate-only selectivity. PSE = Prostate-Specific Enhancer, rPB = rat Probasin promoter, PSAe = Prostate-Specific Antigen enhancer, PSAp = Prostate-Specific Antigen promoter, PSME = Prostate-Specific Membrane protein Enhancer, PSES = Prostate-Specific Antigen enhancer + Prostate-Specific Membrane Antigen enhancer, PSMAe = Prostate-Specific Membrane Antigen enhancer, TARPp = T-cell receptor γ-chain Alternate Reading frame Protein promoter, PSES(M6) = PSES with AP-3 binding site

Prostate-Specific Promoter	Replaced Ad Promoter	Reference
PSE	E1A	19
rPB PSAe/PSAp	E1A E1B	20
PSME	E1A	Lee_2004
PSES	E1A and E4	Li_2005
PSES	E1A and E4	Li_2008
PSAe/PSMAe/TARPp	E1A	Cheng_Cacner_Gene_2006
PSAe/PSMAe/TARPp	E1A	Danielson_2008
PSES(M6)	E1A and E4	Ahn_2009
PSES	E1A and E4	Kim_2013

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CHAPTER FIVE:

Negatively Regulated Selective Oncolytic Virus

Introduction

The use-case for a positively regulated selective oncolytic virus, as described in chapter 4, is straightforward. A positively regulated virus can be targeted to a particular tissue type based on a tissue-specific transcription factor, the prostate for example. The use-case for a negatively regulated virus is not so obvious. What feature of a tumor is missing in tumor cells, but found in all normal cells such that a negatively regulated virus would replicate in a tumor cell by not normal cells? The quintessential example is p53. All normal cells contain transcriptionally active p53, while a large fraction of tumor cells are either deleted for p53 entirely or express mutant forms that are no longer transcriptionally active. An oncolytic virus that replicates only in the absence of transcriptionally active p53 would be a useful clinical tool against nearly all cancer types.

Previous work Toward p53-Selective Oncolytic Viruses

Because of the tremendous promise of a p53-selective oncolytic virus, considerable work by a number of researchers has gone into its development. The earliest work involved deletion of the E1B-55k gene from the Ad5 genome[1,2] because it has been shown that E1B-55k mediates degradation of p53[3]. It was thought that deletion of E1B-55k would make Ad5 replication dependent on the status of p53 in the infected cell. This predicted p53-dependence was not borne out[4,5]. Work continues to this day toward a set of mutations within the Ad5 genome that would render it p53-selective.

As an alternate approach, researchers have added exogenous p53-dependent repressive control to the Ad in an effort to impart p53-selective replication. One such

example is expressing an E2F antagonist driven by a p53-dependent promoter[6]. Since both the E1A and E2 promoters are activated by the E2F transcription factor, it was thought that expressing an E2F antagonist in a p53-dependent fashion would render the Ad5 replication dependent on the absence of p53 in the infected cell. Another, similar, example is to express an shRNA against one of the endogenous Ad proteins and drive expression of this shRNA with a p53-dependent promoter[7]. A final example in this space is to express the I-secI meganuclease driven by a p53-dependent promoter plus adding the 18-base pair I-secI target sequence into the Ad genome[8,9]. Since the I-secI target sequence is not found in the human genome[10], this meganuclease should not cut any DNA except for the Ad genome and do so in a p53-dependent manner. If the Ad genome is cleaved by I-secI in a p53-depenent fashion, then Ad replication would become dependent on the absence of transcriptionally active p53.

Another general strategy found in the literature is similar to the TSTA system described in chapter 4, except in repressor mode rather than activating mode. In this scheme, a p53-sensitive promoter drives expression of a factor that represses another promoter within the Ad genome. One example of this scheme is a p53-dependent promoter driving a gal4-KRAB fusion which represses the CMV-gal4 promoter inserted into the Ad5 genome in place of the E1A promoter[11]. A similar example employs the same repression mechanism, but in this case applied to a promoter consisting of gal4 binding sites surrounding the hTERT promoter, again replacing the E1A promoter[12].

All of these examples exhibit the same problem as the positively regulated virus examples described in chapter 4, potency is sacrificed for selectivity. Some of the viruses

in the examples above exhibit nearly wildtype replication kinetics, but show limited or no selectivity. And some viruses show strong selectivity, but are considerably slower than wild type when infecting $p53^{-/-}$ target cells. The question is, can we develop a virus with negatively regulated selectivity based on p53 while maintaining near wild type kinetics?

Virus Regulated by the TetR System

As a starting point, we used the Tet-On system demonstrated in chapter 4. When controlling the Ad DBP with the Tet-On system, an approximate 1000X difference in TCID50 (Tissue Culture Infective Dose, 50%) between +doxycycline and –doxycycline is observed (see Fig. 4.17 of chapter 4). To test what level of control is possible with the TetR system, we replaced the Tet-On gene with TetR and replaced the TRE3G promoter with the CMV-Tet-O promoter. This promoter was optimized for high expression in the absence of TetR and maximum repression by the TetR protein[13]. A schematic of this circuit and the resulting control authority is shown in Fig 5.1.

Comparing the TetR system performance to that of the Tet-On system, as shown in Fig 5.2, highlights two differences. The off-state of the TetR system is not as good as that of the Tet-On system, and the on-state of the Tet-On system is not as good as that of the TetR system. Both of these observations make sense in light of the control mechanisms of the Tet-On and TetR systems. To generate the off-state in the TetR system, the TetR protein must fist be transcribed, translated, and accumulate in the cell, causing an initial delay in suppression of the CMV-Tet-O promoter. During this delay, some amount of DBP mRNA is transcribed. Conversely, the Tet-O system begins in the off-state and there

is a time delay associated with the transcription, translation, and accumulation of the Tet-On protein required for activating the TRE3G promoter, thus the on-state of the Tet-On system is slower than that of the TetR system.

p53-Sensitive Promoter

To render the TetR-controlled virus into a p53-selective virus, we must replace the CMV promoter driving TetR expression with a p53-sensitive promoter. The literature describes an artificial p53-sensitive promoter, called PrMin-RGC[14]. This promoter consists of 13 p53-binding sites in combination with a minimal CMV promoter. We have reconstituted this promoter on a plasmid driving YPet expression. Transfecting this plasmid into A549 and A549p53KO cell lines demonstrates the p53-selective nature of this promoter, as shown in Fig 5.3. The A549p53KO cell line is a $p53^{-/-}$ version of the A549 cell line generated by Jingwen Yin using CRISPR. The PrMin-RGC promoter exhibits a dynamic range of approximately 200X between $p53^{+/+}$ and $p53^{-/-}$ cell lines.

To measure the performance of this promoter in the context of an Ad infection, we cloned PrMin::YPet into the region between L5 poly-A and E4 poly-A, as described in chapter 4. Two so-called "sensor viruses" were produced, one with wt E1B-55k and another with ΔE1B-55k. When infecting A549 cells, a large YPet signal is produced only with the ΔE1B-55k version, as shown in Fig 5.4. When infecting A549p53KO cells, neither version of the sensor virus produces significant YPet signal. With ΔE1B-55k, the PrMin promoter exhibits a dynamic range of about 80X. This data shows how efficiently the wt E1B-55k protein of Ad5 degrades p53. No significant signal is produced by the

sensor virus containing wt E1B-55k. With this result in hand, the limited success in this space described in the cited references is better understood. Many of the p53-selective viruses described in the literature were constructed with wt E1B55k. Because of the efficient degradation of p53 by wt E1B-55k, one should not expect high expression of any Ad5 gene using a p53-sensitive promoter and thus the repressive effects of these p53-driven gene products would be minimal.

There is a high cost in replication kinetics when deleting E1B-55k from Ad5. Each of the sensor viruses described in the previous paragraph also contain mCherry-P2A-ADP as a kinetics readout. Fig 5.5 shows the measured replication kinetics for the sensor viruses when infecting A549 and A549p53KO. This data confirms that a significant kinetic defect occurs with deletion of E1B-55k.

Degradation of p53 by E1B-55k must be mitigated in order achieve activation of a p53-sensitive promoter placed within the Ad5 genome, but wholesale deletion of E1B-55k incurs an unacceptably high cost in kinetics. The literature describes point mutations in E1B-55k that abrogate to some extent the interaction between E1B-55k and p53 while mostly maintaining the other functions of E1B-55k[15]. The two most promising mutations described are: E1B-55k[H260A] and E1B-55k[R240A]. These mutations were cloned into sensor viruses and the resulting p53 transcriptional activity vs. virus kinetics was measured, as shown in Fig 5.6. These point mutations represent a compromise between p53 transcriptional activity and virus kinetics. The ideal mutation would produce a data point located in the upper right corner of the graph of Fig. 5.6 with maximum p53 transcriptional activity simultaneous with maximum virus kinetics.

p53-Selective, Negatively-Regulated Ad

The sensor virus results do not highlight a clear choice in E1B-55k mutation. As the p53-transcriptional activity is increased, the virus kinetics is decreased. This trade-off is unavoidable with the E1B-55k mutations available to us at this time. It is unknown what level of TetR expression is required to fully suppress the CMV-Tet-O promoter driving DBP in the negatively-regulated virus. Because there is no clear choice, all four versions of E1B-55k were cloned into the p53-selective virus shown schematically in Fig 5.7. The p53 selectivity of these viruses is shown in Fig 5.8 using a cell viability assay to determine cell killing versus initial MOI when infecting A549 and A549p53KO cells. The virus with wt E1-55k shows no selectivity, as expected, because wt E1B-55k is so effective at degrading p53. The virus with ΔE1B-55k shows some differential between A549 and A549p53KO. The virus with ΔE1B-55k exhibits an excellent off state, but a very weak on state, as expected due to the kinetics hit caused by deletion of E1B-55k. The virus with E1B-55k[H260A] shows an improved on state compared to the ΔE1B-55k virus, but a slightly worse off state. The best results were obtained with the E1B-55k[R240A] virus. Though the off state for this virus is slightly worse that the H260A mutation, the on state is greatly improved. The TCID50 for this virus is about 100X different between $p53^{+/+}$ and $p53^{-/-}$ cell lines.

Ultimate Performance of a p53-selective Ad

The 100X differential exhibited by the E1B-55k[R240A] virus is highly promising, but still has room for improvement. Fig 5.9 shows the performance of this virus in both A549p53KO and A549 cells overlaid with that of the wt E1B-55k virus in A549p53KO cells and ΔE1B-55k virus in A549 cells. These two extremes show the potential of this selective virus if the "unicorn" mutation in E1B-55k can be found. Such a mutation would exhibit kinetics equal to a virus with wt E1B-55k and p53 transcriptional activity of a virus with ΔE1B-55k.

Conclusions

An oncolytic virus with selectivity based on the status of p53 in tumor cells has been a goal of the field for nearly three decades. Many clever approaches toward such a virus have been attempted. Few researchers appreciated the efficiency at which E1B-55k degrades p53 and prevents transcriptional activation by p53 in Ad5 infected cells. The sensor viruses described in this chapter, in combination with a TP53-knock out version of A549, provided us with the key insight that any p53-dependent Ad must first address p53 degradation by E1B-55k. In addition, the viral kinetics assay developed as part of this dissertation allows accurate measurement of the kinetics cost associated with modifications made to E1B-55k. With these tools in hand, the "unicorn" version of E1B-55k could be found and its positive impact on both the safety and efficacy of the p53-selective virus described in this chapter will be dramatic.

Figures

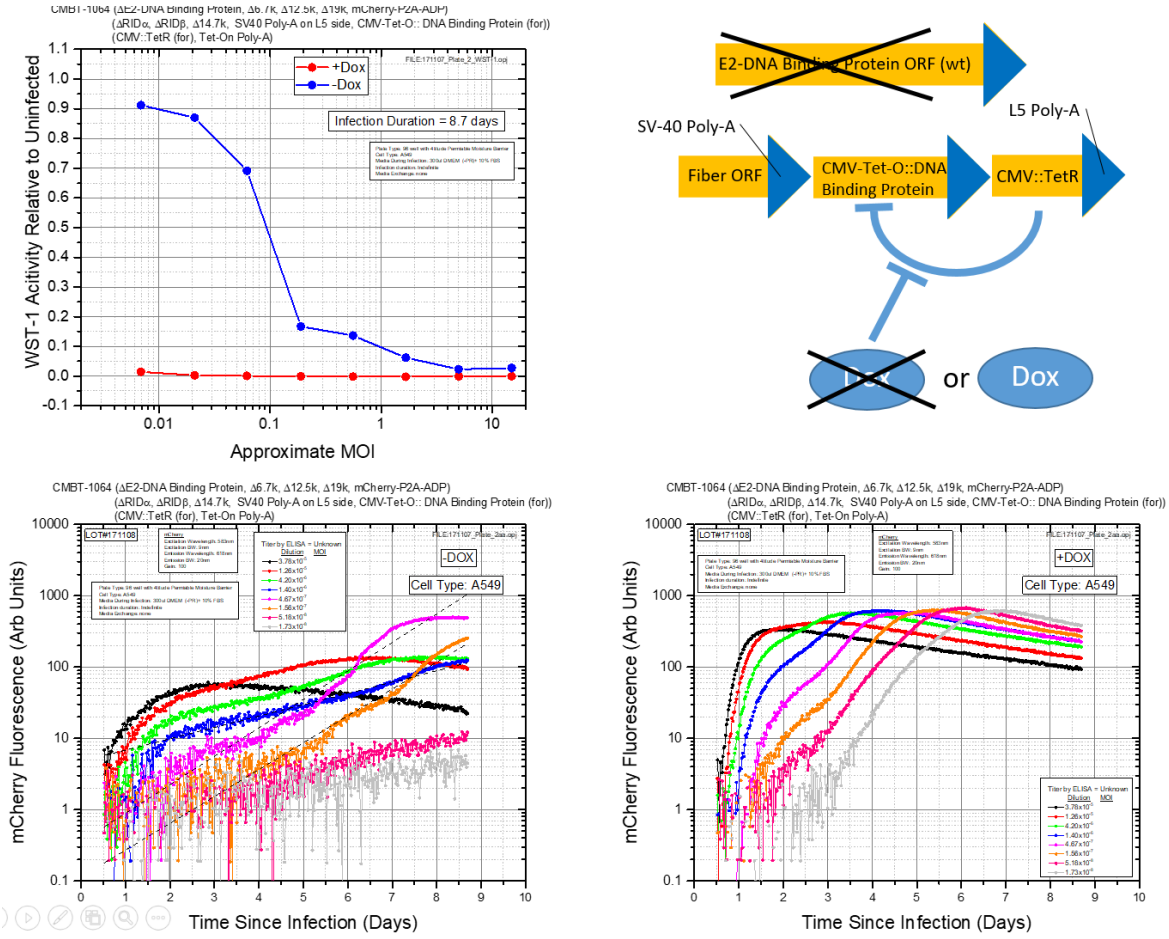

Figure 5.1. Cell viability assay results (upper left), schematic diagram (upper right), and kinetics curves +/- Dox (lower left and right) for virus with DBP placed under direct control of CMV-Tet-O promoter. The TetR protein is driven by the CMV promoter.

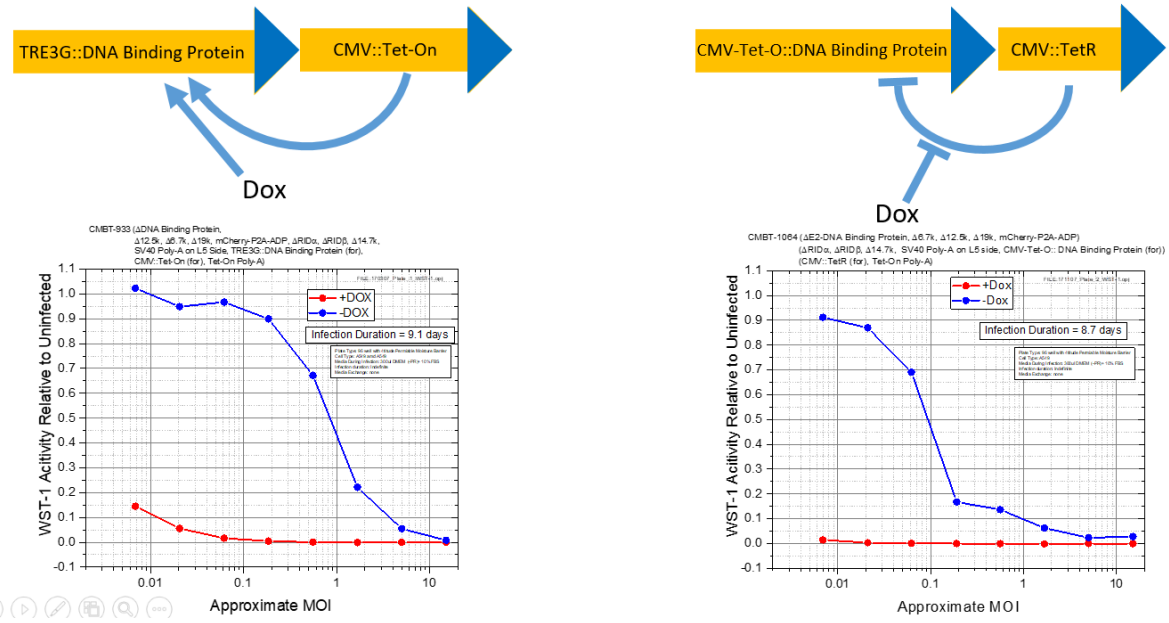

Figure 5.2. A side-by-side comparison of the Tet-On and TetR systems used to control Ad5 replication.

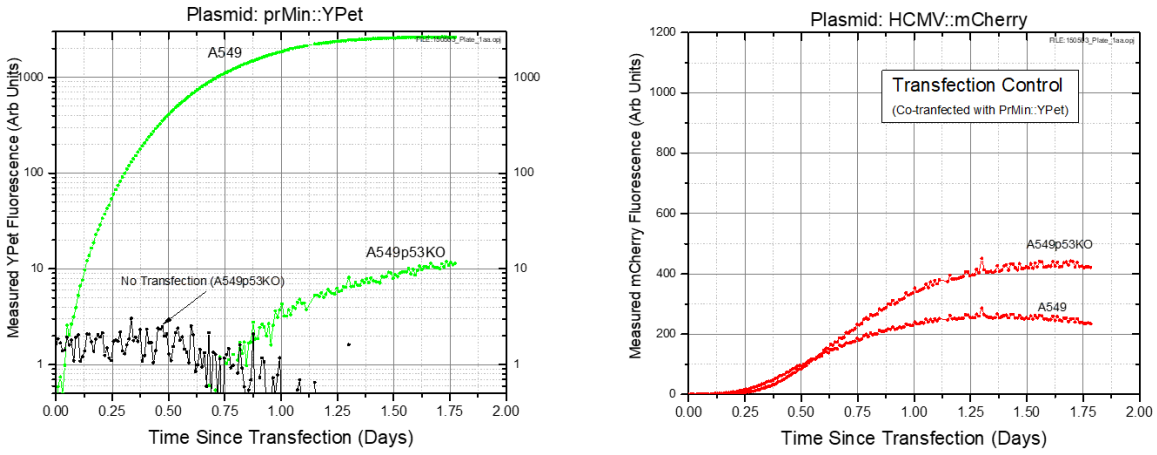

Figure 5.3. Measured YPet fluorescence produced by PrMin::YPet plasmid transfected into A549 and A549p53KO cells. Also shown is measured mCherry fluorescence produced by a co-transfected CMV::mCherry plasmid.

