Exploring the Species Specificity of Lambda Red Recombination

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Project Goals: To extend high-efficiency recombineering beyond *E. coli* and closely related organisms.

The single-stranded annealing protein (SSAP) "Beta" enables a wide variety of genomic manipulations in *E. coli*, including easy generation of single base pair changes, deletions, insertions, memory storage using retrons, and recoding of large 50kb+ segments. However, this protein is not active in all organisms, limiting its use to applications involving *E. coli* and closely related organisms such as *Salmonella*. Finding SSAPs that are active in new organisms is laborious and requires screening of candidate protein variants in the target host organism. This method may not identify highly active SSAPs, as only proteins from closely related species would normally be screened. Ideally, high-activity SSAPs could be engineered to function effectively in any host organism. Here, we use *E. coli* and *L. lactis* as model systems to examine the species specificity requirements of single stranded annealing proteins. *E. coli* is a gram negative bacterium from the phylum Proteobacteria, while *L. lactis* is a gram positive bacterium from the phylum Firmicutes. Active SSAP variants have been identified for both species, but with much higher activity in *E. coli*. We screen hundreds of protein variants and thousands of SSAP fusions to elucidate the features enabling species-specific programming of single-stranded annealing proteins.

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