Red recombination of linear dsDNAs at the replication fork

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Over the past few years, recombination-mediated in vivo genetic engineering, termed Recombineering, has been developed to provide a simple and highly efficient method for DNA modification in E. coli. Especially, a simple method known as "Red recombination" based on the interplay of phage-encoded proteins, "Exo and Beta," allows the efficient tools for Recombineering. Despite many attempts to expand its application, lack of the information on the recombination mechanism still remains as a major hurdle for the accurate design of the recombination templates.

In this study, we report the empirical evidence that linear dsDNAs are recombined through single-strand annealing at the replication fork when the target is denatured during DNA replication. Using the synthetic dsDNA containing the endogeneous homology between the two different antibiotic resistance genes, Cm^r and Km^r, in addition to both terminal homologies, recombination was found to be lagging-strand biased. This strongly suggests that dsDNAs were recombined at the replication fork and consequently, help us for the error-proof design for Recombineering.