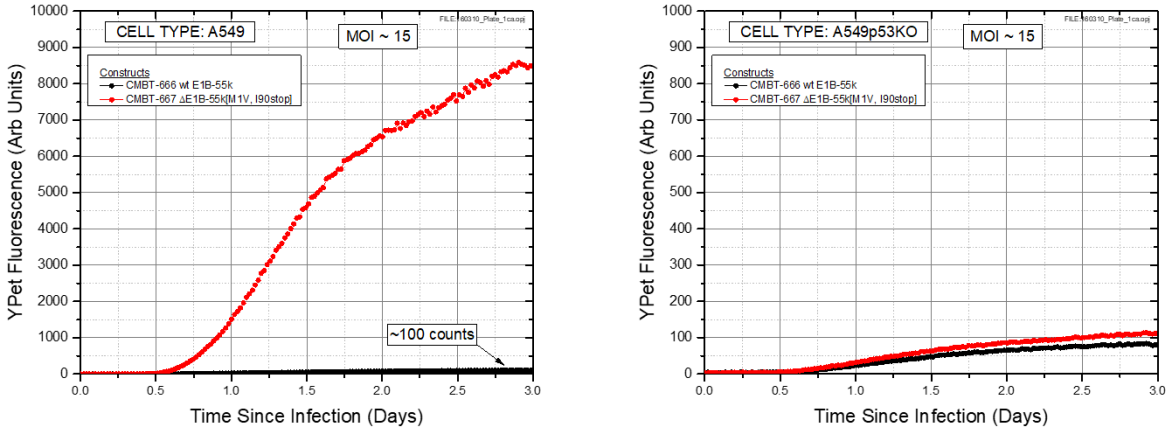

Figure 5.4. Measured YPet fluorescence produced by sensor viruses infecting A549 and A549p53KO cell lines. The black lines represent data for virus with wt E1B-55k and red lines represent data for virus with ΔE1B-55k.

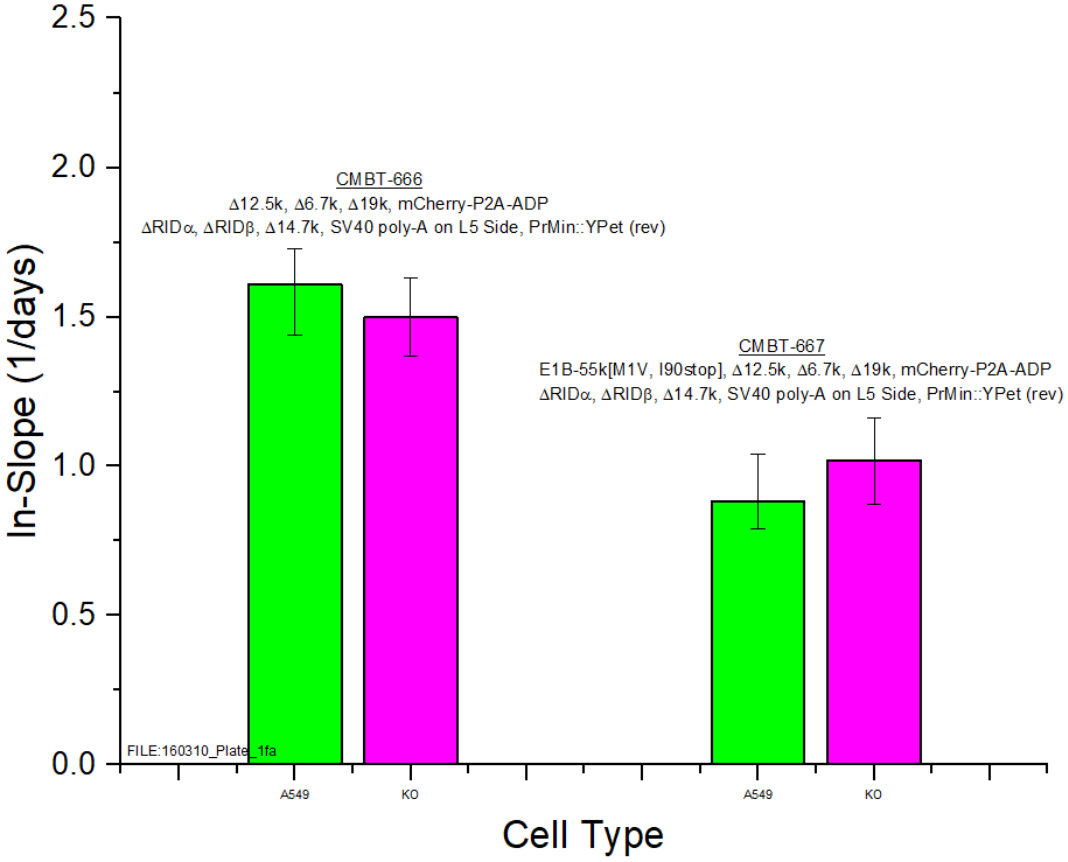

Figure 5.5. Measured replication kinetics for sensor viruses with wt E1B-55k and ΔE1B-55k, when infecting A549 and A549p53KO cell lines.

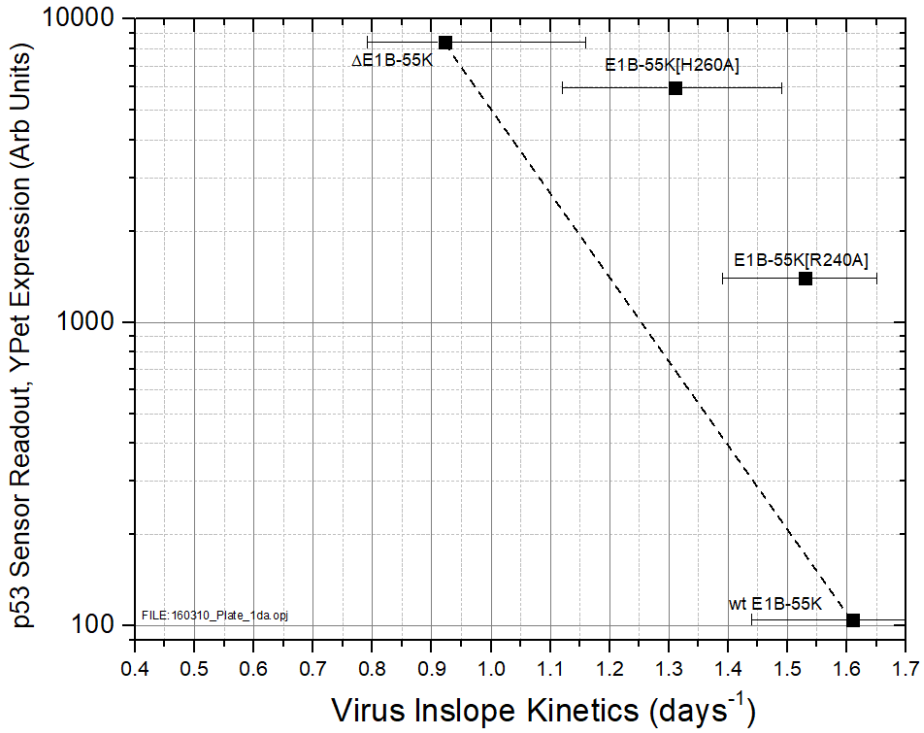

Figure 5.6. Measured p53 transcriptional activity (YPet signal) vs. virus replication kinetics for sensor viruses when infecting A549 cells. Each sensor virus has the following E1B-55k mutations: wt, full deletion, H260A, and R240A.

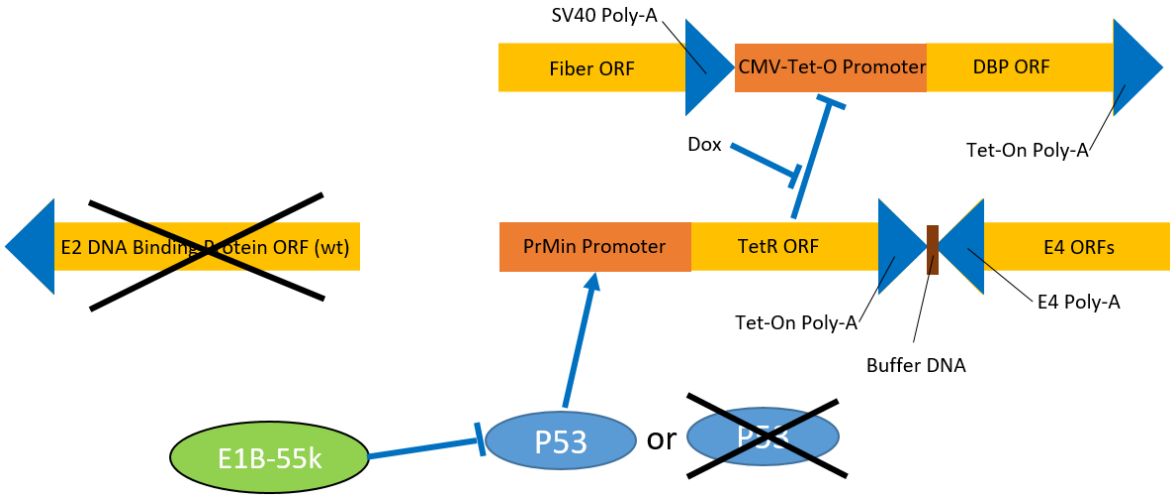

Figure 5.7. Schematic of p53-selective, negative control of Ad5 replication. The E1B-55k protein is kept as wt, completely deleted, or contains An H260A or R240A point mutation. Testing is done in $p53^{+/+}$ or $p53^{-/-}$ cell lines.

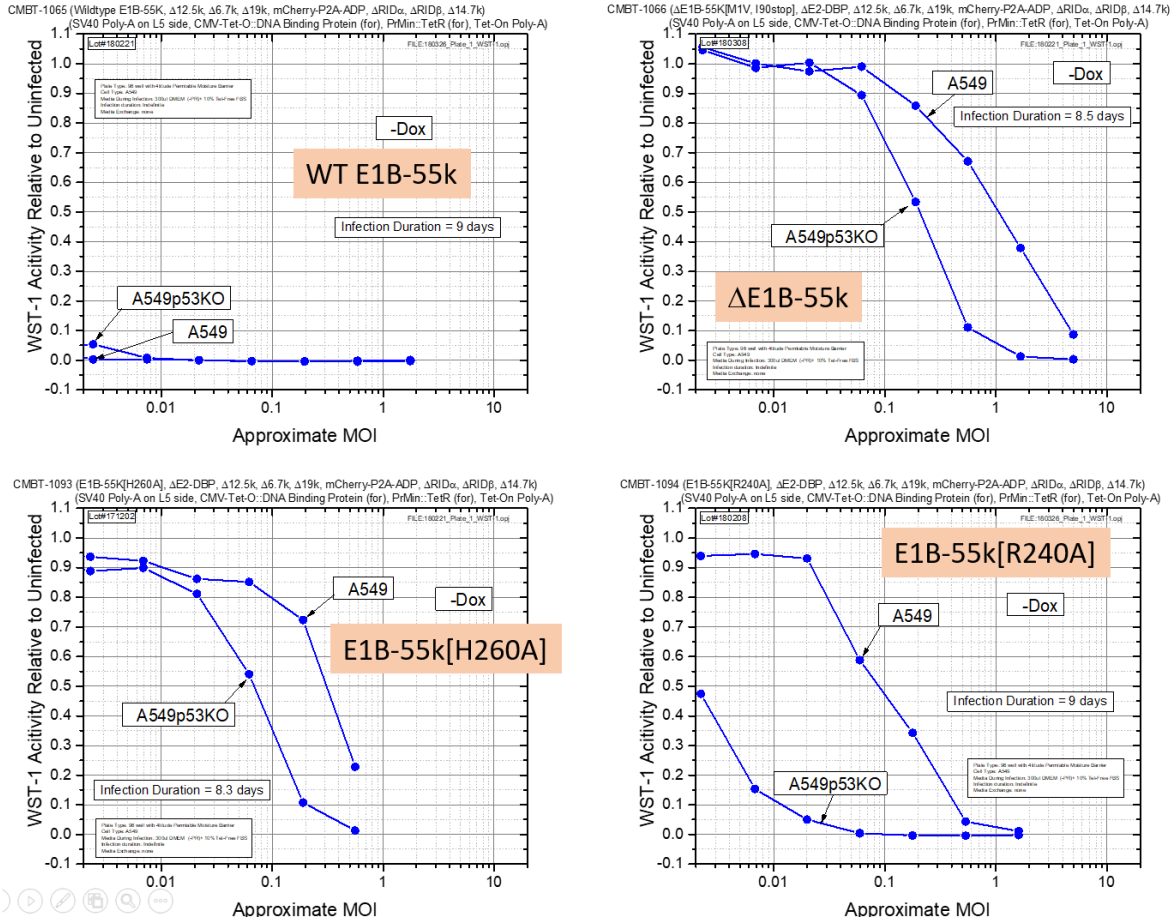

Figure 5.8. Measured cell viability 9 days post infection in A549 and A549p53KO cells infected with p53-selective viruses containing various mutations in E1B-55k.

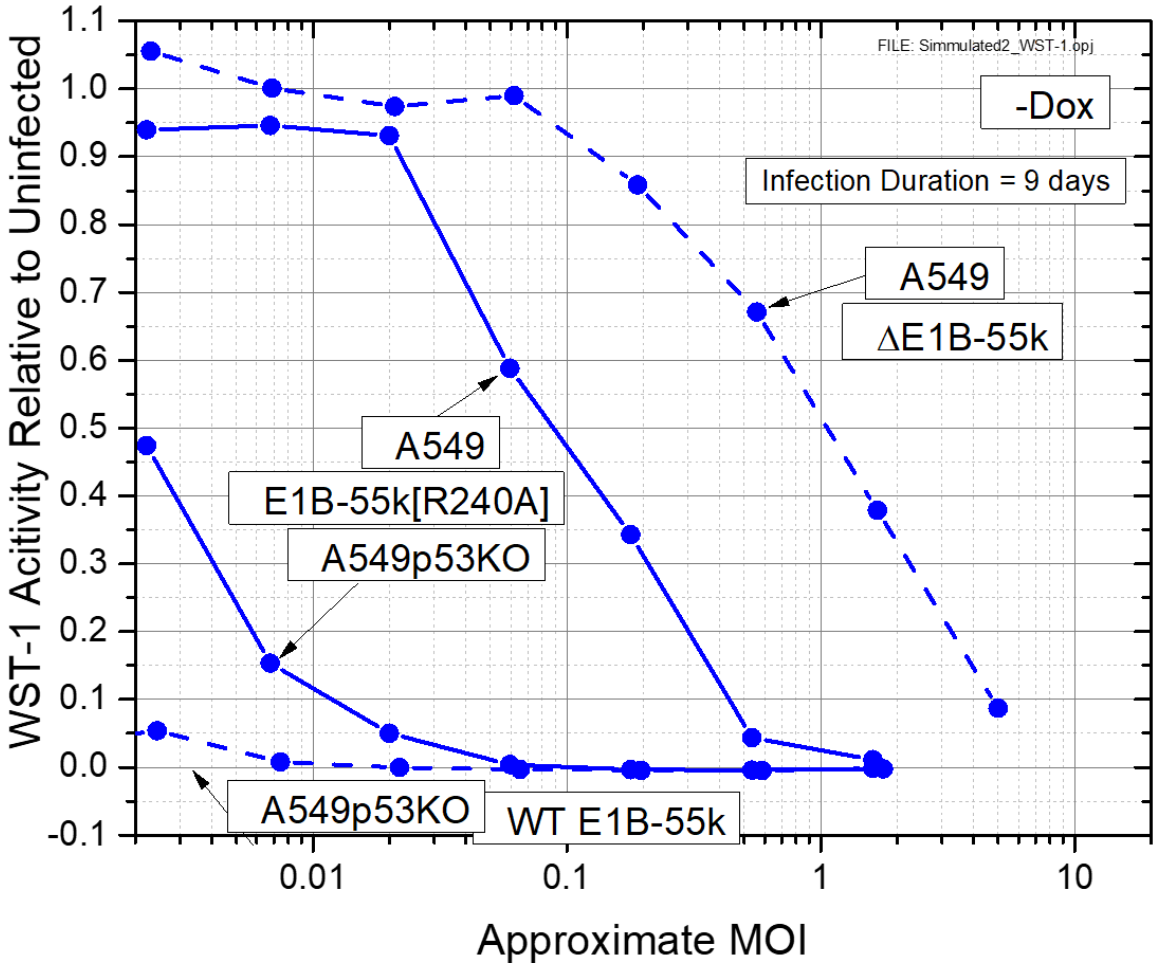

Figure 5.9 Comparison of cell viability between E1B-55k[R240A] virus shown with solid lines and that of wt E1B-55k virus in A549p53KO cells and ΔE1B-55k virus in A549 cells shown with dashed lines. The collapse of the solid line TCID50 compared to the dashed lines is the sacrifice made in the differential when using an imperfect version of mutated E1B-55k.

