Title
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DISCLAIMER

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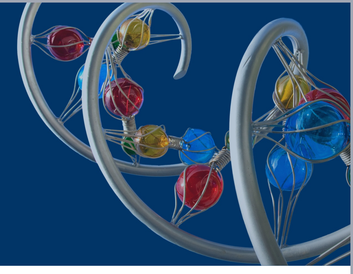
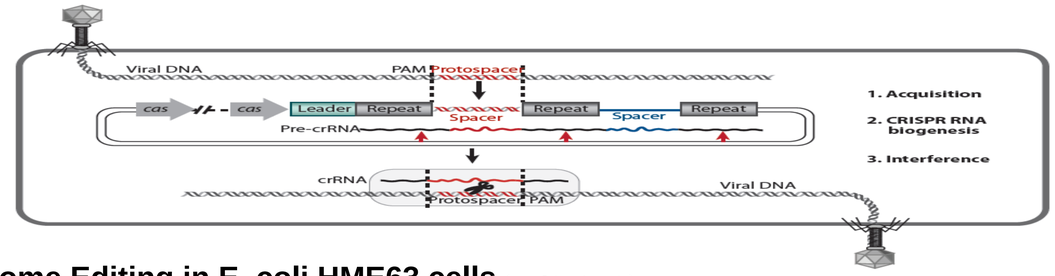


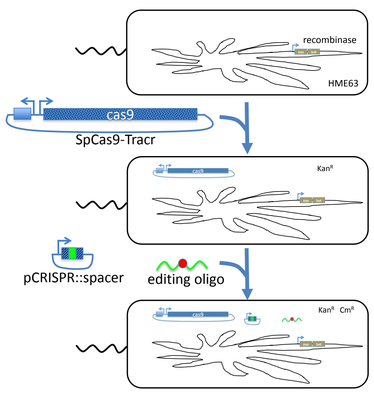
CRISPR systems are prokaryotic adaptive immune systems which integrate short fragments of foreign DNA into the host chromosome at one end of repetitive element known as a CRISPR (clustered regularly interspaced short palindromic repeat). The CRISPR serves as memory, which associated proteins (e.g. Cas9) then read in order to recognize and destroy invasive DNA.

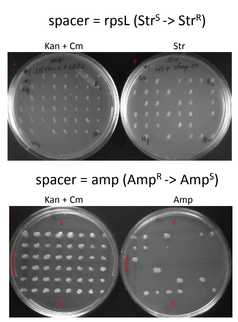
Recently, the Cas9-CRISPR system has proven to be a useful tool for genome editing in eukaryotes, which repair the double stranded breaks made by Cas9 with non-homologous end joining or homologous recombination. *Escherichia coli* lacks non-homologous end joining and has a very low homologous recombination rate, effectively rendering targeted Cas9 activity lethal. We have developed a heat curable, serializable, plasmid based system for selectionless Cas9 editing in arbitrary *E. coli* strains that uses synthetic CRISPRs for targeting and λ-red to effect repairs of double stranded breaks. We have demonstrated insertions, substitutions, and multi-target deletions with our system, which we have tested in several strains.



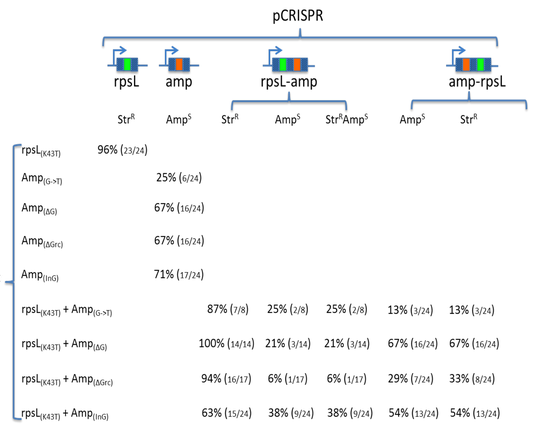
Genome Editing in E. coli HME63 cells (one & two targets)

We have tested several editing conditions of rpsL and Amp in HME63 host cells which have genome integrated λ-red. We have found that: rpsL is easier to edit than amp; oligos causing deletions have the highest success rate; and the first spacer in CRISPR has a higher editing rate than the second.



