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Recombineering: An overview of, and using it to dissect cellular processes

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Abstract

For nearly15 years, we have been developing the λRed homologous recombination system for genetic engineering *in vivo* in *Escherichia coli,* a process known as recombineering. Recombineering is a highly efficient and precise method for genetically engineering chromosomal, plasmid, phage or BAC DNA. With recombineering one can make gene replacements, deletions, insertions, inversions, and single and multiple point mutations. Importantly, recombineering requires ~50 bases of homology and can utilizePCR products, or single strand oligos for efficient recombination.With optimized conditions, oligo recombination can yield over 50% of the colonies as recombinants. The basic principles of how recombineering works, how it is done, and how it can be utilized will be discussed. During the course of studying and optimizingthe recombineering process, we have discovered that recombineering is an excellent tool to study central biological processes *in vivo* including: methyl-directed mismatch repair, DNA replication, and chromosome segregation. Some of these findings will be discussed to emphasize the utility of recombineering. For additional background, protocols, strain requests, references and more, see: http://redrecombineering.ncifcrf.gov/

Biography

Jim received his PhD from the Institute of Molecular Biology at the University of Oregon in 1993 where he studied E. coli and phage lambda recombination systems with Frank Stahl. His postdoctoral studies were on chromosome segregation in E. coli with Stuart Austin at NCI before resuming studies on phage-encoded recombination systems when he joined Don Court's laboratory at NCI in 2001. Jim has published more than 20 papers, the majority of them on recombination and using recombination systems for in vivo genetic engineering. He also is on the editorial board of Advancements in Genetic Engineering.