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CHAPTER SIX:

Experimental Procedures

Cell Culture

HEK293, 293-E4, and A549 cells were cultured in DMEM + 10% Fetal Bovine Serum (FBS). The FBS is heat inactivated at 55C for one hour, passed through a 0.22μm filter, then aliquoted into 50ml volumes for freezing. Cell passage was performed as follows:

Prepare Trypsin

- Mix 1 part Trypsin/TD with 4 parts VE (EDTA at 0.02%)

Release and Collect Cells

- Aspirate media
- Wash cells with PBS -/- (10ml for 10cm plate, 20ml for 15cm plate)
- Add Trypsin (3ml for 10cm plate, 5ml for 15cm plate)
- Incubate at 37C for 5 min
- Use fresh media to wash cells from plate surface (5-10ml for 10cm plate, 10-20ml for 15cm plate)

Spread cells on fresh plates

- Spread media/cells onto fresh plates
  - 1 confluent 10cm plate splits to 4X10cm plates
  - 1 confluent 10cm plate splits to 2x15cm plates
  - 1 confluent 15cm plate splits to 4X15cm plates

Virus Expansion

Virus production and expansion is done with HEK293 cells or 293-E4 cells + dexamethasone, depending on the need to rescue deletions in the E4 region of the Ad genome. The expansion steps typically used are: 2X6 well, 1X10cm, 2X15cm, 15X15cm. The steps for expansion to 15X15cm are show below. All other expansion steps are similar, but scaled appropriately:

- 24 hours prior to infection:
  - Replace media on all plates with MEM + 10% FBS + Dex
  - Dex is supplied at 10,000X
  - Add sufficient 10,000X Dex to reach 1X Dex concentration in media prior to application
- Return plates to 37C incubator
- On day of infection:
- Prepare freezing medium by placing dry ice in insulated bucket and adding 95% EtOH
  - 95% EtOH is located in cabinet in Hunter lab (cabinet just opposite the tissue culture room)
  - Dry ice located in the Shaw lab
  - As an alternate, may use liquid nitrogen
- Perform freeze/thaw 3 times total
  - If taking virus from -80C, count this as the first freeze step
  - Thaw in the 37C water bath
- Spin virus solution at 3200RPM for 10 minutes

- Replace media on 15X15cm plates with 16ml of fresh MEM + 2%FBS + Dex
  - Dex is supplied at 10,000X
  - Add sufficient 10,000X Dex to reach 1X Dex concentration in media prior to application
- Distribute supernatant from spin evenly among the 15X15cm plates
  - Take care to avoid the cell debris pellet

Virus harvest

Harvest of virus are the last expansion stage, 15X15cm plates, attempts to capture the cells prior to viral lysis, so called cell-associated. The following steps describe the harvest protocol:

Harvest Cells

- Cells are ready for harvest when they exhibit full cytopathic effect (CPE) and are rounded up, but not yet completely detached from the plate
  - Typically takes 48-72 hours if infected with a multiplicity of infection (MOI) of 5-10
- Repeatedly wash the plate with 10ml pipette and collect supernatant
- Pool supernatant from all plates into a new, sterile 250ml conical centrifuge tube
- Pellet the cells at 2,500RPM 4C for 5 minutes
- Aspirate supernatant
- Re-suspend pellet in 10ml of 1X TMN (Cracking Buffer)
- Transfer to a 15ml centrifuge tube

- Can store this suspension at -80C for later processing
- 10X TMN (Cracking Buffer)
  - 100mM Tris pH 7.5
  - 1.5M NaCl
  - 10mM $MgCl_2$
- Recipe for 1 liter of 10X TMN
  - 100ml 1M Tris pH 7.5
  - 300ml 5M NaCl
  - 10ml 1M $MgCl_2$
  - Bring to 1 liter total volume with ddH_2O
  - Autoclave using cycle 1 (liquid for 40 minutes)

Virus Purification

All purified virus used in the experiments described in this dissertation were two stage CsCl purified. The details of this purification protocol are as follows:

Release Viral Particles

- If freeze/thaw protocol has already been applied to virus, skip to CsCl loading section
- Prepare freezing medium by placing dry ice inside insulated bucket and adding 95% EtOH
  - 95% EtOH is located in cabinet in Hunter lab (cabinet just opposite the tissue culture room)

    - Dry ice located in the Shaw lab
- As an alternate, use a thermos filled with liquid nitrogen
- Freeze solution in dry ice/95% EtOH bath or liquid nitrogen
- Thaw in the 37C water bath
- Perform freeze/thaw 3 times total
    - Mix well between each freeze/thaw cycle

Viral Solution Preparation

- Pellet cellular debris at 3,200RPM for 10 minutes
- Transfer supernatant to clean 15ml centrifuge tube
    - Supernatant will still be cloudy
- Pellet any remaining cellular debris at 3,600RPM for 10 minutes
- Transfer supernatant to a new 50ml tube
    - Supernatant may still be cloudy
- Dilute the volume to 18ml with 10mM Tris pH8

Set up Gradient #1

- To an SW32 tube (Beckman #344058) add 10ml of light CsCl solution
    - Tubes are located on shelf across from high speed centrifuge
    - Buckets and SW32 rotor are located in Shaw lab 4C, below east side bench
    - Use Styrofoam tube holder for more secure mounting
- Underlay with 10ml of heavy CsCl solution
    - Insert pipet tip along the wall of the tube
    - Avoid air bubbles

  - You should be able to see the interface between CsCl layers
- Carefully and slowly layer the 18ml of virus solution on top of the CaCl
  - Top off the tube with 10mM Tris pH8 if necessary. Stop 2-3mm from lip of tube.
  - Hold pipet tip against tube wall, immersed in meniscus of top CsCl layer
- Load tubes into buckets
  - Bucket cover release is 1/4 turn
  - Check that cone-shaped adapter is removed from bucket (do not discard)
  - Wipe debris and excess moisture from bucket
  - Ease tube into bucket while holding very top of tube. Tube falls quickly in this bucket type.

<u>Spin #1</u>

- Spin the gradient at 20k RPM for at least 3 hours at 4C
  - This spin can go overnight if needed
  - Release vacuum to allow opening of centrifuge lid
  - Install all buckets in appropriate numbered locations
  - Install rotor by removing from rotor stand in a vertical motion and placing on centrifuge spindle in a vertical motion. There is no mechanical detent.
  - Close lid
  - Engage vacuum
  - Set temperature to 4C
  - Set time to "hold" (press "other options" button located below screen)

- Set acceleration to "max"
- Set deceleration to "max"
- Enter rotor number from list shown on display
    - The SW32 rotor is number 15 and is called "other1"
- Wait under pressure drops below 500 microns before starting spin
- Make appropriate entries to sign-in sheet

Pull Virus Band after Spin #1

- Setup apparatus in TC hood
    - Place large tube clamp in TC hood (tube clamp located on floor next to north-east TC hood)
    - Fill beaker with 100ml of 1X Rocadyne and place below tube clamp
    - Mount black paper behind tube holder for better viewing (paper located in drawer next to north-east TC hood)
- Wear double gloves
- Transfer tube from centrifuge bucket to tube holder. Use forceps to lift out.
    - The infectious virus will be the lowest band formed on the gradient (see example figures below)
- Using an 18 gauge needle and 5ml syringe, puncture the tube about 1cm below the band
- Pull the band out, trying to avoid any bands sitting above the infectious band.
- The volume should be about 2ml
- Squirt the virus into a labeled 15ml conical tube

- Dilute the virus to 4ml total volume with 10mM Tris pH8
- Clean all surfaces with SDS and dry with paper towel
- When returning centrifuge buckets, make sure to include conical adapters

Set up Gradient #2

- To an SW41 tube (Beckman #344059) add 4ml of light CsCl solution
  - Tubes are located in drawer next to north-east TC hood
  - Buckets and SW41 rotor are located in Shaw lab 4C, below east side bench
  - Use metal tube rack to hold tubes (located in drawer next to north-east TC hood)
- Underlay with 4ml of heavy CsCl solution
  - Insert pipet tip along the wall of the tube
  - Avoid air bubbles
  - You should be able to see the interface between CsCl layers
- Carefully and slowly layer the 4ml of virus solution on top of the CaCl
  - Top off the tube with 10mM Tris pH8 if necessary. Stop 2-3mm from lip of tube.
  - Hold pipet tip against tube wall, immersed in meniscus of top CsCl layer
- Load tubes into buckets
  - Bucket cover release is requires special screwdriver located in drawer next to north-east TC hood
  - Wipe debris and excess moisture from bucket

- Ease tube into bucket while holding very top of tube. Tube falls slowly in this bucket type.

Spin #2

- Spin the gradient at 20k RPM for at least 3 hours at 4C
  - This spin can go overnight if needed
  - Release vacuum to allow opening of centrifuge lid
  - Install all buckets in appropriate numbered locations
  - Install rotor by removing from rotor stand in a vertical motion and placing on centrifuge spindle in a vertical motion. There is no mechanical detent.
  - Close lid
  - Engage vacuum
  - Set temperature to 4C
  - Set time to "hold"
  - Set acceleration to "max"
  - Set deceleration to "max"
  - Enter rotor number from list shown on display
  - Wait under pressure drops below 500 microns before starting spin

Prepare Dialysis Buffer

- Measure out 300ml of 100% glycerol in a graduated cylinder and pour into a 3 liter beaker (glycerol located on floor across from lab sink)
- Wash remaining glycerol from the graduated cylinder using ddH_2O as part of the 2400ml required for final solution

  - Do not exceed 2500ml total volume at this point
- Add 300ml of 10X TMN
- Bring to 3000ml total volume with ddH_2O
- Add stir bar
  - Sterilize with 70% EtOH
- Mix on stir station to ensure homogeneity
- Store in 4C cold room
- Equilibrate dialysis cassette by placing in dialysis buffer (only ~5-20 minutes before use)
  - Label each cassette for each viral purification
  - Use floating cassette buoys to float the cassette in buffer (buoys are located on table across from Jason). Sterilize with 70% EtOH.
  - Use Pierce brand 10,000 MW cutoff cassette #66380 with 0.5-3ml capacity (Cassette located on shelf across from Jason)

<u>Prepare for viral band extraction</u>

- Assemble small tube holder and background inside TC hood
- All components are located in drawer next to north east TC hood
- Preposition one 5ml syringe and one 18Ga x 25.4mm needle for each virus inside the TC hood
- Syringes located in general stock cabinets and needles located on shelf across from Jason

- Fill a 1 liter beaker with 100ml of 1X Rocadyne and place under tube holder (Rocadyne located at sink near TC room)

Extract Viral Band

- Wear double gloves
- Load ultra-centrifuge tube into tube holder
  - If virus band is located high in tube, clamp below band
  - If virus band is located low in tube, clamp above band
- Pull 2ml volume of air into the 18G needle/syringe
- Orient needle with beveled opening pointed down and puncture side of tube at a position slightly below the virus band
  - To puncture the tube requires considerable force
  - Support the tube with your free hand
  - Be sure to position your hand to avoid accidental puncture
- Rotate the beveled opening up, toward the virus band
- Draw solution from the tube while moving the needle tip left to right and in and out to extract all of the virus band
  - Avoid extracting any of the higher bands
- Pull needle from tube while supporting with your freed hand
  - Avoid fluid that will drip from the punctured tube

Load Dialysis Cassette

- Insert the needle into the cassette with the beveled opening rotated in-plane with the cassette frame

    - Insert slowly and to a distance just sufficient to penetrate the inner volume
- Rotate cassette/needle/syringe so that syringe is positioned above the cassette
- Slowly insert plunger to transfer all fluid from syringe to cassette
- Once fluid transfer is complete, slowly draw back syringe to extract all remaining air from cassette volume
- Either mark the puncture position or remember its orientation relative to cassette label to avoid its reuse
- Each of the four corners allows a single puncture

Dialyze

- Place the cassette/buoy assembly in the dialysis buffer and place beaker in 4C cold room on a stir plate
- Dialyze overnight

Removal from Dialysis Cassette

- Place the dialysis cassette in the TC hood
- Assemble a 5ml syringe with a 18Ga needle
- Draw 3ml of air into the syringe
- Carefully insert the needle in to the cassette, stopping just as the needle extends past the frame of the cassette
    - Be sure to use a different puncture hole from the one used to load the viral solution
- Rotate cassette/needle/syringe so that syringe is positioned above the cassette

- Inject sufficient air to spread the cassette membranes and allow free flow of the viral fluid
- Gently shake the cassette to ensure all viral particles are in solution
    - During dialysis, the viral particles may form a precipitate
- Rotate cassette/needle/syringe so that syringe is positioned below the cassette
- Slowly draw the viral fluid from the cassette while adjusting its tilt to force all fluid in to the corner of the cassette
- Extract the syringe/needle and transfer all fluid into a 1.5ml centrifuge tube

Aliquot Viral Solution

- Load the white centrifuge tube rack (with bottom basin) with dry ice and 90% EtoH
    - Place in the TC hood
    - White/basin tube rack is located on shelf across from Aaron's desk
- Aliquot 150µl volumes into separate tubes until all viral solution is distributed
- Place in chilled centrifuge tube rack
- Label each tube with viral identifier and lot#
- Make appropriate entries in the CERF database “AA Pure Virus Stocks” on the “Large Stocks” sheet of the workbook
- Place all tubes in their appropriate box and positions in the horizontal -80C freezer located one floor above the lab

Clean up

- Dump dialysis buffer solution in sink
- Rinse the 4 liter breaker with deionized water

- Autoclave the 4 liter beaker on cycle 3 with aluminum foil as cover

10X TMN (Cracking Buffer)

100mM Tris pH 7.5

1.5M NaCl

10mM $MgCl_2$

Recipe for 1 liter of 10X TMN

100ml 1M Tris pH 7.5

300ml 5M NaCl

10ml 1M $MgCl_2$

Bring to 1 liter total volume with ddH_2O

Autoclave using cycle 1 (liquid for 40 minutes)

Large Aliquot of Heavy CsCl Solution

- CsCl 423.3g
- 10mM Tris pH8 577.7ml

Large Aliquot of Light CsCl Solution

- CsCl 223.9g
- 10mM Tris pH8 776.1ml

Virus Titration

The FBVK assay is tolerant to inaccurate knowledge of the viral titer, thus much of the work described in this dissertation was done without a carefully measured titer. In

some cases, a well measured titer was desired and these cases the Limiting Dilution Assay (LDA) with 293 cells was used to obtain a titer. The protocol for LDA titer is as follows:

- ~24 hours prior, seed 96 well plate with 25,000 cells per well using 100ul of media per well.
- After ~24 hours inspect wells to ensure that cells are achieved at least 85% confluency.
- Add 100ul of fresh media to all wells of cell-containing 96 well plate
- Dilute small aliquot virus are 1E+06 in a 1.5ml Eppendorf tube. Likely will require 2-3 steps of serial dilution to achieve this value.
- Place 250ul of diluted virus in all wells of column number 1 of a fresh (no cells) 96 well plate.
- Place 160ul of fresh media in all other wells of same 96 well plate.
- Transfer 80ul of virus/media from all wells of column 1 to all wells of column 2.
- Exchange pipet tips and repeat transfer from column 2 to column 3.
- Repeat dilution transfer across the 96 well plate, changing tips between columns. DO NOT TRANSFER VIRUS/MEDIA TO COLUMN 12. These wells will be used for blanking during the fluorescence scan after 9-10 days.
- Once dilution plate is complete, transfer 100ul of virus/media from each well of the dilution plate to the corresponding well of the cell-containing plate.
- Place cell-containing plate in incubator at 37C, 5% CO2 for 9-10 days.
- After 9-10 days, read fluorescence of each well using a plate reader with settings appropriate for the fluorophore expressed by the given virus.

- Score each well as positive if the fluorescence level is 3 standard deviations above the average of all the uninfected wells (column 12).
- Apply the Spearman & Karber algorithm for calculating titer as described in reference #1.

Virus Whole Genome Assembly: Adsembly Protocol

The following protocol for producing whole genome plasmids using the multisite gateway Adsembly process is taken from unpublished work by Colin Powers:

For standard Adsembly reactions, combine 20fmol of dual-DEST core plasmid, 50fmol of E1 module plasmid, 10fmol of E3 module plasmid, and 10fmol of E4 module plasmid in 8µl total volume. Add 2µl of LR clonase II enzyme mix (Life Technologies) and incubate 8-16hrs at room temp. The reaction can then be transformed into bacteria for genome screening or directly transfected into mammalian cells to reconstitute virus.

Virus Whole Genome Assembly: AdSLIC Protocol

The following protocol for producing whole genome plasmids using the AdSLIC protocol is taken from unpublished work by Colin Powers:

To complete AdSLIC from a core module, 5µg of a core module is linearized by restriction digest on either end in a 20µl reaction. After digesting for >2hrs, the enzyme is heat inactivated according to manufacturers instructions. 4µl (1µg total) is then treated for SLIC in a 20µl volume using our standard SLIC method. At the same time, the module(s) for insertion (E1, E3, E4, or E3+E4) are PCR amplified (see AdSLIC primers list) and gel

purified. 400ng is typically treated for SLIC in a 20µl volume using our standard SLIC method, however amounts down to 100ng have been successful. 10µl of SLIC-ready vector and 10µl of each insert is then mixed together and annealed using our standard SLIC method. After annealing, 5µl is transformed into NEB10β competent cells and screened by restriction digest and sequencing.

The successful joining of a core module with either an E1, E3, or E4 module or any combination of two of those creates a "macro-module" that must be subsequently linearized by digest again and joined with the absent modules in the same fashion as the first. Macro-modules are useful when making frequent manipulations within a single part of the genome.

References

1. Hierholzer and Killington (1996). Virology Methods Manual. San Diego CA: Harcourt Brace & Company.

APPENDIX A:

Detailed Map of the Adenovirus Serotype 5 (Ad5) Genome

Need for a Detailed Genomic Map

During development of the engineered virus genomes described in the previous chapters, the greatest concern was producing a false negative result caused by placement of exogenous DNA in locations that disrupted the Ad lifecycle. Because the Ad genome is so compact and employs both positive and negative strands for encoding multiple genes, the addition of exogenous DNA is fraught with risk. A few examples of mistakes one could make are the following; Interruption of a critical ORF or promoter located on the opposite strand. Introduction of a cryptic splice site. Disruption of a full length poly-A sequence.

Detailed Map of the Ad5 Genome

In order to best avoid mistakes during genome design, a detailed map of the Ad5 genome was assembled from a variety of sources. The complete Ad5 genome was first published by Chroboczek, Bieber, and Jacrot[1] in 1992. As a starting point for this detailed map, we used the Ad5 genome available from GenBank with Ascension #AY339865.1. This sequence was created and submitted by Sugarman et. al[2] in 2003. The annotation available with #AY339865.1 includes all of the ORFs and promoters, but only some of the poly-A sites and none of the splice sites. The references listed in table 6.1 were used to verify and enhance the annotation provided by #AY339865.1. The resulting map is shown graphically in Fig 6.1. This map was made using Vector NTI Advance® version 11.5.2.

