Aplicaciones biotecnológicas de la recombinación sitio-específica: *"tissue-specific gene knock out"*

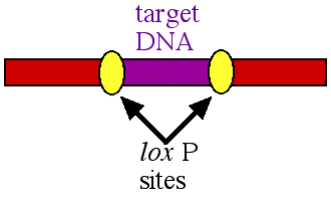
Cre-*lox* P Recombination

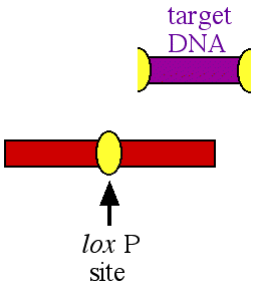
In the early 1990's a new method was developed to delete a specific portion of DNA. The procedure took advantage of the basic research performed on the bacteriophage called P1. In this virus, there is an enzyme called **cre** and particular DNA sequences called ***lox* P sites**. The *lox* P sites work in pairs and they flank a segment of DNA called a target (figure 1).

Figure 1. A pair of *lox* P sites (yellow ovals) flanking the target DNA (purple) to be deleted.
When the cre enzyme binds to the *lox* P sites, it cuts the *lox* sites in half and then splices together the two halves after the target DNA has been removed (figure 2).

Figure 2. After the cre enzyme has excised the target DNA, one lox P site is left behind and the two flanking fragments of DNA are spliced together. The target DNA is excised and degraded.
Molecular biologists recognized the specificity and utility of this viral recombination system and put it to good use. Now if you want to excise a piece of DNA at a particular time, all you need to do is to flank the target DNA with a pair of *lox* P sites and introduce the cre enzyme when you want the target excised.
Mike Snyder's group used this to added epitope tags onto yeast proteins (Proteomics Chapter).
An additional twist is to express a Cre transgene under control of an inducible promoter so you can delete the target DNA inside selected cells of a transgenic organism when you want it deleted.

recombinación sitio-específica:





Tissue-specific knock-out mice

While "housekeeping" genes are expressed in all types of cells at all stages of development, other genes are normally expressed in only certain types of cells when turned on by the appropriate signals (e.g. the arrival of a hormone). To study such genes, one might expect that the methods described above would work. However, it turns out that genes that are only expressed in certain adult tissues may nonetheless be vital during embryonic development. In such cases, the animals do not survive long enough for their knockout gene to be studied. Fortunately, there are now techniques with which transgenic mice can be made where a particular gene gets knocked out in only one type of cell.

The Cre/*loxP* System

One of the bacteriophages that infects E. coli, called P1, produces an enzyme — designated Cre — that cuts its DNA into lengths suitable for packaging into fresh virus particles. Cre cuts the viral DNA wherever it encounters a pair of sequences designated *loxP*. All the DNA between the two *loxP* sites is removed and the remaining DNA ligated together again (so the enzyme is a recombinase).

Using "Method 1" (above), mice can be made transgenic for

the gene encoding Cre attached to a promoter that will be activated only when it is bound by the same transcription factors that turn on the other genes required for the unique function(s) of that type of cell;

a "target" gene, the one whose function is to be studied, flanked by *loxP* sequences.

In the adult animal,

those cells that

- receive signals (e.g., the arrival of a hormone or cytokine)
- to turn on production of the transcription factors needed
- to activate the promoters of the genes whose products are needed by that particular kind of cell

will also turn on transcription of the Cre gene. Its protein will then remove the "target" gene under study.

All other cells will lack the transcription factors needed to bind to the Cre promoter (and/or any enhancers) so the target gene remains intact.

The result: a mouse with a particular gene knocked out in only certain cells. The Cre/*loxP* system can also be used to remove DNA sequences that block gene transcription. In such a "knockin" mouse, the "target" gene is turned on in only certain cells.

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/T/TransgenicAnimals.html

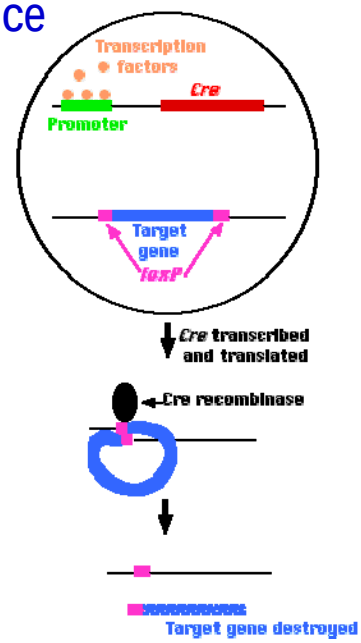


Figure 21.7. **Structure of the loxP recognition sequence**. *Note* that the central 8 bp sequence which is flanked by the 13 bp inverted repeats is asymmetric and confers orientation.