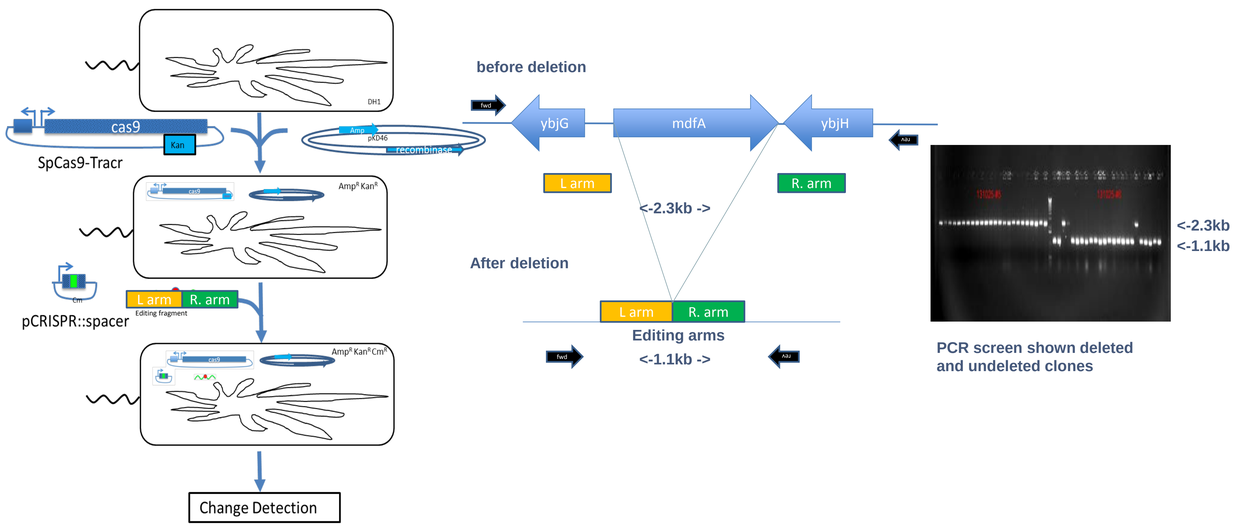


Up to 96% of randomly selected colonies carry the desired phenotype



mdfA gene deletion in DH1 cells

We made mdfA gene deletions in DH1 cells transformed with vector pKD46(λ-red) and vector pBHK-Cas9. The basic procedure, gene and arms structures before and after deletion and results are shown below:

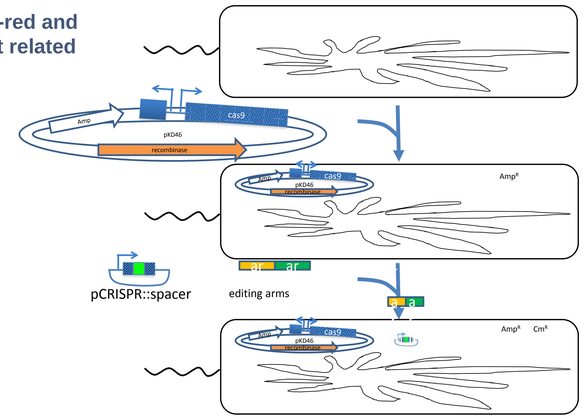


PCR screen shown deleted and undeleted clones

Ten cell membrane transport related genes have been deleted in *E. coli* DH1 cells

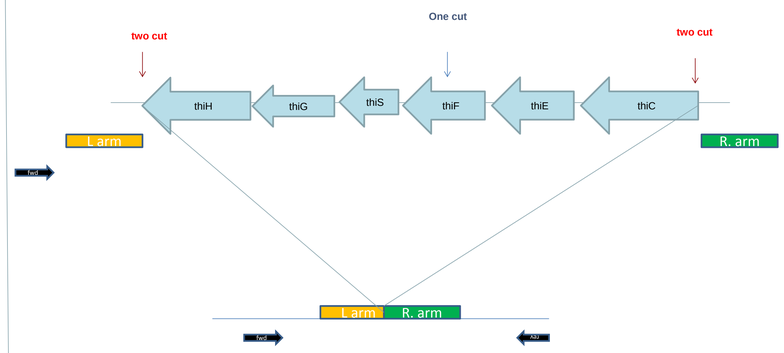
We constructed a new pKD46 derived vector which contains both λ-red and Cas9. We created ten knock out strains for cell membrane transport related protein genes using this new vector, which retains the temperature sensitivity of the original plasmid.

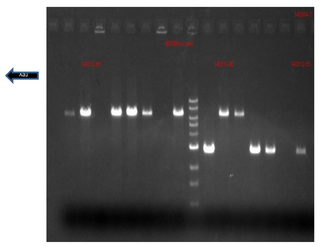
#	gene	deletions	gene deleted clone
1	mdfA	(8/19)	EC112
2	basB	(2/11)	EC096
3	exbB	(2/24)	EC097
4	glpT	(1/3)	EC098
5	mdlB	(2/7)	EC099
6	tehA	(3/12)	EC100
7	ycjF	(5/12)	EC101
8	yhjD	(1/13)	EC102
9	yhjG	(4/20)	EC104
10	yhjX	(1/14)	EC103



A long gene cluster has been deleted in *E. coli* DH1 cells

We knocked out the 4kb thiamine synthesis gene cluster in DH1 cells, with single and double spacer CRISPRs.





<-6.7 kb (undeleted)
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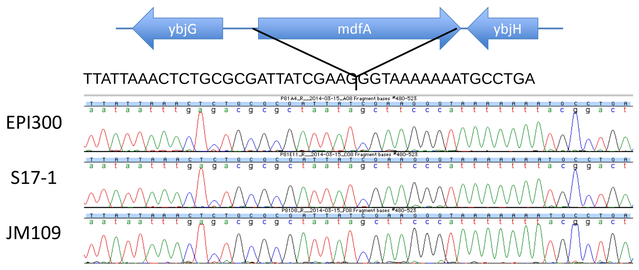
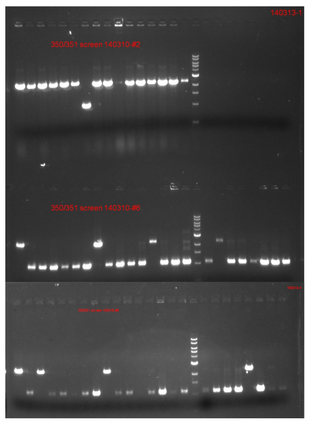
Cas9 Editing Also Works in Other *E. coli* strains

Besides HME63 and DH1, we have attempted CRISPR-based editing with the strains EPI300 (top), S17-1 (middle) and JM109 (bottom).

Given our success with *E. coli*, we hope to move into other useful prokaryotic model systems.





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