The following text, starting on the next page, is the detailed Ad5 genome map exported as a GenBank file:

```
LOCUS       AD5\Genome\Annotated\171130aa       35938 bp    DNA     linear   VRL 30-NOV-2017
DEFINITION  TPA: Human adenovirus type 5, complete genome.
ACCESSION   BK000408
VERSION     BK000408.1  GI:33694637
KEYWORDS    Third Party Annotation; TPA.
SOURCE      Human adenovirus type 5.
  ORGANISM  Human adenovirus type 5
            Viruses; dsDNA viruses, no RNA stage; Adenoviridae; Mastadenovirus.
REFERENCE   1  (bases 1 to 35938)
  AUTHORS   Davison,A.J., Benko,M. and Harrach,B.
  TITLE     Genetic content and evolution of adenoviruses
  JOURNAL   J. Gen. Virol. 84 (Pt 11), 2895-2908 (2003)
REFERENCE   2  (bases 1 to 35938)
  AUTHORS   Davison,A.J.
  TITLE     Direct Submission
  JOURNAL   Submitted (03-MAY-2002) MRC Virology Unit, Church Street, Glasgow
            G11 5JR, U.K.
COMMENT     This file is created by Vector NTI
            http://www.invitrogen.com/
COMMENT     ORIGDB|GenBank
COMMENT     VNTDATE|-12050614|
COMMENT     VNTDBDATE|-12050614|
COMMENT     LSOWNER|
COMMENT     VNTNAME|AD5 Genome Annotated 171130aa|
COMMENT     VNTAUTHORNAME|William Partlo|
COMMENT     VNTOAUTHORNAME|UNKNOWN|
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /serotype="Human adenovirus type 5"
                     /db_xref="taxon:28285"
                     /vntifkey="98"
                     /note="Human adenovirus C"
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                     /vntifkey="34"
                     /label=Inverted\Terminal\Repeat\1\(ITR1)
                     /note="ITR"
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                     /product="E1A"
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                     /db_xref="GI:33694638"
                     /vntifkey="4"
                     /label=E1A\(13S)
                     /note="other spliced forms exist with larger introns"
     CDS             1714..2244
                     /codon_start=1
                     /product="E1B 19K"
                     /protein_id="DAA00640.1"
                     /db_xref="GI:33694639"
                     /vntifkey="4"
                     /label=E1B-19K
     CDS             2019..3509
                     /codon_start=1
                     /product="E1B 55K"
                     /protein_id="DAA00641.1"
                     /db_xref="GI:33694640"
                     /vntifkey="4"
                     /label=E1B-55K
     CDS             3609..4031
                     /codon_start=1
                     /product="IX"
                     /protein_id="DAA00642.1"
                     /db_xref="GI:33694641"
                     /vntifkey="4"
                     /label=Protein\IX\(pIX)
     CDS             complement(join(4091..5427,5706..5718))
                     /codon_start=1
                     /product="IVa2"
                     /protein_id="DAA00643.1"
                     /db_xref="GI:33694642"
                     /vntifkey="4"
                     /label=Protein\IVa2\(pIVa2)\exon\#2
```

```
CDS             complement(join(5197..8784,14111..14119))
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                /product="52K"
                /protein_id="DAA00646.1"
                /db_xref="GI:33694645"
                /vntifkey="4"
                /label=L1\52kDa\Protein
CDS             12318..14075
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                /product="pIIIa"
                /protein_id="DAA00647.1"
                /db_xref="GI:33694646"
                /vntifkey="4"
                /label=L1\Capsid\Vertex\Protein\IIIa\(pIIIa)
CDS             14156..15871
                /codon_start=1
                /product="III"
                /protein_id="DAA00648.1"
                /db_xref="GI:33694647"
                /vntifkey="4"
                /label=L2\Penton\Base\(pIII)
CDS             15878..16474
                /codon_start=1
                /product="pVII"
                /protein_id="DAA00649.1"
                /db_xref="GI:33694648"
                /vntifkey="4"
                /label=L2\Major\Core\Protein\(pVII)
CDS             16544..17650
                /codon_start=1
                /product="V"
                /protein_id="DAA00650.1"
                /db_xref="GI:33694649"
                /vntifkey="4"
                /label=L2\Minor\Core\Protein\V\(pV)
CDS             17678..17920
                /codon_start=1
                /product="pX"
                /protein_id="DAA00651.1"
                /db_xref="GI:33694650"
                /vntifkey="4"
                /label=L2\Core\Protein\Precursor\X\(pX,\mu)
CDS             18003..18755
                /codon_start=1
                /product="pVI"
                /protein_id="DAA00652.1"
                /db_xref="GI:33694651"
                /vntifkey="4"
                /label=L3\Protease\Cofactor;\Capsid\Protein\Precursor\VI\(pVI)
CDS             18842..21700
                /codon_start=1
                /product="hexon"
                /protein_id="DAA00653.1"
                /db_xref="GI:33694652"
                /vntifkey="4"
                /label=L3\Hexon
CDS             21733..22347
                /codon_start=1
                /product="protease"
                /protein_id="DAA00654.1"
                /db_xref="GI:33694653"
                /vntifkey="4"
                /label=L3\Endoprotease\23KDa
CDS             complement(22443..24032)
                /codon_start=1
                /product="DBP"
                /protein_id="DAA00655.1"
                /db_xref="GI:33694654"
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                /vntifkey="4"
                /label=E2A\DNA\Binding\72kDa\Protein
CDS             24061..26484
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                /product="100K"
                /protein_id="DAA00656.1"
                /db_xref="GI:33694655"
                /vntifkey="4"
                /label=L4\Hexon-assembly\Associated\100kDa\Protein
CDS             join(26195..26510,26713..27086)
                /codon_start=1
                /product="33K"
                /protein_id="DAA00657.1"
                /db_xref="GI:33694656"
                /vntifkey="4"
                /label=L4\Splicing\Factor\33KDa\Protein\Exon\#1
CDS             26195..26785
                /codon_start=1
                /product="22K"
                /protein_id="DAA00658.1"
                /db_xref="GI:33694657"
                /vntifkey="4"
                /label=L4\Packaging\22kDa\Protein
CDS             27174..27857
                /codon_start=1
                /product="pVIII"
                /protein_id="DAA00659.1"
                /db_xref="GI:33694658"
                /vntifkey="4"
                /label=L4\Hexon\Linking\Protein;\Caspid\Protein\Precursor\(pVIII)
CDS             27858..28181
                /codon_start=1
                /product="E3 12.5K"
                /protein_id="DAA00660.1"
                /db_xref="GI:33694659"
                /vntifkey="4"
                /label=Early\E3A\Immune\Modulating\12.5kDa\Protein
CDS             28547..28738
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                /product="E3 CR1-alpha0"
                /protein_id="DAA00661.1"
                /db_xref="GI:33694660"
                /vntifkey="4"
                /label=E3A\CR1-alpha\Membrane\6.7kDa\Protein
CDS             28735..29217
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                /product="E3 gp19K"
                /protein_id="DAA00662.1"
                /db_xref="GI:33694661"
                /vntifkey="4"
                /label=E3A\Inhibitor\of\MHC;\Membrane\Glycoprotein\19kDa
CDS             29413..29772
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                /product="E3 CR1-beta0"
                /protein_id="DAA00663.1"
                /db_xref="GI:33694662"
                /vntifkey="4"
                /label=ADP
CDS             29784..30059
                /codon_start=1
                /product="E3 RID-alpha"
                /protein_id="DAA00664.1"
                /db_xref="GI:33694663"
                /vntifkey="4"
                /label=E3B\RiD\alpha
CDS             30062..30460
                /codon_start=1
                /product="E3 RID-beta"
                /protein_id="DAA00665.1"
                /db_xref="GI:33694664"
                /vntifkey="4"
                /label=E3B\RiD-Beta
CDS             30453..30839
                /codon_start=1
                /product="E3 14.7K"
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                /protein_id="DAA00666.1"
                /db_xref="GI:33694665"
                /vntifkey="4"
                /label=E3B\14.7kDa
CDS             31042..32787
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                /protein_id="DAA00668.1"
                /db_xref="GI:33694667"
                /vntifkey="4"
                /label=L5\Fiber
CDS             complement(join(32914..33192,33904..34077))
                /codon_start=1
                /product="E4 ORF6/7"
                /protein_id="DAA00669.1"
                /db_xref="GI:33694668"
                /vntifkey="4"
                /label=E4\Transactivator\of\Host\E2F\(E4-ORF6/7)
CDS             complement(33193..34077)
                /codon_start=1
                /product="E4 34K"
                /protein_id="DAA00670.1"
                /db_xref="GI:33694669"
                /vntifkey="4"
                /label=EarlyE4\Inhibitor\of\Nuclear\Immunity\2\(E4-ORF6)
CDS             complement(33998..34342)
                /codon_start=1
                /product="E4 ORF4"
                /protein_id="DAA00671.1"
                /db_xref="GI:33694670"
                /vntifkey="4"
                /label=E4-ORF4
CDS             complement(34353..34703)
                /codon_start=1
                /product="E4 ORF3"
                /protein_id="DAA00672.1"
                /db_xref="GI:33694671"
                /vntifkey="4"
                /label=E4-ORF3
CDS             complement(34700..35092)
                /codon_start=1
                /product="E4 ORFB"
                /protein_id="DAA00673.1"
                /db_xref="GI:33694672"
                /vntifkey="4"
                /label=E4-ORF2
variation       34935^34936
                /experiment="experimental evidence, no additional details
                recorded"
                /citation=[1]
                /replace="t"
                /vntifkey="96"
                /label=Variation_2
                /note="compared to sequence presented in GenBank Accession Number M73260"
CDS             complement(35140..35526)
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                /product="E4 ORF1"
                /protein_id="DAA01570.1"
                /db_xref="GI:33694673"
                /vntifkey="4"
                /label=E4-ORF1
repeat_region   35836..35938
                /rpt_type=inverted
                /vntifkey="34"
                /label=Inverted\Terminal\Repeat\2\(ITR2)
                /note="ITR"
enhancer        195..320
                /vntifkey="9"
                /label=E1A\Enhancer
                /note="http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=209889"
misc_RNA        10620..10779
                /vntifkey="53"
                /label=Virus\Associated\RNA\I
                /note="VA RNAI"
misc_RNA        10876..11036
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                /vntifkey="53"
                /label=Virus\Associated\RNA\II
                /note="VA RNAII"
misc_marker     29491..29772
                /vntifkey="22"
                /label=E3A\Adenovirus\Death\Protein\(ADP)
CDS             join(560..974,1229..1545)
                /vntifkey="4"
                /label=E1A\(12S)
CDS             join(560..637,1229..1318)
                /vntifkey="4"
                /label=E1A\(9S)
CDS             join(7978..8391,9644..9667)
                /vntifkey="4"
                /label=i-leader\Protein
promoter        5986..6049
                /vntifkey="29"
                /label=Major\Late\Promoter
TATA_signal     6018..6024
                /vntifkey="41"
                /label=Major\Late\Promoter\TATA
misc_marker     6049..6049
                /vntifkey="22"
                /label=Major\Late\Transcription\Start\Site
polyA_site      14097..14102
                /vntifkey="26"
                /label=L1\Poly-A
polyA_site      17951..17956
                /vntifkey="26"
                /label=L2\Poly-A
polyA_site      22372..22377
                /vntifkey="26"
                /label=L3\Poly-A
polyA_site      28164..28169
                /vntifkey="26"
                /label=L4\Poly-A
polyA_site      32783..32788
                /vntifkey="26"
                /label=L5\Poly-A
polyA_site      1611..1616
                /vntifkey="26"
                /label=E1A\Poly-A
polyA_site      4038..4043
                /vntifkey="26"
                /label=E1B\Poly-A
polyA_site      29772..29777
                /vntifkey="26"
                /label=E3A\Poly-A
polyA_site      complement(32816..32821)
                /vntifkey="26"
                /label=E4\Poly-A
misc_feature    6049..6089
                /vntifkey="21"
                /label=Tripartite\Leader\Element\#1
misc_feature    7111..7181
                /vntifkey="21"
                /label=Tripartite\Leader\Element\#2
misc_feature    9644..9731
                /vntifkey="21"
                /label=Tripartite\Leader\Element\#3
misc_feature    194..358
                /vntifkey="21"
                /label=Packaging-Enhancer\Sequence
misc_marker     499..499
                /vntifkey="22"
                /label=E1A\Transcription\Start\Site
misc_marker     1702..1702
                /vntifkey="22"
                /label=E1B\Transcription\Start\Site
promoter        1637..1702
                /vntifkey="29"
                /label=E1B\Promoter
TATA_signal     1674..1678
                /vntifkey="41"
                /label=E1B\Promoter\TATA
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TATA_signal     27539..27543
                /vntifkey="41"
                /label=E3\Promoter\TATA
promoter        27486..27568
                /vntifkey="29"
                /label=E3\Promoter
misc_marker     27568..27568
                /vntifkey="22"
                /label=E3\Transcription\Start\Site
TATA_signal     complement(35634..35641)
                /vntifkey="41"
                /label=E4\TATA
misc_marker     35608..35608
                /vntifkey="22"
                /label=E4\Trascription\Start\Site
promoter        complement(35608..35915)
                /vntifkey="29"
                /label=E4\Promoter
TATA_signal     472..475
                /vntifkey="41"
                /label=E1A\TATA
promoter        199..499
                /vntifkey="29"
                /label=E1A\Promoter
exon            complement(30868..31031)
                /vntifkey="61"
                /label=UXP\Exon\#1
polyA_site      30838..30843
                /vntifkey="26"
                /label=E3B\Poly-A
promoter        complement(27051..27132)
                /vntifkey="29"
                /label=E2\Early\Promoter
misc_marker     complement(27051..27051)
                /vntifkey="22"
                /label=E2\Early\Transcription\Start\Site
TATA_signal     complement(27074..27079)
                /vntifkey="41"
                /label=E2\Early\TATA\#1
TATA_signal     complement(27103..27110)
                /vntifkey="41"
                /label=E2\Early\TATA\#2
misc_marker     complement(5835..5835)
                /vntifkey="22"
                /label=pIVa2\Trascription\Initiation\Site
promoter        complement(5835..5985)
                /vntifkey="29"
                /label=pIVa2\Promoter
promoter        complement(25932..26005)
                /vntifkey="29"
                /label=E2\Late\Promoter
misc_marker     complement(31062..31062)
                /vntifkey="22"
                /label=UXP\Transcription\Start\Site
promoter        complement(31033..31220)
                /vntifkey="29"
                /label=UXP\Promoter
TATA_signal     3551..3554
                /vntifkey="41"
                /label=Protein\IX\(pIX)\TATA
misc_marker     complement(25911..25911)
                /vntifkey="22"
                /label=E2\Late\Transcription\Start\Site
polyA_site      complement(22388..22393)
