Video Article

Principles of Site-Specific Recombinase (SSR) Technology

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Abstract

Site-specific recombinase (SSR) technology allows the manipulation of gene structure to explore gene function and has become an integral tool of molecular biology. Site-specific recombinases are proteins that bind to distinct DNA target sequences. The Cre/lox system was first described in bacteriophages during the 1980's. Cre recombinase is a Type I topoisomerase that catalyzes site-specific recombination of DNA between two loxP (locus of X-over P1) sites. The Cre/lox system does not require any cofactors. LoxP sequences contain distinct binding sites for Cre recombinases that surround a directional core sequence where recombination and rearrangement takes place. When cells contain loxP sites and express the Cre recombinase, a recombination event occurs. Double-stranded DNA is cut at both loxP sites by the Cre recombinase, rearranged, and ligated ("scissors and glue"). Products of the recombination event depend on the relative orientation of the asymmetric sequences.

SSR technology is frequently used as a tool to explore gene function. Here the gene of interest is flanked with Cre target sites loxP ("floxed"). Animals are then crossed with animals expressing the Cre recombinase under the control of a tissue-specific promoter. In tissues that express the Cre recombinase it binds to target sequences and excises the floxed gene. Controlled gene deletion allows the investigation of gene function in specific tissues and at distinct time points. Analysis of gene function employing SSR technology --- conditional mutagenesis -- has significant advantages over traditional knock-outs where gene deletion is frequently lethal.

Video Link

The video component of this article can be found at https://www.jove.com/video/718/