Site-specific recombination systems, notably the Cre-loxP system, extend the power of gene targeting

Several site-specific recombination systems from bacteriophages and yeasts have been characterized and are promising tools for genome engineering (Kilby *et al.*, 1993). Thus far, the Cre-*lox*P recombination system from bacteriophage P1 has been the most widely used. The natural function of the Cre (causes recombination) recombinase is to mediate recombination between two *lox*P sequences that are in the same orientation (the *lox*P sequence consists of 34 bp and comprises two inverted 13 bp repeats separated by a central asymmetric 8 bp spacer; *Figure 21.7*). As a result of recombination, the intervening sequence between the two *lox*P sites is excised (see *Figure 4.15*). Using gene targeting, *lox*P sequences can be stitched into a desired gene or chromosomal location, and the subsequent provision of a gene encoding the Cre product can result in an artificially generated site-directed recombination event

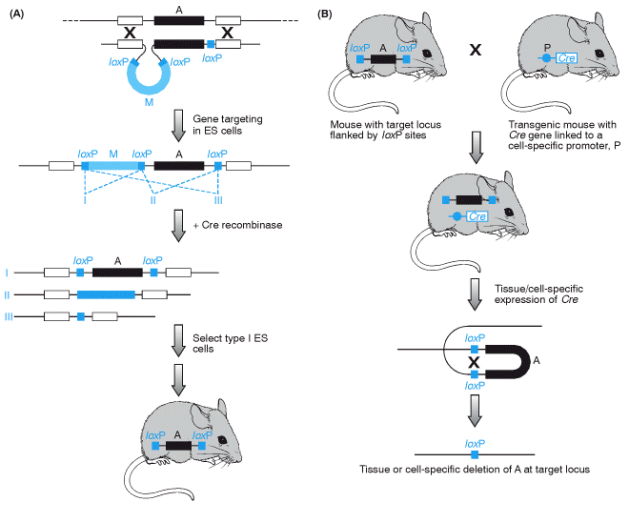


Figure 21.8. Gene targeting using the Cre-*lox*P recombination system can be used to inactivate a gene in a desired cell type. (A) Illustration of a standard homologous recombination method using mouse ES cells, in which three *lox*P sites are introduced along with a marker M at a target locus A (typically a small gene or an internal exon which if deleted would cause a frameshift mutation). Subsequent transfection of a *Cre* recombinase gene and transient expression of this gene results in recombination between the introduced *lox*P sites to give different products. Type I recombinants are used to generate mice in which the target locus is flanked by *lox*P sites. Such mice can be mated with previously constructed transgenic mice (B) which carry an integrated construct consisting of the *Cre* recombinase gene linked to a tissue-specific promoter. Offspring which contain both the *lox*P-flanked target locus plus the *Cre* gene will express the *Cre* gene in the desired tissue type, and the resulting recombination between the *lox*P sites in these cells results in tissue-specific inactivation of the target locus A. http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=hmg.figgrp.2741-

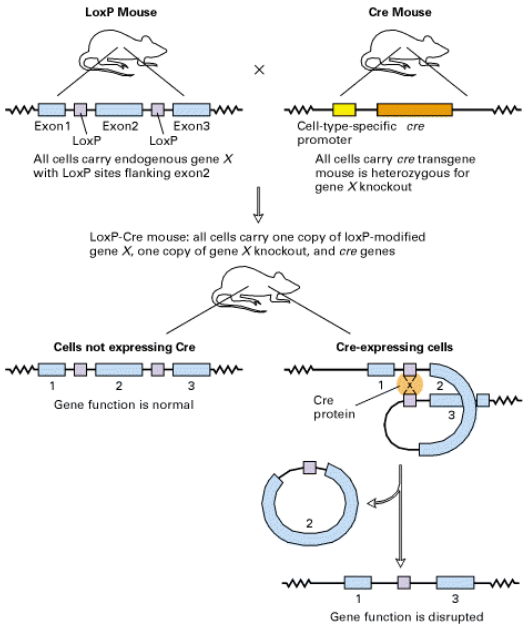


Figure 8-35. Cell-type-specific gene knockouts using the loxP-Cre recombination system. Two loxP sites are inserted on each side of an essential exon (2) of the gene of interest (i.e., gene X) (blue) by homologous recombination. These sites do not disrupt gene function. The loxP-containing mouse is crossed to a transgenic mouse carrying a cell-type-specific promoter controlling expression of the Cre recombinase, which induces recombination between loxP sites. This mouse is heterozygous for a constitutive gene *X* knockout. In the resulting loxP-Cre mouse, Cre protein is produced only in those cells in which the promoter is active, and in those cells recombination therefore occurs between the loxP sites, leading to deletion of exon 2. Since the other allele is a constitutive gene *X* knockout, deletion between the loxP sites results in complete loss of function in all cells expressing Cre.

Lodish