                /vntifkey="26"
                /label=E2A\Poly-A
polyA_site      complement(4090..4095)
                /vntifkey="26"
                /label=E2B\Poly-A
CDS             join(560..637,854..>1112,1229..1545)
                /vntifkey="4"
                /label=E1A\(11S)
CDS             join(560..637,854..>974,1229..1545)
                /vntifkey="4"
                /label=E1A\(10S)
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splicing_signal 632..644
                /vntifkey="38"
                /label=Splice\Donor\Site\(9S)
splicing_signal 969..981
                /vntifkey="38"
                /label=Splice\Donor\Site\(12S)
splicing_signal 1107..1119
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                /label=Splice\Donor\Site\(13S)
splicing_signal 1222..1234
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(9S,\12S,\13S)
splicing_signal 2250..2262
                /vntifkey="38"
                /label=Splice\Donor\Site\(19K,\14.5S,\14S)
splicing_signal 3211..3223
                /vntifkey="38"
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splicing_signal 3505..3517
                /vntifkey="38"
                /label=Splice\Donor\(55K,\14.5S,\14S)
splicing_signal 3588..3600
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(19k,\55k,\14.5S,\14S)
splicing_signal 6084..6096
                /vntifkey="38"
                /label=Splice\Donor\Site\(Tripartite\Leader\Element\#1)
splicing_signal 7177..7189
                /vntifkey="38"
                /label=Splice\Donor\Site\(Tripartite\Leader\Element\#2)
splicing_signal 7104..7116
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(Tripartite\Leader\Element\#2)
splicing_signal 7945..7957
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(I-leader\Protein)
splicing_signal 8386..8398
                /vntifkey="38"
                /label=Splice\Donor\Site\(I-Leader\Protein)
splicing_signal 9728..9740
                /vntifkey="38"
                /label=Splice\Donor\Site\(Tripartite\Leader\Element\#3)
splicing_signal 9637..9649
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(Tripartite\Leader\Element\#3)
splicing_signal 11043..11055
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(52K,\55K)
splicing_signal 12311..12323
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                /label=Splice\Acceptor\Site\(pIIIa)
splicing_signal 14148..14160
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(Penton\Base)
splicing_signal 15722..15734
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(pVII)
splicing_signal 16514..16526
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(pV)
splicing_signal 17995..18007
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(pVI)
splicing_signal 18799..18811
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                /label=Splice\Acceptor\Site\(Hexon)
splicing_signal 21599..21611
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(23KDa)
splicing_signal 24041..24053
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(100kDa)
splicing_signal 26505..26517
                /vntifkey="38"
                /label=Splice\Donor\Site\(33KDa)
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splicing_signal 26706..26718
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(33KDa,\pVIII)
splicing_signal 31035..31046
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(Fiber)
splicing_signal complement(35535..35547)
                /vntifkey="38"
                /label=Splice\Donor\Site\(ORF2,\ORF3,\ORF4,\ORF6,\ORF6/7)
splicing_signal complement(35099..35111)
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(ORF2)
splicing_signal complement(34727..34739)
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(ORF3)
splicing_signal complement(34596..34608)
                /vntifkey="38"
                /label=Splice\Donor\Site\(ORF1,\ORF3/4)
splicing_signal complement(34425..34437)
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(ORF4)
splicing_signal complement(34324..34336)
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                /label=Splice\Acceptor\Site\(ORF6)
splicing_signal complement(34282..34294)
                /vntifkey="38"
                /label=Splice\Donor\Site\(ORF6)
splicing_signal complement(34077..34089)
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(ORF1,\ORF3/4,\ORF6/7)
splicing_signal complement(33897..33909)
                /vntifkey="38"
                /label=Splice\Donor\Site\(ORF1,\ORF3,\ORF3/4,\ORF4,\ORF6/7)
splicing_signal complement(33187..33199)
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(ORF1,\ORF3,\ORF3/4,\ORF4,\ORF6/7)
splicing_signal complement(26977..26989)
                /vntifkey="38"
                /label=Splice\Donor\Site\(1st\Leader\of\E2A\DNA\Binding\72kDa\Protein)
splicing_signal complement(25832..25844)
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                /label=Splice\Donor\Site\(1st\Leader\of\DNA\Binding\72kDa\Protein\from\late\promoter)
splicing_signal complement(24661..24673)
                /vntifkey="38"
                /label=Splice\Donor\Site\(2nd\Leader\of\DNA\Binding\72kDa\Protein)
splicing_signal complement(24739..24751)
                /vntifkey="38"
                /label=Splice\Aceptor\Site\(2nd\Leader\of\DNA\Binding\72kDa\Protein)
splicing_signal complement(24036..24048)
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(DNA\Binding\72kDa\Protein)
splicing_signal complement(14104..14116)
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                /label=Splice\Donor\Site\(E2B)
splicing_signal complement(14292..14304)
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(E2B)
splicing_signal complement(8779..8791)
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(DNA\Ploymerase\140kDa\Protein)
splicing_signal complement(10584..10596)
                /vntifkey="38"
                /label=Splice\Acceptor\Site\(E2B\Terminal\Protein\(pTP)\87kDa)
splicing_signal complement(5699..5711)
                /vntifkey="38"
                /label=Splice\Donor\Site\(pIVa2)
splicing_signal complement(5422..5434)
                /vntifkey="38"
                /label=Splcie\Acceptor\Site\(pIVa2)
CDS             complement(32914..33192,34603..34703)
                /vntifkey="4"
                /label=E4-ORF3/4
CAAT_signal     complement(31148..31152)
                /vntifkey="3"
                /label=I-CCAAT\box
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     exon            complement(24668..24744)
                     /vntifkey="61"
                     /label=UXP\exon\#2
     exon            complement(23629..24041)
                     /vntifkey="61"
                     /label=UXP\exon\#3
     misc_feature    complement(14111..14119)
                     /vntifkey="21"
                     /label=DNA\Poly\and\pTP\Exon
     misc_feature    complement(8583..10589)
                     /vntifkey="21"
                     /label=pTP\Exon
     misc_feature    26713..27086
                     /vntifkey="21"
                     /label=L4\Splicing\Factor\33kDa\Protein\Exon\#2
     misc_feature    complement(5706..5718)
                     /vntifkey="21"
                     /label=Protein\IVa2\(pIVa2)\exon\#1
     splicing_signal 3269..3282
                     /vntifkey="38"
                     /label=Splice\Acceptor\Site?\(14S)
     misc_feature    4070..4071
                     /vntifkey="21"
                     /label=CA
     misc_feature    4090..4096
                     /vntifkey="21"
                     /label=G/T\Rich
     misc_feature    complement(4065..4066)
                     /vntifkey="21"
                     /label=CA
     misc_feature    complement(4041..4046)
                     /vntifkey="21"
                     /label=G/T\Rich
     misc_feature    32809..32810
                     /vntifkey="21"
                     /label=CA
     misc_feature    32816..32824
                     /vntifkey="21"
                     /label=G/T\Rich
     misc_feature    complement(32797..32798)
                     /vntifkey="21"
                     /label=CA
     misc_feature    complement(32781..32788)
                     /vntifkey="21"
                     /label=G/T\Rich
     misc_feature    10632..10643
                     /vntifkey="21"
                     /label=Box\A
     misc_feature    10675..10689
                     /vntifkey="21"
                     /label=Box\B
BASE COUNT     8370 a     10075 c      9761 g      7732 t
ORIGIN
        1 catcatcaat aatatacctt attttggatt gaagccaata tgataatgag ggggtggagt
       61 ttgtgacgtg gcgcggggcg tgggaacggg gcgggtgacg tagtagtgtg gcggaagtgt
      121 gatgttgcaa gtgtggcgga acacatgtaa gcgacggatg tggcaaaagt gacgtttttg
      181 gtgtgcgccg gtgtacacag gaagtgacaa ttttcgcgcg gttttaggcg gatgttgtag
      241 taaatttggg cgtaaccgag taagatttgg ccattttcgc gggaaaactg aataagagga
      301 agtgaaatct gaataatttt gtgttactca tagcgcgtaa tatttgtcta gggccgcggg
      361 gactttgacc gtttacgtgg agactcgccc aggtgttttt ctcaggtgtt ttccgcgttc
      421 cgggtcaaag ttggcgtttt attattatag tcagctgacg tgtagtgtat ttataccgg
      481 tgagttcctc aagaggccac tcttgagtgc cagcgagtag agttttctcc tccgagccgc
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31381 gtggctgccg ccgcacctct aatggtcgcg ggcaacacac tcaccatgca atcacaggcc
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Figures and Tables

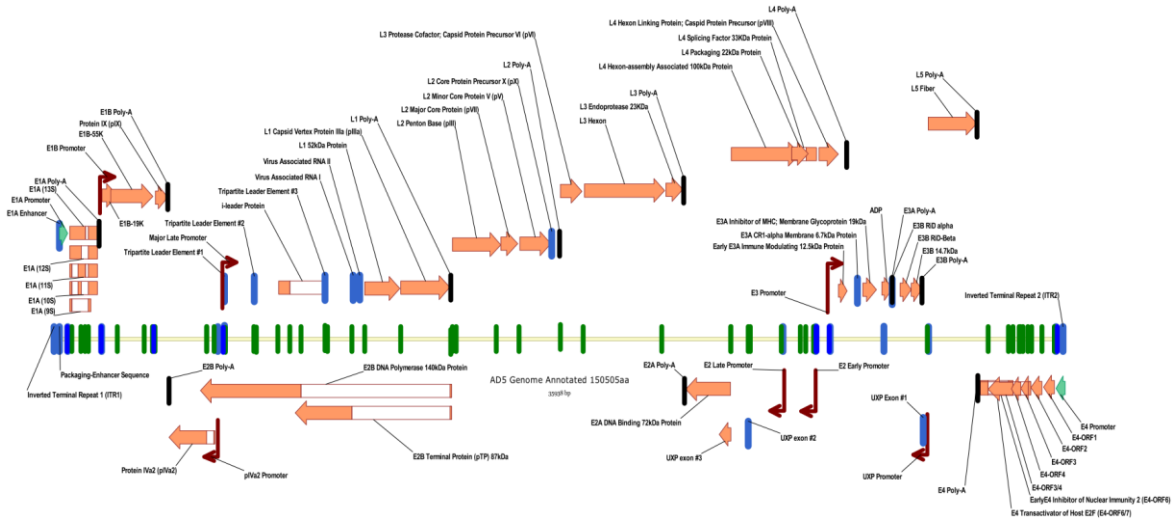

Figure 6.1. Detail map of the Ad5 genome. The green bars along the axis are splice sites. The Blue bars along the axis are miscellaneous features such as TATA boxes, Initiator element sites, packaging sequences, and ITRS.

Table 6.1. References used to annotate features in the Ad5 genome.

Genomic Feature	**Reference(s)**
Inverted Terminal Repeat Sequence (ITRS)	14
Packaging Domain	12
E1A Promoter	4, 22
E1A Splice Sites	20, 26
E1A ORFs	8, 11, 22
E1A Poly-A Site	26
E1B Promoter	4
E1B Splice Sites	20, 26
E1B ORFs	8, 11, 23
E1B Poly-A Site	26
Protein IX Promoter	4
Protein IX ORF	11
VA RNA Genes	13
Major Late Promoter	4, 21
Major Late Transcript Splice Sites	20, 26
Major Late ORFs	11
Major Late Transcript Poly-A Sites	26
E3 Promoter	4
E3 Splice Sites	9, 26
E3 ORFs	6, 9, 11
E3 Poly-A Sites	3, 26
Protein IVa2 Promoter	4
Protein IVa2 Splice Sites	20, 26
Protein IVa2 Poly-A Site	26
E2 Early Promoter	4, 15
E2 Late Promoter	4,5
E2 Splice Sites	20, 26
E2 ORFs	7, 11
E2 Poly-A Sites	19, 26
U exon Promoter	17
U exon ORF	16
E4 Promoter	4, 18
E4 Splice Sites	20, 26
E4 ORFs	10, 11, 24, 25
E4 Poly-A Site	26

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Appendix B:

Table of Adenovirus Whole Genome Plasmids

Plasmid Name	Serotype	Description
CMBT-324	Ad5	GFP-E1A, Fiber-GS-EGFRVHH but with incorrect poly-A tail on the Fiber-GS-EGFRVHH ORF
CMBT-325	Ad5	GFP-E1A, Fiber-GS-EGFRVHH but with correct poly-A tail on the Fiber-GS-EGFRVHH ORF
CMBT-326	Ad5	GFP-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, EGFRVHH-GS-EGFRVHH-GS-FKBP
CMBT-327	Ad5	GFP-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, EGFRVHH-GS-FKBP-GS-EGFRVHH
CMBT-341	Ad5	GFP-E1A, everything else wt
CMBT-342	Ad5	GFP-E1A, mCherry-ADP
CMBT-343	Ad5	GFP-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, mCherry-GS-FKBP
CMBT-344	Ad5	GFP-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, tdTomato-GS-FKBP
CMBT-345	Ad5	GFP-E1A, Fiber-GS-mCherry
CMBT-346	Ad5	GFP-E1A, Fiber-GS-tdTomato
CMBT-347	Ad5	GFP-E1A, mCherry-ADP, Fiber-GS-EGFRVHH but with incorrect poly-A tail
CMBT-348	Ad5	GFP-E1A, mcherry-ADP, Fiber-GS-EGFRVHH but with correct version of poly-A tail
CMBT-349	Ad5	GFP-E1A, mCherry-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EGFRVHH-GS-EGFRVHH-GS-FKBP, FRB-Fiber
CMBT-350	Ad5	GFP-E1A, mCherry-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EGFRVHH-GS-FKBP-GS-EGFRVHH, FRB-Fiber
CMBT-351	Ad5	GFP-E1A, mCherry-ADP, FRB-Fiber
CMBT-352	Ad5	YPet-E1A, everything else wt
CMBT-353	Ad5	YPet-E1A, mCherry-ADP
CMBT-354	Ad5	YPet-E1A, mCherry-ADP, FRB-Fiber
CMBT-355	Ad5	YPet-E1A, mCherry-ADP, Fiber-GS-EGFRVHH (Incorrect Poly-A)
CMBT-356	Ad5	YPet-E1A, mCherry-ADP, Fiber-GS-EGFRVHH

CMBT-357	Ad5	YPet-E1A, mCherry-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EGFRVHH-GS-EGFRVHH-GS-FKBP, FRB-Fiber
CMBT-358	Ad5	YPet-E1A, mCherry-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, EGFRVHH-GS-FKBP-GS-EGFRVHH
CMBT-360	Ad5	mCherry-ADP, everything else wt
CMBT-366	Ad5	ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, mCherry-GS-FKBP
CMBT-367	Ad5	ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, tdTomato-GS-FKBP
CMBT-368	Ad5	Fiber-GS-mCherry
CMBT-369	Ad5	Fiber-GS-tdTomato
CMBT-370	Ad5	YPet-E1A, Fiber-GS-EGFRVHH (Incorrect Poly-A)
CMBT-371	Ad5	YPet-E1A, Fiber-GS-EGFRVHH
CMBT-372	Ad5	YPet-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, EGFRVHH-GS-EGFRVHH-GS-FKBP
CMBT-373	Ad5	YPet-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, EGFRVHH-GS-FKBP-GS-EGFRVHH
CMBT-374	Ad5	Ypet-E1A, mCherry, ΔADP
CMBT-375	Ad5	YPet-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, EGFRVHH-GS-FKBP
CMBT-376	Ad5	YPet-E1A, FRB-Fiber
CMBT-377	Ad5	Ypet-E1A, ΔADP[M1I, S15(stop)]
CMBT-378	Ad5	YPet-E1A, mCherry-P2A-ADP
CMBT-379	Ad5	YPet-P2A-E1A
CMBT-385	Ad5	YPet-E1A, FRB*-Fiber
CMBT-386	Ad5	YPet-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB*-Fiber, EGFRVHH-GS-FKBP
CMBT-387	Ad5	VPet-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB*-Fiber, EGFRVHH-GS-EGFRVHH-GS-FKBP
CMBT-388	Ad5	YPet-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB*-Fiber, EGFRVHH-GS-FKBP-GS-EGFRVHH

PCMN-389	Ad5	YPet-E1A
PCMN-390	Ad5	Ypet-E1A, FRB-Fiber
PCMN-391	Ad5	Ypet-E1A, ΔRIDα/β/14.7K, FRB-Fiber, EGFRVHH-GS-FKBP
PCMN-392	Ad5	Ypet-E1A, ΔRIDα/β/14.7K, FRB-Fiber, EGFRVHH-GS-EGFRVHH-GS-FKBP
PCMN-393	Ad5	Ypet-E1A, ΔRIDα/β/14.7K, FRB-Fiber, EGFRVHH-GS-FKBP-GS-EGFRVHH
PCMN-394	Ad5	Ypet-E1A, FRB*-Fiber
PCMN-395	Ad5	Ypet-E1A, ΔRIDα/β/14.7K, FRB*-Fiber, EGFRVHH-GS-FKBP
PCMN-396	Ad5	Ypet-E1A, ΔRIDα/β/14.7K, FRB*-Fiber, EGFRVHH-GS-EGFRVHH-GS-FKBP
PCMN-397	Ad5	Ypet-E1A, ΔRIDα/β/14.7K, FRB*-Fiber, EGFRVHH-GS-FKBP-GS-EGFRVHH
CMBT-401	Ad5	Ypet-E1A, "attB Linker"-ADP
CMBT-402	Ad5	YPet-E1A, mCherry-GS-ADP
CMBT-403	Ad5	YPet-P2A-ADP
CMBT-404	Ad5	mCherry-ADP, YPet-P2A-Fiber
CMBT-405	Ad5	mCherry-P2A-ADP, Ypet-P2A-Fiber
PA-406	Ad34	Ypet-P2A-Fiber
CMBT-407	Ad5	Ypet-P2A-Fiber
CMBT-408	Ad5	Ypet-P2A-ADP, ∆E1B-55K[M1V,I90stop]
CMBT-409	Ad5	Ypet-P2A-ADP, E1B-55K[H260A]
CMBT-410	Ad5	Ypet-P2A-ADP, E4-ORF3[E52L,E53L]
CMBT-411	Ad5	Ypet-P2A-ADP, E4-ORF3[E52P,E53P]
CMBT-412	Ad5	Ypet-P2A-ADP, E1B-Δ55k[M1V, I90stop], E4-ORF3[E52L,E53L]
CMBT-413	Ad5	Ypet-P2A-ADP, E1B-Δ55k[M1V, I90stop], E4-ORF3[E52P,E53P]
CMBT-414	Ad5	Ypet-P2A-ADP, E1B-55K[H260A], E4-ORF3[E52L,E53L]

CMBT-415	Ad5	Ypet-P2A-ADP, E1B-55K[H260A], E4-ORF3[E52P,E53P]
CMBT-416	Ad5	Ypet-P2A-ADP, E1B-55K[R240A]
CMBT-417	Ad5	Ypet-P2A-ADP, E1B-55K[R240A], E4-ORF3[E52L,E53L]
CMBT-418	Ad5	Ypet-P2A-ADP, E1B-55K[R240A], E4-ORF3[E52P,E53P]
PA-420	Ad34	wt
PCMN-421	Ad5	Ypet-P2A-ADP, all else wt
PCMN-422	Ad5	YPet-P2A-E1A, all else wt
PCMN-423	Ad5	Ypet-P2A-E1A, ΔRIDα/β/14.7K, FRB-Fiber, EGFRVHH-GS-FKBP
CMBT-424	Ad5	YPet-P2A-E1A, Fiber-P2A (incorrect poly-A)
CMBT-425	Ad5	Ypet-P2A-E1A, Fiber-GS-P2A (incorrect poly-A)
CMBT-426	Ad5	Fiber-P2A-YPet (incorrect poly-A)
CMBT-427	Ad5	Fiber-GS-P2A-YPet (incorrect poly-A)
CMBT-428	Ad5	YPet-P2A-E1A, ADP-P2A
CMBT-429	Ad5	ADP-P2A-YPet
CMBT-430	Ad5	YPet-P2A-E1A, ΔRIDα/β/14.7K, ADP-P2A-mCherry-GS-FKBP, FRB-Fiber
CMBT-431	Ad5	YPet-P2A-E1A, ΔRIDα/β/14.7K, mCherry-GS-FKBP, FRB-Fiber
CMBT-432	Ad5	E1A-P2A-YPet, all else wt
CMBT-433	Ad5	YPet-E1A, mCherry-P2A-ADP-GS-Flag
PCMN-434	Ad5	YPet-P2A-ADP, E1B-55K[H260A]
PCMN-435	Ad5	YPet-P2A-ADP, E1B-Δ55k[M1V, I90stop]
CMBT-436	Ad5	YPet-E1A, ADP-GS-Flag
CMBT-437	Ad5	YPet-E1A, Flag-GS-ADP
PCMN-438	Ad5	E1B-55K-P2A-YPet, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber

PCMN-439	Ad5	E1B-55K-P2A-YPet, ΔRIDα, ΔRIDβ, Δ14.7k, E3::EGFRVHH-GS-FKBP, FRB-Fiber
PCMN-440	Ad5	E1B-55K-P2A-YPet, ΔRIDα, ΔRIDβ, Δ14.7k, EGFRVHH-GS-FKBP-P2A-ADP, FRB-Fiber
CMBT-443	Ad5	YPet-P2A-E1A, Fiber-P2A (correct poly-A)
CMBT-444	Ad5	YPet-P2A-E1A, Fiber-GS-P2A (correct poly-A)
CMBT-445	Ad5	Fiber-P2A-YPet (correct poly-A)
CMBT-446	Ad5	Fiber-GS-P2A-YPet (correct poly-A)
CMBT-447	Ad5	ΔRIDα, ΔRIDβ, Δ14.7k, ADP-P2A-YPet-GS-FKBP, FRB-Fiber
CMBT-448	Ad5	ΔRIDα, ΔRIDβ, Δ14.7k, E3::YPet-GS-FKBP, FRB-Fiber
CMBT-449	Ad5	ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber-GS-P2A-YPet-GS-FKBP
CMBT-450	Ad5	ΔRIDα, ΔRIDβ, Δ14.7k, YPet-GS-FKBP-P2A-ADP, FRB-Fiber
