Genome Editing in Escherichia coli with Cas9 and synthetic CRISPRs

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DISCLAIMER

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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.