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Oligo-mediated recombination for genetic engineering: Optimization, DNA replication requirement and DNA polymerase involvement in *E. coli*

Recombineering, homologous recombination-mediated genetic engineering, is an efficient, simple and rapid way to engineer large replicons, especially so using a single-strand oligonucleotide (oligo).Previously it has been shown that using a lagging-strand oligo and avoiding the methyl-directed mismatch repair (MMR) system are two methods to enhance oligo-mediated recombination. Creating mismatches at 4 or more consecutive wobble positions leads to high frequency recombination because the MMR system is avoided. This proves useful for making high frequency changes without altering the amino-acid sequence and can be applied to essential genes. Other methods tooptimize oligo recombination will be discussed. These optimizations provide a good starting point for developing recombineering in other organisms. We investigated the dependence of oligo recombination on DNA replication. When replication of the target sequence was prevented, recombinant formation was reduced up to 2000-fold indicating that DNA replication functions may be involved in oligo recombination. This was investigated using oligos that contained multiple nucleotide changes: a single base change allowing recombinant selection, and silent changes serving as genetic markers to determine the extent of oligo processing. Such oligos were often partially degraded in the process of recombination. The position and number of the silent nucleotide changes within the oligo strongly affected both oligo processing and recombination frequency. Exonucleases, especially those associated with DNA Polymerases I and III, and DNA ligase were found to affect inheritance of the silent nucleotide changes in the oligos and their mutants reduced recombination frequencies.

Biography

Jim Sawitzke 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

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