CMBT-451	Ad5	YPet-P2A-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, EGFRVHH-GS-FKBP-P2A-ADP, FRB-Fiber
CMBT-455	Ad5	E1A-P2A-mCherry, all else wt
CMBT-456	Ad5	E1B-55k-P2A-YPet, all else wt
CMBT-457	Ad5	YPet-P2A-E4-ORF2, all else wt
CMBT-458	Ad5	E1A-P2A-mCherry, ΔRIDα, ΔRIDβ, Δ14.7k, ADP-P2A-YPet-GS-FKBP, FRB-Fiber
CMBT-459	Ad5	E1A-P2A-mCherry, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber-GS-P2A-YPet-GS-FKBP
CMBT-460	Ad5	E1A-P2A-mCherry, ΔRIDα, ΔRIDβ, Δ14.7k, YPet-GS-FKBP-P2A-ADP, FRB-Fiber
CMBT-461	Ad5	E1A-P2A-mCherry, ΔRIDα, ΔRIDβ, Δ14.7k, E3::YPet-GS-FKBP, FRB-Fiber
CMBT-462	Ad5	YPet-P2A-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, E3::EGFRVHH-GS-FKBP, FRB-Fiber
CMBT-464	Ad5	YPet-P2A-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber-GS-P2A-EGFRVHH-GS-FKBP
CMBT-465	Ad5	YPet-P2A-E1A, FRB-Fiber

CMBT-466	Ad5	YPet-P2A-E1A, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-467	Ad5	YPet-P2A-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber
CMBT-468	Ad5	YPet-P2A-E1A, ΔRIDα, ΔRIDβ, Δ14.7k, E3::EGFRVHH-GS-FKBP, FRB-Fiber
CMBT-477	Ad5	E1A-P2A-mCherry, ΔRIDα, ΔRIDβ, Δ14.7k, E3::YPet
CMBT-478	Ad5	E1A-P2A-mCherry, YPet-P2A-ADP
CMBT-479	Ad5	E1A-P2A-mCherry, Fiber-GS-P2A-YPet
CMBT-480	Ad5	Partial duplication of E1A poly-A and E1B promoter, YPet-P2A-ADP
CMBT-481	Ad5	E1A-P2A-mCherry, ADP-P2A-YPet
CMBT-482	Ad5	E1A(wt), EF1alpa::Tet-on,E1B(wt), YPet-P2A-ADP
CMBT-483	Ad5	E1A(wt),E1B-promoter(partial), EF1alpha::Tet-on, E1A-ploy-A(Partial), E1B(wt), YPet-P2A-ADP
CMBT-484	Ad5	TRE3G::E1A (with cloning remnant), EF1alpha::Tet-On, E1B(wt), YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-485	Ad5	TREG3G::E1A,E1B-promoter(partial), EF1alpha::Tet-On, E1A-pol-A(partial), E1B(wt), YPet-P2A-ADP
CMBT-486	Ad5	YPet-P2A-E1A, ΔRIDα/RIDβ/14.7k, E3::EGFRVHH-GS-FKBP
PCMN-487	Ad5	Fiber-P2A-YPet (correct poly-A)
PCMN-488	Ad5	Fiber-GS-P2A-YPet (correct poly-A)
CMBT-489	Ad5	E1A(wt), EF1alpa::Tet-on,E1B(wt), YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
PCMN-490	Ad5	E1B-55K-P2A-YPet, ΔRIDα/β/14.7K, E3::EGFRVHH-GS-FKBP
PCMN-491	Ad5	E1B-55k-P2A-YPet, all else wt
CMBT-492	Ad5	E1A(wt), EF1alpa::Tet-on,E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-499	Ad5	E1B-55K-P2A-mCherry
CMBT-500	Ad5	E1B-55K-P2A-mCherry, PVIII-P2A-YPet

CMBT-501	Ad5	E1B-55K-P2A-mCherry, YPet-P2A-ADP
CMBT-502	Ad5	E1B-55K-P2A-mCherry, E3::YPet, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-503	Ad5	E1B-55K-P2A-mCherry, Fiber-P2A-YPet
CMBT-504	Ad5	TRE3G::E1A (with cloning remnant), YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-On 3G
CMBT-505	Ad5	TRE3G::E1A (with cloning remnant), YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On 3G
CMBT-506	Ad5	TRE3G::E1A (with cloning remnant), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-On 3G
PCMN-513	Ad5	YPet-P2A-ADP readout with ΔE4ORF6/7
PCMN-514	Ad5	E1A[ΔLXCXE], YPet-P2A-ADP, ΔE4ORF6/7
PCMN-515	Ad5	YPet-P2A-ADP readout with E1A[C124G] and ΔE4ORF6/7
PCMN-516	Ad5	YPet-P2A-ADP readout with E1A[Δ2-11] and ΔE4ORF6/7
PCMN-517	Ad5	YPet-P2A-ADP readout with E1A[Y47H, C124G] and ΔE4ORF6/7
PCMN-518	Ad5	YPet-P2A-ADP readout with E1A[Δ2-11, Y47H, C124G] and ΔE4ORF6/7
CMBT-519	Ad5	TRE3G::E1A (with cloning remnant), EF1alpa::Tet-On, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-520	Ad5	YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-521	Ad5	E1B-55k-P2A-mCherry, YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-522	Ad5	TRE3G::E1A (with cloning remnant), CMV::Tet-On, E1B(wt), YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-523	Ad5	TRE3G::E1A (with cloning remnant), E2F1::Tet-On, E1B(wt), YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-524	Ad5	TRE3G::E1A (with cloning remnant), YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On 3G
CMBT-525	Ad5	TRE3G::E1A (with cloning remnant), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On 3G
CMBT-526	Ad5	TRE3G::E1A (with cloning remnant), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On 3G
CMBT-527	Ad5	TRE3G::E1A (with cloning remnant), CMV::Tet-On, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k

CMBT-528	Ad5	TRE3G::E1A (with cloning remnant), E2F1::Tet-On, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-529	Ad5	E1A-ERT2, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-530	Ad5	YPet-P2A-DNA Polymerase
CMBT-534	Ad5	PVIII-P2A-YPet
CMBT-535	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
PCMN-536	Ad5	E1B-55K-P2A-YPet, ΔRIDα, ΔRIDβ, Δ14.7k, E3::EGFRVHH-GS-EGFRVHH-GS-FKBP, FRB-Fiber
PCMN-537	Ad5	E1B-55K-P2A-YPet, ΔRIDα, ΔRIDβ, Δ14.7k, E3::EGFRVHH-GS-FKBP-GS-EGFRVHH, FRB-Fiber
CMBT-541	Ad5	TRE3G::YPet-P2A-E1A (with cloning remnant), Δ12.k, mCherry-P2A-ADP, ΔRIDa, ΔRIDb, Δ14.7k, EF1α::Tet-On 3G
CMBT-542	Ad5	TRE3G::YPet-P2A-E1A (with cloning remnant), Δ12.k, mCherry-P2A-ADP, ΔRIDa, ΔRIDb, Δ14.7k, CMV::Tet-On 3G
CMBT-543	Ad5	TRE3G::YPet-P2A-E1A (with cloning remnant), Δ12.k, mCherry-P2A-ADP, ΔRIDa, ΔRIDb, Δ14.7k, E2F1::Tet-On 3G
CMBT-544	Ad5	TRE3G::YPet-P2A-E1A (with cloning remnant), EF1α::Tet-on, E1B(wt)
CMBT-545	Ad5	TRE3G::YPet-P2A-E1A (with cloning remnant), E2F1::Tet-on, E1B(wt)
CMBT-546	Ad5	TRE3G::YPety-P2A-E1A (with cloning remnant), CMV::Tet-on, E1B(wt)
PCMN-547	Ad5	YPet-P2A-ADP, ΔE4-ORF3[M1V, M19stop, M44stop, M50stop]
PCMN-548	Ad5	YPet-P2A-ADP, ΔE1B-55K[M1V,I90stop], ΔE4-ORF3[M1V, M19stop, M44stop, M50stop]
CMBT-549	Ad5	YPet-P2A-ADP, ΔRIDα,ΔRIDβ, Δ14.7k, EF1α::Tet-on, TRE3G:E4
CMBT-550	Ad5	YPet-P2A-ADP, ΔRIDα,ΔRIDβ, Δ14.7k, CMV::Tet-on, TRE3G:E4
CMBT-551	Ad5	YPet-P2A-ADP, ΔRIDα,ΔRIDβ, Δ14.7k, E2F1::Tet-on, TRE3G:E4
CMBT-552	Ad5	E1B-55K-P2A*-mCherry
CMBT-553	Ad5	E1B-55K-P2A*-mCherry, PVIII-P2A-YPet
CMBT-554	Ad5	E1B-55K-P2A*-mCherry, YPet-P2A-ADP

CMBT-555	Ad5	E1B-55K-P2A*-mCherry, E3::YPet, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-556	Ad5	E1B-55K-P2A*-mCherry, Fiber-P2A-YPet
CMBT-557	Ad5	TRE3G::E1B, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-On
CMBT-558	Ad5	TRE3G::E1B, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On
CMBT-559	Ad5	TRE3G::E1B, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On
CMBT-560	Ad5	mCherry-P2A-ADP, all else wt
CMBT-561	Ad5	E1B-55K-P2A-YPet, ΔADP[M1I, S15stop]
PCMN-562	Ad5	YPet-P2A-ADP, E1B-55K[H260A], E4-ORF3[E52P,E53P]
PCMN-563	Ad5	YPet-P2A-ADP, E1B-55K[R240A]
PCMN-564	Ad5	YPet-P2A-ADP, E1B-55K[R240A], E4-ORF3[E52P,E53P]
CMBT-566	Ad5	YPet-P2A-ADP, ΔRIDα[M1K]
CMBT-567	Ad5	YPet-P2A-ADP, ΔRIDβ[M1K, C30G, M60stop]
CMBT-568	Ad5	YPet-P2A-ADP, Δ14.7k[M1K, M9stop, M31stop, M39stop]
CMBT-569	Ad5	E1A-P2A-YPet-PEST
CMBT-570	Ad5	E1A-P2A-YPet-PEST, ΔADP[mCherry]
CMBT-583	Ad5	ΔE1B-55K[M1V,I90stop], YPet-P2A-ADP, ΔRIDα, ΔRIDβ, 114.7k, EF1α::Tet-On, TRE3G::E4
CMBT-584	Ad5	ΔE1B-55K[M1V,I90stop], YPet-P2A-ADP, ΔRIDα, ΔRIDβ, 114.7k, CMV::Tet-On, TRE3G::E4
CMBT-585	Ad5	ΔE1B-55K[M1V,I90stop], YPet-P2A-ADP, ΔRIDα, ΔRIDβ, 114.7k, E2F1::Tet-On, TRE3G::E4
CMBT-586	Ad5	TRE3G::E1B, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, 114.7k, EF1α::Tet-On, E4orf3[E52P/E53P]
CMBT-587	Ad5	TRE3G::E1B, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, 114.7k, CMV::Tet-On, E4orf3[E52P/E53P]

CMBT-588	Ad5	TRE3G::E1B, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, 114.7k, E2F1::Tet-On, E4orf3[E52P/E53P]
CMBT-590	Ad5	YPet-GS-(DNA Polymerase)
CMBT-593	Ad5	4X G-to-C mutations in the ITRS to elliminate transcription binding sites. Includes YPet-P2A-ADP readout.
CMBT-594	Ad5	4X G-to-C mutations in the ITRS to elliminate transcription binding sites. Includes YPet-P2A-ADP readout. Replace E4 promoter with TRE3G promoter and express Tet-On from EF1a promoter located in E3 region.
CMBT-595	Ad5	4X G-to-C mutations in the ITRS to elliminate transcription binding sites. Includes YPet-P2A-ADP readout. Replace E4 promoter with TRE3G promoter and express Tet-On from CMV promoter located in E3 region.
CMBT-596	Ad5	4X G-to-C mutations in the ITRS to elliminate transcription binding sites. Includes YPet-P2A-ADP readout. Replace E4 promoter with TRE3G promoter and express Tet-On from E2F1 promoter located in E3 region.
CMBT-598	Ad5	mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-599	Ad5	ΔADP[mCherry]
CMBT-600	Ad5	ΔE1B-55k[M1V, I90 stop], mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-601	Ad5	ΔE1B-55k[M1V, I90 stop], ΔADP[mCherry], ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-602	Ad5	E1A-P2A-YPet-PEST , Fiber-mCherry
CMBT-603	Ad5	ΔADP[mCherry], ΔRIDα, ΔRIDβ, and Δ14.7k
CMBT-604	Ad5	mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A
CMBT-605	Ad5	ΔE1B-55k[M1V, I90stop], mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A
CMBT-606	Ad5	ΔADP[mCherry], ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A
CMBT-607	Ad5	ΔE1B-55k[M1V, I90stop], ΔADP[mCherry], ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A
CMBT-608	Ad5	mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A, PrMin::YPet

CMBT-609	Ad5	ΔE1B-55k[M1V, I90stop], mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A, PrMin::YPet
CMBT-610	Ad5	ΔADP[mCherry], ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A, PrMin::YPet
CMBT-611	Ad5	ΔE1B-55k[M1V, I90stop], ΔADP[mCherry], ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A, PrMin::YPet
CMBT-612	Ad5	DBP-GS-BFP
CMBT-614	Ad5	YPet-P2A-ADP, ΔE4[E4::mCherry]
CMBT-615	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-on, ΔE4[TRE3G::mCherry]
CMBT-616	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-on, ΔE4[TRE3G:mCherry]
CMBT-617	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-on, ΔE4[TRE3G:mCherry]
CMBT-618	Ad5	4X G-to-C mutations in ITRS, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-on, ΔE4[TRE3G::mCherry]
CMBT-619	Ad5	4X G-to-C mutations in ITRS, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-on, ΔE4[TRE3G:mCherry]
CMBT-620	Ad5	4X G-to-C mutations in ITRS, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-on, ΔE4[TRE3G:mCherry]
CMBT-621	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-On
CMBT-622	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-on
CMBT-623	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On
CMBT-624	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-Off, TRE3G::E4
CMBT-625	Ad5	YPet-P2A-ADP, ΔRIDα,ΔRIDβ, Δ14.7k, CMV::Tet-off, TRE3G::E4
CMBT-626	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-Off, TRE3G::E4
CMBT-629	Ad5	E1A(wt), EF1α::Tet-On, E1B(wt), YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G::E4
CMBT-630	Ad5	E1A-P2A-YPet-PEST, Fiber-T2A-mCherry
CMBT-631	Ad5	Fiber-GS-BFP

CMBT-632	Ad5	Fiber-P2A-BFP
CMBT-633	Ad5	mCherry-P2A-E4-ORF2
CMBT-639	Ad5	YPet-P2A-ADP, TRE3G::E4
CMBT-640	Ad5	YPet-P2A-ADP, ΔRIDα,ΔRIDβ, Δ14.7k, TRE3G::E4
CMBT-641	Ad5	E1A(wt), EF1α::Tet-On, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k , TRE3G::E4
CMBT-642	Ad5	E1A(wt), CMV::Tet-On, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k , TRE3G::E4
CMBT-643	Ad5	E1A(wt), E2F1::Tet-On, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k , TRE3G::E4
CMBT-644	Ad5	YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k , EF1a::Tet-On
CMBT-645	Ad5	YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k , CMV::Tet-On
CMBT-646	Ad5	YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k , E2F1::Tet-On
~~CMBT-647~~	~~Ad5~~	~~E1A(wt), EF1α::Tet-On, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k~~
CMBT-648	Ad5	E1A(wt), CMV::Tet-On, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-649	Ad5	E1A(wt), E2F1::Tet-On, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-650	Ad5	YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k , EF1a::Tet-On, TRE3G::E4
CMBT-651	Ad5	YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k , CMV::Tet-On, TRE3G::E4
CMBT-652	Ad5	YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k , E2F1::Tet-On, TRE3G::E4
CMBT-653	Ad5	E1A(wt), EF1α:Tet-On, minimal SV40 Poly-A, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-654	Ad5	E1A(wt), CMV:Tet-On, Mimimal SV40 Poly-A, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-655	Ad5	E1A(wt), E2F1:Tet-On, Minimal SV40 Poly-A, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-656	Ad5	E1A(wt), EF1α:Tet-On, minimal SV40 Poly-A, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G::E4
CMBT-657	Ad5	E1A(wt), CMV:Tet-On, Mimimal SV40 Poly-A, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G::E4

CMBT-658	Ad5	E1A(wt), E2F1:Tet-On, Minimal SV40 Poly-A, E1B(wt), YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G::E4
CMBT-659	Ad5	E1B-55k[H260A], mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A, PrMin::YPet
CMBT-660	Ad5	E1B-55k[R240A], mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A, PrMin::YPet
CMBT-661	Ad5	E1B-55k[H260A], ΔADP[mCherry], ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A, PrMin::YPet
CMBT-662	Ad5	E1B-55k[R240A], ΔADP[mCherry], ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A, PrMin::YPet
CMBT-663	Ad5	TRE3G::E1A (with cloning remnant), all else wt
CMBT-664	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDa, ΔRIDb, Δ14.7k
CMBT-665	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A
CMBT-666	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A, PrMin::YPet (rev)
CMBT-667	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A, PrMin::YPet (rev)
CMBT-668	Ad5	E1B-55k[H260A], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A, PrMin::YPet (rev)
CMBT-669	Ad5	E1B-55k[R240A], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A, PrMin::YPet (rev)
CMBT-684	Ad5	ΔADP[mCherry], ΔRIDa, ΔRIDb, Δ14.7k, SV40 Poly-A, PrMin::Tet-On, TRE3G::E4
CMBT-685	Ad5	ΔADP[mCherry], ΔRIDa, ΔRIDb, Δ14.7k, SV40 Poly-A, E2F1::Tet-On, TRE3G::E4
CMBT-686	Ad5	ΔADP[mCherry], ΔRIDa, ΔRIDb, Δ14.7k, SV40 Poly-A, CMV::Tet-On, TRE3G::E4
CMBT-687	Ad5	ΔADP[mCherry], ΔRIDa, ΔRIDb, Δ14.7k, SV40 Poly-A, EF1α::Tet-On. TRE3G::E4
PCMN-688	Ad5	E1A-P2A-YPet-PEST, C to T mutation in DNA Poly to make H5Ts149
PCMN-689	Ad5	E1A-P2A-YPet-PEST, C to T mutation in DNA Poly to make H5Ts149, Fiber-GS-mCherry
PCMN-690	Ad5	C to T mutation in DNA Poly to make H5Ts149, YPet-P2A-ADP
CMBT-691	Ad5	mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A on E4 side

CMBT-692	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on E4 side
CMBT-693	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet, SV40 Poly-A on E4 side
CMBT-694	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet, SV40 Ploy-A on E4 side
CMBT-695	Ad5	E1B-55k[H260A], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet, SV40 Poly-A on E4 side
CMBT-696	Ad5	E1B-55k[H240A], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet, SV40 Poly-A on E4 side
~~PCMN-697~~	~~Ad34~~	~~PVIII-P2A-YPet~~
~~PA-698~~	~~Ad5/Ad34~~	~~pVIII-P2A-YPet, ΔAd5 Fiber[Ad34 Fiber]~~
CMBT-699	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-700	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-701	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-702	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On (rev), SV40 Poly-A on E4 side, TRE3G::E4
CMBT-703	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On (rev), SV40 Poly-A on E4 side, TRE3G::E4
CMBT-704	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-On (rev), SV40 Poly-A on E4 side, TRE3G::E4
CMBT-705	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side, TRE3G::E4
CMBT-706	Ad5	TRE3G::E2A, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1:Tet-On
CMBT-707	Ad5	E1A(wt), EF1α:Tet-On, minimal SV40 Poly-A, E1B(wt), TRE3G::E2A, YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-708	Ad5	E1A(wt), CMV:Tet-On, Mimimal SV40 Poly-A, E1B(wt), TRE3G::E2A, YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-709	Ad5	E1A(wt), E2F1:Tet-On, Minimal SV40 Poly-A, E1B(wt), TRE3G::E2A, YPet-P2A-ADP, Δ12.5k, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-710	Ad5	TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-711	Ad5	TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On (rev), SV40 Poly-A on E4 side

CMBT-712	Ad5	TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Ef1α::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-713	Ad5	TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-714	Ad5	PrMin::YPet placed inside E1A intron (forward) Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-715	Ad5	PrMin::YPet placed inside E1A intron (reverse) Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-716	Ad5	PrMin::YPet placed inside E1A intron (forward), ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-717	Ad5	PrMin::YPet placed inside E1A intron (reverse), ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-718	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On(rev), SV40 Poly-A on E4 side, TRE3G::E4
