

PRECISE CONSTRUCTION OF CHROMOSOMAL LOCI USING A CRE-LOXP SYSTEM

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Our lab has focused on developing a model system for chromosomal engineering using the Cre-loxP system. Cre recombinase is an enzyme that catalyzes the site-specific recombination of DNA by recognizing specific sites known as lox sites. The loxP site possesses two 13bp inverted repeats separated by an 8bp spacer. Functional variants of the loxP site can also be made by changing only the sequence of the spacer. Cre can mediate either excision or inversion between two target lox sites on the same DNA molecule depending on their arrangement. Lox sites containing different spacer sequences should not recombine with each other, and are referred to as heterospecific lox sites.

We have incorporated single copies of five different lox sites into a single plasmid construct, which is useful for facilitating future gene exchanges. Five additional plasmid constructs, each containing one additional copy of each lox site are also available. These will be used to test the function of each site by its ability to facilitate gene fragment inversion in the presence of Cre. Verification that only predicted inversions occur without extraneous inversion or deletion involving heterospecific lox sites would serve to validate our approach and set the stage for future gene exchange reactions.