

Exploring Species Specificity of Lambda Red Recombination

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Project Goals: To extend recombineering methods to organisms other than *E. coli* by identifying and overcoming the host-specificity of Beta recombinases.

Recombination using the lambda red protein Beta has been extensively used in *E. coli* to incorporate insertions, deletions, and point mutations into the genome at chosen loci. Although Beta increases the rate of ssDNA recombination in *E. coli* 10,000 fold, this catalytic recombination activity has not been observed in other bacteria such as *Lactobacillus* or *Corynebacterium*. Interestingly, the family of single-stranded annealing proteins that includes Beta has been found to have species-specific activities and unpredictable efficiencies, making it difficult to design a generalized method for porting Beta recombination across organisms. In order to explore mechanisms of generalizing the recombinase activity, we use *Lactobacillus* and *E. coli* as two model organisms with orthogonal recombination systems to probe the source of species specificity.

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