CMBT-719	Ad5	ΔE1B-55k[M1V, I90stop], TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On(rev), SV40 Poly-A on E4 side
CMBT-720	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, PrMin::YPet(for), ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-722	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, PrMin::YPet(for), ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-723	Ad5	ΔE2 Transcription Binding Sites, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on E4 side
CMBT-724	Ad5	TRE3G::E2, ΔE2 Transcription Binding Sites, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-725	Ad5	TRE3G::E2, ΔE2 Transcription Binding Sites, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-726	Ad5	TRE3G::E2, ΔE2 Transcription Binding Sites, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Ef1α::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-727	Ad5	TRE3G::E2, ΔE2 Transcription Binding Sites, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side

CMBT-728	Ad5	ΔE1B-55k[M1V, I90stop], TRE3G::E2, ΔE2 Transcription Binding Sites, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-729	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On (rev), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-730	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On (rev), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-731	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-On (rev), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-732	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-733	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-734	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On (for), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-735	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On (for), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-736	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-On (for), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-737	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (for), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-738	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (for), SV40 Poly-A on L5 side, TRE3G::E4
CMBT-739	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On (rev), SV40 Poly-A on L5 side
CMBT-740	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On (rev), SV40 Poly-A on L5 side
CMBT-741	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Ef1α::Tet-On (rev), SV40 Poly-A on L5 side
CMBT-742	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on L5 side
CMBT-743	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on L5 side
CMBT-744	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E2F1::Tet-On (for), SV40 Poly-A on L5 side
CMBT-745	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::Tet-On (for), SV40 Poly-A on L5 side

CMBT-746	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EF1α::Tet-On (for), SV40 Poly-A on L5 side
CMBT-747	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (for), SV40 Poly-A on L5 side
CMBT-748	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (for), SV40 Poly-A on L5 side
CMBT-749	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on E4 side, TRE3G::E4
CMBT-754	Ad5	ΔE1B-55K[M1V, I90stop, ΔAA91-307], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: E1B-55k (for), CMV::Tet-On (for), Tet-On Poly-A, ΔE4orf3
CMBT-755	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, E2F1::Tet-On (for), Tet-On Poly-A
CMBT-756	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV::Tet-On (for), Tet-On Poly-A
CMBT-757	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, EF1α::Tet-On (for), Tet-On Poly-A
CMBT-758	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, PrMin::Tet-On (for), Tet-On Poly-A
CMBT-759	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, PrMin::Tet-On (for), Tet-On Poly-A
CMBT-760	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, E2F1::Tet-On (for), Tet-On Poly-A, TRE3G::E4
CMBT-761	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV::Tet-On (for), Tet-On Poly-A, TRE3G::E4
CMBT-762	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, EF1α::Tet-On (for), Tet-On Poly-A, TRE3G::E4
CMBT-763	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, PrMin::Tet-On (for), Tet-On Poly-A, TRE3G::E4
CMBT-764	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, PrMin::Tet-On (for), Tet-On Poly-A, TRE3G::E4
CMBT-765	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, PrMin::YPet (rev), ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-766	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, PrMin::YPet (rev), ΔRIDα, ΔRIDβ, Δ14.7k

CMBT-767	Ad5	ΔE1B-55k[M1V, I90stop, ΔAA91-307], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet (rev), SV40 Poly-A on E4 side
CMBT-768	Ad5	PrMin::YPet placed in front of E1A intron (forward) Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-769	Ad5	PrMin::YPet placed in front of E1A intron (reverse) Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-770	Ad5	PrMin::YPet placed in front of E1A intron (forward), ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-771	Ad5	PrMin::YPet placed in front of E1A intron (reverse), ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-772	Ad5	Δ12.5k, Δ6.7k, Δ19k, TRE3G (rev), mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-773	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G(rev), PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-774	Ad5	Δ12.5k, Δ6.7k, Δ19k, TRE3G(rev), mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G(rev), PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-775	Ad5	Δ12.5k, Δ6.7k, Δ19k, TRE3G (for), mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-776	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G(for), PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-777	Ad5	Δ12.5k, Δ6.7k, Δ19k, TRE3G(for), mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G(for), PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-778	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, TRE3G (rev), mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-779	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G(rev), PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-780	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, TRE3G(rev), mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G(rev), PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-781	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, TRE3G (for), mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side

CMBT-782	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G(for), PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-783	Ad5	ΔE1B-5k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, TRE3G(for), mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, TRE3G(for), PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-784	Ad5	E1A(wt), PrMin::YPet (for),minimal SV40 poly-A,E1B(wt), Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-785	Ad5	E1A(wt), PrMin::Tet-On (for),minimal SV40 poly-A,E1B(wt), Δ12.5k, Δ6.7k,Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-787	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet (rev), SV40 Poly-A on E4 side, ΔE4-ORF3
CMBT-790	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, E2F1::Tet-On (for),SV-40 Poly-A plus termination sequence
CMBT-791	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV::Tet-On (for),SV-40 Poly-A plus termination sequence
CMBT-792	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, EF1α::Tet-On (for),SV-40 Poly-A plus termination sequence
CMBT-793	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, PrMin::Tet-On (for),SV-40 Poly-A plus termination sequence
CMBT-794	Ad5	ΔE1B-55K[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, PrMin::Tet-On (for),SV-40 Poly-A plus termination sequence
CMBT-795	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, E2F1::Tet-On (for),SV-40 Poly-A plus termination sequence, TRE3G::E4
CMBT-796	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV::Tet-On (for),SV-40 Poly-A plus termination sequence, TRE3G::E4
CMBT-797	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, EF1α::Tet-On (for),SV-40 Poly-A plus termination sequence, TRE3G::E4
CMBT-798	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, PrMin::Tet-On (for),SV-40 Poly-A plus termination sequence, TRE3G::E4

CMBT-799	Ad5	ΔE1B-55K[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, PrMin::Tet-On (for),SV-40 Poly-A plus termination sequence, TRE3G::E4
CMBT-812	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, YPet (for)
CMBT-813	Ad5	TRE3G (rev) placed before pVIII ORF, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-814	Ad5	ΔE1B-55K[M1V, I90stop], TRE3G (rev) placed before pVIII ORF, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-815	Ad5	E1B-55k[H260A], TRE3G (rev) placed before pVIII ORF, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-816	Ad5	E1B-55k[R240A], TRE3G (rev) placed before pVIII ORF, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On (rev), SV40 Poly-A on E4 side
CMBT-817	Ad5	E1A(wt), PrMin::YPet, minimal SV40 poly-A, ΔE1B-55K[M1V, I90stop,ΔAA91-307], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-818	Ad5	E1A(wt), PrMin::Tet-On, minimal SV40 poly-A, ΔE1B-55K[M1V, I90stop,ΔAA91-307], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-828	Ad5	E1B-55k[H260A], TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On(rev), SV40 Poly-A on E4 side
CMBT-829	Ad5	E1B-55k[R240A], TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::Tet-On(rev), SV40 Poly-A on E4 side
CMBT-830	Ad5	E1A(wt), PrMin::Tet-On, minimal SV40 poly-A, TRE3G(rev) placed between E2 early promoter and pVIII ORF, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-831	Ad5	E1A(wt), PrMin::Tet-On, minimal SV40 poly-A, ΔE1B-55K[M1V, I90stop,ΔAA91-307], TRE3G(rev) placed between E2 early promoter and pVIII ORF, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-832	Ad5	E1A(wt), PrMin::Tet-On, minimal SV40 poly-A, TRE3G::E2, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-833	Ad5	E1A(wt), PrMin::Tet-On, minimal SV40 poly-A, ΔE1B-55K[M1V, I90stop,ΔAA91-307], TRE3G::E2, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k

CMBT-840	Ad5	E1A(wt), PrMin::YPet,minimal SV40 poly-A, ΔE1B-55K[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-841	Ad5	E1A(wt), PrMin::Tet-On,minimal SV40 poly-A, ΔE1B-55K[M1V, I90stop], TRE3G::E2, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-842	Ad5	E1A(wt), PrMin::YPet,minimal SV40 poly-A, E1B-55K[H260A], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-843	Ad5	E1A(wt), PrMin::Tet-On,minimal SV40 poly-A, E1B-55K[H260A], TRE3G::E2, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-844	Ad5	E1A(wt), PrMin::YPet,minimal SV40 poly-A, E1B-55K[R240A], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-845	Ad5	E1A(wt), PrMin::Tet-On,minimal SV40 poly-A, E1B-55K[R240A], TRE3G::E2, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
~~CMBT-846~~	~~Ad5~~	~~E1A(wt), PrMin::Tet-On,minimal SV40 poly-A, TRE3G::E2, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k~~
CMBT-847	Ad5	Hexon-P2A-YPet, all else wt
CMBT-848	Ad5	Hexon-P2A-Ypet, Δ12.5k, Δ6.7k, Δ19k, mChery-P2A-ADPΔRIDα, ΔRIDβ, Δ14.7k
CMBT-851	Ad5	mCherry-P2A-ADP, SV40 poly-A on E4 side
PCMN-852	Ad5	pVIII-P2A-YPet
PCMN-853	Ad5/34	pVIII-P2A-YPet, Fiber = Ad5 tail + Ad34 Shaft + Ad34 Knob
PCMN-854	Ad34	all wt
~~PCMN-855~~	~~Ad34~~	~~pVIII-P2A-YPet, all else wt~~
PCMN-856	Ad9	all wt
~~PCMN-857~~	~~Ad9~~	~~pVIII-P2A-YPet, all else wt~~
PCMN-858	Ad5	Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα/β/14.7K + EGFRVHH-GS-FKBP, FRB-TtoL-Fiber
PCMN-859	Ad5	Δ12.5k, Δ6.7k, Δ19k, ΔRIDα/β/14.7K + EGFRVHH-GS-FKBP, FRB-TtoL-Fiber
PCMN-860	Ad5	YPet-P2A-ADP, ΔRIDα/β/14.7K + EGFRVHH-GS-FKBP, FRB-TtoL-Fiber

PCMN-862	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDa, ΔRIDb, Δ14.7k
CMBT-863	Ad5	YPet-P2A-Hexon, all else wt
CMBT-864	Ad5	YPet-P2A-Hexon, Δ12.5k, Δ6.7k, Δ19k, mChery-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
PCMN-866	Ad5/Ad34	E1B-55k-P2A-YPet, Fiber = Ad5 Tail + Ad34 Shaft + Ad34 Knob
PCMN-867	Ad9	E1B-55k-P2A-YPet, all else wt
PCMN-868	Ad34	E1B-55k-P2A-YPet, all else wt
PCMN-869	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
PCMN-870	Ad5	mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
PCMN-871	Ad5	mCherry-P2A-ADP
PCMN-872	Ad5	E1B-55K-P2A-YPet, ΔRIDα, ΔRIDβ, Δ14.7k, E3::EGFRVHH-GS-FKBP, FRB-TotL-Fiber
CMBT-873	Ad5	YPet-P2A-pVIII
PCMN-874	Ad5	Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
PCMN-875	Ad5/Ad9	E1B-55k-P2A-YPet, Fiber = Ad5 Tail + Ad9 Shaft + Ad9 Knob
CMBT-876	Ad5	E1A-P2A-YPet-PEST, mCherry-P2A-ADP
CMBT-877	Ad5	E1A-P2A-YPet-PEST, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-878	Ad5	E1A-P2A-YPet-PEST, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-879	Ad5	E1A-P2A-PEST, mCherry-P2A-ADP
CMBT-880	Ad5	E1A-P2A-PEST, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-881	Ad5	E1A-P2A-PEST, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-882	Ad5	E1A-PEST, mCherry-P2A-ADP
CMBT-883	Ad5	E1A-PEST, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-884	Ad5	E1A-PEST, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k

CMBT-885	Ad5	Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-886	Ad5	DBP-P2A-YPet
PCMN-887	Ad5	E3-14.7k-P2A-YPet
PCMN-888	Ad9	E3-14.8k-P2A-YPet
PCMN-889	Ad34	E3-15k-P2A-YPet
CMBT-890	Ad5	E3-14.7k-P2A-YPet
~~CMBT-891~~	~~Ad5~~	~~YPet-GS-pVII~~
CMBT-892	Ad5	YPet-P2A-pVII
CMBT-893	Ad5	pVII-GS-YPet
CMBT-894	Ad5	pVII-P2A-YPet
CMBT-895	Ad5	TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::ADP-P2A-Tet-On(rev), SV40 Poly-A on E4 side
CMBT-896	Ad5	ΔE1B-55k[M1V, I90stop], TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::ADP-P2A-Tet-On(rev), SV40 Poly-A on E4 side
CMBT-897	Ad5	E1B-55k[H260A], TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::ADP-P2A-Tet-On(rev), SV40 Poly-A on E4 side
CMBT-898	Ad5	E1B-55k[R240A], TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::ADP-P2A-Tet-On(rev), SV40 Poly-A on E4 side
CMBT-899	Ad5	YPet[G25D]-GS-pVII
CMBT-900	Ad5	E4-ORF3-P2A-YPet
PCMN-901	Ad5	E1B-55K-P2A-YPet + 2AA's from c-term of E1B-55k
PCMN-902	Ad9	E1B-55K-P2A-YPet + 2AA's from c-term of E1B-55k
PCMN-903	Ad34	E1B-55K-P2A-YPet + 2AA's from c-term of E1B-55k
CMBT-904	Ad5	YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, plus RIDα fragment

CMBT-905	Ad5	E1A[ΔLXCXE], Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k
CMBT-906	Ad5	Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, ΔE4-ORF6/7
CMBT-907	Ad5	E1A[ΔLXCXE], Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, ΔE4-ORF6/7
CMBT-908	Ad5	Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EGFRVHH-GS-FKBP
CMBT-909	Ad5	Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-TtoL-Fiber
CMBT-910	Ad5	Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, EGFRVHH-GS-FKBP, FRB-TtoL-Fiber
CMBT-916	Ad5	TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet-P2A-Tet-On (rev), SV40 Poly-A on E4 side
CMBT-917	Ad5	ΔE1B-55k[M1V, I90stop], TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet-P2A-Tet-On (rev), SV40 Poly-A on E4 side
CMBT-918	Ad5	E1B-55k[H260A], TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet-P2A-Tet-On (rev), SV40 Poly-A on E4 side
CMBT-919	Ad5	E1B-55k[R240A], TRE3G::E2A, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet-P2A-Tet-On (rev), SV40 Poly-A on E4 side
PCMN-920	Ad5 5/9/9	14.7k-P2A-YPet, Fiber = Ad5 tail + Ad9 Shaft + Ad9 Knob
PCMN-921	Ad5 5/34/34	14.7k-P2A-YPet, Fiber = Ad5 tail + Ad34 Shaft + Ad34 Knob
PCMN-922	Ad5	E1A[ΔLXCXE], YPet-P2A-ADP
CMBT-925	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::ADP-P2A-Tet-On, SV40 Poly-A on E4 side
CMBT-926	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::ADP-P2A-Tet-On, SV40 Poly-A on E4 side
CMBT-927	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet-P2A-Tet-On, SV40 Poly-A on E4 side
CMBT-928	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::YPet-P2A-Tet-On, SV40 Poly-A on E4 side

CMBT-929	Ad5	ΔE1B-55K[M1V, I90stop, ΔAA91-307], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: E1B-55k (for), E2F1::Tet-On (for), Tet-On Poly-A, ΔE4orf3
CMBT-930	Ad5	ΔE1B-55K[M1V, I90stop, ΔAA91-307], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: E1B-55k (for), EF1A::Tet-On (for), Tet-On Poly-A, ΔE4orf3
CMBT-931	Ad5	ΔE1B-55K[M1V, I90stop, ΔAA91-307], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV::E1B-55k (for), Tet-On Poly-A, ΔE4orf3
CMBT-932	Ad5	ΔL3-Endoprotease, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: Endoprotease (for), CMV::Tet-On (for), Tet-On Poly-A
CMBT-933	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), CMV::Tet-On (for), Tet-On Poly-A
CMBT-934	Ad5	YPet-GS-ADP
CMBT-935	Ad5	ΔADP[YPet]
CMBT-936	Ad5	14.7k-P2A-YPet
CMBT-937	Ad5	ΔRIDα, ΔRIDβ, Δ14.7k, E3B::YPet
CMBT-938	Ad5	Fiber-GS-YPet
PCMN-945	Ad3	all wt
PCMN-946	Ad3	15.3k-P2A-YPet
PCMN-949	Ad5 5/3/3	14.7k-P2A-YPet, Fiber = Ad5 tail + Ad3 Shaft + Ad3 Knob
PCMN-950	Ad5	E1A-P2A-YPet
PCMN-951	Ad3 3/5/5	15.3k-P2A-YPet, Fiber = Ad3 tail + Ad5 Shaft + Ad5 Knob
PCMN-952	Ad9 9/5/5	14.8k-P2A-YPet, Fiber = Ad9 tail + Ad5 Shaft + Ad5 Knob
PCMN-953	Ad34 34/5/5	15k-P2A-YPet, Fiber = Ad34 tail + Ad5 Shaft + Ad5 Knob
PCMN-962	Ad5 5/5/3	14.7k-P2A-YPet, Fiber = Ad5 tail + Ad5 Shaft + Ad3 Knob

PCMN-963	Ad5 5/5/9	14.7k-P2A-YPet, Fiber = Ad5 tail + Ad5 Shaft + Ad9 Knob
PCMN-964	Ad5 5/5/34	14.7k-P2A-YPet, Fiber = Ad5 tail + Ad5 Shaft + Ad34 Knob
PCMN-965	Ad3 3/3/5	15.3k-P2A-YPet, Fiber = Ad3 tail + Ad3 Shaft + Ad5 Knob
PCMN-966	Ad9 9/9/5	14.8k-P2A-YPet, Fiber = Ad9 tail + Ad9 Shaft + Ad5 Knob
PCMN-967	Ad34 34/34/5	15k-P2A-YPet, Fiber = Ad34 tail + Ad34 Shaft + Ad5 Knob
PCMN-968	Ad5	DBP-P2A-YPet
PA-969	Ad5 5/3/3	ΔE1-EF1α-[luc-GFP]-miR122; fiber chimera Ad5/3/3
PA-970	Ad5 5/9/9	ΔE1-EF1α-[luc-GFP]-miR122; fiber chimera Ad5/9/9
PA-971	Ad5 5/34/34	ΔE1-EF1α-[luc-GFP]-miR122; fiber chimera Ad5/34/34
PA-972	Ad34 34/34/5	ΔE1-EF1α-[luc-GFP]-miR122; fiber chimera Ad34/34/5
PA-973	Ad34 34/5/5	ΔE1-EF1α-[luc-GFP]-miR122; fiber chimera Ad34/5/5
~~PA-979~~	~~Ad34~~	~~ΔE1-EF1α-[luc-GFP]-miR122~~
CMBT-991	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), E2F1::Tet-On (for), Tet-On Poly-A
CMBT-992	Ad5	ΔE1A[ΔAA6-285], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: E1A (for), CMV::Tet-On (for), Tet-On Poly-A
CMBT-993	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV::DNA Bindnig Protein (for), Tet-On Poly-A
CMBT-994	Ad5	ΔE1A[ΔAA6-285], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV::E1A, Tet-On Poly-A
PCMN-995	Ad34 34/5/34	15k-P2A-YPet, Fiber = Ad34 Tail + Ad5 Shaft + Ad34 Knob
PCMN-996	Ad5	YPet-P2A-ADP, ΔRIDα[M1K], ΔRIDβ[M1K, C30G, M60stop], Δ14.7k[M1K, M9stop, M31stop, M39stop]
PCMN-997	Ad5	ΔRIDα[M1K], ΔRIDβ[M1K, C30G, M60stop], 14.7k-P2A-YPet
PCMN-998	Ad34 34/5/5	Δ10.1k[M1K, V17I], Δ14.9k[M1K, C33G, M57Stop], 15k-P2A-YPet, Fiber = Ad34 tail + Ad5 Shaft + Ad5 Knob

PCMN-999	Ad5	E1A[ΔLXCXE], hexon[E451Q], ΔE3-12.5k, ΔE3-6.7k, ΔE3-19k, YPet-P2A-ADP, ΔE4-ORF6/7
PCMN-1000	Ad5	E1A[ΔLXCXE], hexon[E451Q], ΔE3-6.7k, ΔE3-12.5k, ΔE3-19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, ΔE4-ORF6/7
PCMN-1001	Ad34 34/5/5	Δ10.1k, Δ14.9k, 15k-P2A-YPet, Fiber = Ad34 tail + Ad5 Shaft + Ad5 Knob
PCMN-1002	Ad5	ΔE3-12.5k, ΔE3-6.7k, ΔE3-19k, YPet-P2A-ADP
PCMN-1003	Ad5	E1A[ΔLXCXE], hexon[E451Q], YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, ΔE4-ORF6/7
PCMN-1010	Ad5	E1A[ΔLXCXE], hexon[E451Q], YPet-P2A-ADP, ΔE3-RIDα, ΔRIDβ, Δ14.7k, EGFRVHH-GS-FKBP, ΔE4-ORF6/7
PCMN-1011	Ad5	YPet-P2A-ADP, ΔE3-RIDα, ΔRIDβ, Δ14.7k, EGFRVHH-GS-FKBP
PCMN-1012	Ad5	E1A[ΔLXCXE], hexon[E451Q], YPet-P2A-ADP, FRB-TtoL-Fiber, ΔE4-ORF6/7
PCMN-1013	Ad5	YPet-P2A-ADP, FRB-TtoL-Fiber
PCMN-1014	Ad34/Ad5	ΔpVIII[Replace with Ad34 version of pVIII], YPet-P2A-ADP, Fiber = Ad34 tail + Ad5 shaft + Ad5 knob (35,990bp, 1.035)
PCMN-1015	Ad34/Ad5	ΔpVIII[Replace with Ad34 version of pVIII], YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad34 tail + Ad5 shaft + Ad5 knob (34,937bp, 1.005)
PCMN-1016	Ad34/Ad5	ΔpVIII[Replace with Ad34 version of pVIII], Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad34 tail + Ad5 shaft + Ad5 knob (33,433bp, 0.961)
PCMN-1017	Ad34/Ad5	ΔpVIII[Replace with Ad34 version of pVIII], YPet-P2A-ADP, Fiber = Ad34 tail + Ad34 shaft + Ad34 knob (35,222bp, 1.013)
PCMN-1018	Ad34/Ad5	ΔpVIII[Replace with Ad34 version of pVIII], YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad34 tail + Ad34 shaft + Ad34 knob (34,169bp, 0.983)
PCMN-1019	Ad34/Ad5	ΔpVIII[Replace with Ad34 version of pVIII], Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad34 tail + Ad34 shaft + Ad34 knob (32,665bp, 0.939)
PCMN-1020	Ad5	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, EGFRVHH-GS-FKBP-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, FRB-Fiber, ΔE4-ORF6/7
PCMN-1021	MAV1	wt
PCMN-1022	MAV1	E3-Glycoprotein-P2A-YPet

PCMN-1023	MAV1	E3-Glycoprotein-P2A-Renilla_Luciferase
PCMN-1024	Ad34	ΔE1B-54.9k[M1V, V89STOP], 15k-P2A-YPet
PCMN-1025	Ad34	15k-P2A-YPet, ΔE4-ORF3
PCMN-1026	Ad34	ΔE1B-54.9k[M1V, V89STOP], 15k-P2A-YPet, ΔE4-ORF3
PCMN-1027	Ad5	ΔE4-ORF2, all else WT
PCMN-1028	Ad5	Flag-E4-Orf2, all else WT
PCMN-1031	Ad5	E1A-YPet, all else WT
PCMN-1032	Ad5	Δ12.5k[M1K], Δ6.7k[M1K], Δ19k[M1K, V31stop, E39stop, C46stop, E51stop], YPet-P2A-ADP, ΔRIDα[M1K], ΔRIDβ[M1K, C30G, M60stop], Δ14.7k[M1K, M9stop, M31stop, M39stop]
PCMN-1033	Ad5	YPet-P2A-ADP, Ad9 E4-ORF2
PCMN-1034	Ad5	Ad9 E4-ORF2
PCMN-1035	Ad5	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, EGFRVHH-GS-YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, ΔE4-ORF6/7
PCMN-1036	Ad5	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, EGFRVHH-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, ΔE4-ORF6/7
PCMN-1037	Ad5	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, E3B::EGFRVHH, ΔE4-ORF6/7
PCMN-1038	Ad5	ΔRIDα, ΔRIDβ, 14.7k-P2A-YPet
PCMN-1039	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, ΔE4-ORF3
PCMN-1041	Ad5/Ad34	Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob
PCMN-1042	Ad5/Ad34	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, YPet-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, ΔE4-ORF6/7
PCMN-1047	Ad5/Ad34	E1A[ΔLXCXE], hexon[E451Q], (Renilla Luc)-P2A-ADP, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, ΔE4-ORF6/7
PCMN-1048	Ad5/Ad34	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, (Renilla Luc)-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, ΔE4-ORF6/7

PCMN-1055	Ad5/Ad34	YPet-P2A-ADP, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob
PCMN-1056	Ad5/Ad34	E1A[ΔLXCXE], hexon[E451Q], YPet-P2A-ADP, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, ΔE4-ORF6/7
CMBT-1057	Ad5	E1A[ΔLXCXE], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), E2F1::Tet-On (for), Tet-On Poly-A, ΔE4-ORF6/7
CMBT-1058	Ad5	E1A-YPet, all else WT
CMBT-1059	Ad5	Duplicate section of L4-33kDa protein after E2 early promoter (Contains all AAs with alternate CODONs). Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k (34,267bp, 0.954)
CMBT-1060	Ad5	Duplicate section of L4-33kDa protein after E2 early promoter (Contains all AAs with alternate CODONs). E2 Early promoter Inr flanked by Tet-O binding sequences. Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k (34,286bp, 0.954)
CMBT-1061	Ad5	Duplicate section of L4-33kDa protein after E2 early promoter (Contains all AAs with alternate CODONs). E2 Early promoter Inr flanked by Tet-O binding sequences. Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::TetR (rev), SV40 Poly-A on E4 side (35,553bp, 0.989)
CMBT-1062	Ad5	ΔE1B55k[M1V, I90stop], Duplicate section of L4-33kDa protein after E2 early promoter (Contains all AAs with alternate CODONs). E2 Early promoter Inr flanked by Tet-O binding sequences. Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::TetR (rev), SV40 Poly-A on E4 side (35,553bp, 0.989)
CMBT-1063	Ad5	Duplicate section of L4-33kDa protein after E2 early promoter (Contains all AAs with alternate CODONs). E2 Early promoter Inr flanked by Tet-O binding sequences. Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, CMV::TetR (rev), SV40 Poly-A on E4 side (35,684bp, 0.993)
CMBT-1064	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV-Tet-O:: DNA Binding Protein (for), CMV::TetR (for), Tet-On Poly-A (36,509bp, 1.016)
CMBT-1065	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV-Tet-O:: DNA Binding Protein (for), PrMin::TetR (for), Tet-On Poly-A (36,299bp, 1.010)

CMBT-1066	Ad5	ΔE1B55k[M1V, I90stop], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV-Tet-O:: DNA Binding Protein (for), PrMin::TetR (for), Tet-On Poly-A (36,299bp, 1.010)
PCMN-1070	Ad5	mCherry-P2A-ADP, 14.7k-P2A-YPet (37,494, 1.043)
CMBT-1076	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A on L5 side, CMV::YPet (rev)
CMBT-1077	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A on L5 side, E2F1::YPet (rev)
CMBT-1078	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A on L5 side, MUC1::YPet (rev)
CMBT-1079	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A on L5 side, Ptf1aRE::YPet (rev)
CMBT-1080	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), MUC1::Tet-On (for), Tet-On Poly-A
PCMN-1087	Ad5	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, EGFRVHH-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, ΔE4-ORF6/7 (33,570bp, 0.934)
PCMN-1091	Ad5	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, EGFRVHH-GS-FKBP-P2A-ADP, ΔRIDα, ΔRIDβ, 14.7k-P2A-YPet, FRB-Fiber, ΔE4-ORF6/7 (35,310bp, 0.982)
PCMN-1092	Ad5	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, EGFRVHH-P2A-ADP, ΔRIDα, ΔRIDβ, 14.7K-P2A-YPet, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, ΔE4-ORF6/7 (34,737bp, 0.966)
CMBT-1093	Ad5	E1B55k[H260A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV-Tet-O:: DNA Binding Protein (for), PrMin::TetR (for), Tet-On Poly-A (36,299bp, 1.010)
CMBT-1094	Ad5	E1B55k[R240A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV-Tet-O:: DNA Binding Protein (for), PrMin::TetR (for), Tet-On Poly-A (36,299bp, 1.010)
CMBT-1105	Ad5	ΔE1A/E1B + EF1α::YPet
CMBT-1106	Ad5	ΔE1A/E1B + EF1α::YPet, Fiber = 5/5/34
CMBT-1107	Ad5	ΔE1A/E1B + EF1α::YPet, Fiber = 5/5/3

CMBT-1108	Ad5	ΔE1A/E1B + EF1α::YPet, Fiber = 5/5/9
CMBT-1120	Ad5	E1A[ΔLXCXE], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, E2F1::YPet (rev), ΔE4-ORF6/7
~~PCMN-1121~~	~~Ad5~~	~~E1A-P2A-YPet, 14.7k-P2A-mCherry~~
CMBT-1131	Ad5	ΔE1B-55k[M1V, I90stop], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), PrMin::Tet-On (for), Tet-On Poly-A
CMBT-1132	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), Tet-On Poly-A (rev), MUC1::Tet-On (rev)
CMBT-1133	Ad5	ΔE1B-55k[M1V, I90stop], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), Tet-On Poly-A (rev), PrMin::Tet-On (rev)
CMBT-1134	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV-Tet-O:: DNA Binding Protein (for), PrMin::TetR (Rev), Tet-On Poly-A
CMBT-1135	Ad5	ΔE1B-55k[M1V, I90stop], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV-Tet-O:: DNA Binding Protein (for), PrMin::TetR (Rev), Tet-On Poly-A
CMBT-1136	Ad5	E1B-55k[H260A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV-Tet-O:: DNA Binding Protein (for), PrMin::TetR (Rev), Tet-On Poly-A
CMBT-1137	Ad5	E1B-55k[R240A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, CMV-Tet-O:: DNA Binding Protein (for), PrMin::TetR (Rev), Tet-On Poly-A
CMBT-1138	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: YPet (for), CMV::Tet-On (for), Tet-On Poly-A
PCMN-1139	Ad5	E1A[ΔLXCXE], hexon[E451Q], Δ12.5k, Δ6.7k, Δ19k, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, ΔE4-ORF6/7
PCMN-1142	Ad5	E1A-P2A-YPet, 14.7k-P2A-mCherry
PCMN-1143	Ad5	E1A-P2A-YPet, ΔRIDα, ΔRIDβ, Δ14.7k

PCMN-1144	Ad5	E1A-P2A-YPet, ΔRIDα[M1K], ΔRIDβ[M1K, C30G, M60stop], Δ14.7k[M1K, M9stop, M31stop, M39stop]
PCMN-1145	Ad5	E1A-P2A-YPet, Δ12.5k, Δ6.7k, Δ19k, ΔRIDα, ΔRIDβ, Δ14.7k
PCMN-1146	Ad5	E1A-P2A-YPet, Δ12.5k[M1K], Δ6.7k[M1k], Δ19k[M1K, V31stop, E39stop, C46stop, E51stop], ΔRIDα[M1K], ΔRIDβ[M1K, C30G, M60stop], Δ14.7k[M1K, M9stop, M31stop, M39stop]
CMBT-1154	Ad5	E1B-55k[H260A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), Tet-On Poly-A (rev), PrMin::Tet-On (rev)
CMBT-1155	Ad5	E1B-55k[R240A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), Tet-On Poly-A (rev), PrMin::Tet-On (rev)
PCMN-1162	Ad5	NanoLuc-P2A-ADP
PCMN-1163	Ad5	E3-14.7k-P2A-NanoLuc
CMBT-1171	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 poly-A on L5 side, PSES::YPet (rev)
CMBT-1172	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), PSES::Tet-On (for), Tet-On Poly-A
CMBT-1173	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), Tet-On Poly-A (rev), PSES::Tet-On (rev)
CMBT-1180	Ad5	E1B-55k[H260A], Duplicate section of L4-33kDa protein after E2 early promoter (Contains all AAs with alternate CODONs). E2 Early promoter Inr flanked by Tet-O binding sequences. Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::TetR (rev), SV40 Poly-A on E4 side
CMBT-1181	Ad5	E1B-55k[R240A], Duplicate section of L4-33kDa protein after E2 early promoter (Contains all AAs with alternate CODONs). E2 Early promoter Inr flanked by Tet-O binding sequences. Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, PrMin::TetR (rev), SV40 Poly-A on E4 side
CMBT-1187	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), CMV::Tet-On (rev), TRE3G:: YPet (rev)

CMBT-1188	Ad5	ΔE1B-55k[M1V, I90stop], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PrMin::TetR (Rev), , CMV-Tet-O:: DNA Binding Protein (rev)
CMBT-1189	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PSES::Tet-On (rev), TRE3G:: DNA Binding Protein (rev)
CMBT-1190	Ad5	ADP-YPet
CMBT-1194	Ad5	ΔE1B-55k[M1V, I90stop], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PrMin::Tet-On (rev), TRE3G:: DNA Binding Protein (rev)
CMBT-1195	Ad5	E1B-55k[H260A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PrMin::Tet-On (rev), TRE3G:: DNA Binding Protein (rev)
CMBT-1196	Ad5	E1B-55k[R240A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PrMin::Tet-On (rev), TRE3G:: DNA Binding Protein (rev)
CMBT-1197	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad4 Tail + Ad5 Shaft + Ad34 Knob, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), PSES::Tet-On (for), Tet-On Poly-A
CMBT-1198	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber= Ad5 Tail + Ad5 Shaft + Ad34 Knob, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PSES::Tet-On (rev), TRE3G:: DNA Binding Protein (rev)
CMBT-1199	Ad5	E1B-55K[H260A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), PrMin::Tet-On (for), Tet-On Poly-A
CMBT-1200	Ad5	E1B-55K[R240A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), PrMin::Tet-On (for), Tet-On Poly-A
CMBT-1202 CMBT-AA	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, SV40 poly-A on L5 side, PSES::YPet (rev)

CMBT-1203 CMBT-AB	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, SV40 poly-A on L5 side, CMV::YPet (rev)
CMBT-1204 CMBT-AC	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, SV40 poly-A on L5 side, E2F1::YPet (rev)
CMBT-1205 CMBT-AD	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, SV40 poly-A on L5 side, MUC1::YPet (rev)
CMBT-1206 CMBT-AE	Ad5	ΔE1B-55k[M1V, I90stop], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, SV40 poly-A on L5 side, PrMin::YPet (rev)
CMBT-1207 CMBT-AF	Ad5	E1A[ΔLXCXE], Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, SV40 Poly-A on L5 side, E2F1::YPet (rev), ΔE4-ORF6/7
CMBT-1208 CMBT-BA	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PrMin::TetR (Rev), , CMV-Tet-O:: DNA Binding Protein (rev)
CMBT-1209 CMBT-BB	Ad5	E1B-55k[H260A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PrMin::TetR (Rev), , CMV-Tet-O:: DNA Binding Protein (rev)
CMBT-1210 CMBT-BC	Ad5	E1B-55k[R240A], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PrMin::TetR (Rev), , CMV-Tet-O:: DNA Binding Protein (rev)
CMBT-1211 CMBT-DA	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, SV40 poly-A on L5 side, PSES::YPet (rev) Corrected version of PSES
CMBT-1212 CMBT-EA	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber = Ad5 Tail + Ad5 Shaft + Ad34 Knob, SV40 Poly-A on L5 side, TRE3G:: DNA Binding Protein (for), PSES::Tet-On (for), Tet-On Poly-A, corrected version of PSES
CMBT-1213 CMBT-EB	Ad5	ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, Fiber= Ad5 Tail + Ad5 Shaft + Ad34 Knob, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PSES::Tet-On (rev), TRE3G:: DNA Binding Protein (rev), corrected version of PSES
CMBT-1214	Ad5	ΔE1B-55k[M1V, I90stop], , Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, TRE3G:: YPet (for), PrMin::Tet-On (for), Tet-On Poly-A

CMBT-1215	Ad5	Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, Tet-On Poly-A (rev), PrMin::Tet-On (rev), TRE3G:: YPet (rev)
CMBT-1216	Ad5	ΔE1B-55k[M1V, I90stop], ΔE2-DNA Binding Protein, Δ12.5k, Δ6.7k, Δ19k, mCherry-P2A-ADP, ΔRIDα, ΔRIDβ, Δ14.7k, SV40 Poly-A on L5 side, PrMin::DNA Binding Protein (rev)

PCMN-1250	Ad5/Ad34	E1A[ΔLXCXE], hexon[E451Q], Fiber = Ad5 tail + Ad5 shaft + Ad34 knob, ΔE4-ORF6/7