What is a conditional knockout mouse model?

A conditional knockout model initially expresses the wild-type sequence. The target gene is only knocked out in cells where a specific recombinase is active. You control where knockout occurs by crossing two lines together: your conditional knockout line and a recombinase-expressing line.

Cre recombinase is the enzyme most commonly used for conditional genetic rearrangements. It recognizes specific sequences known as lox sequences, e.g. loxP. Other recombinase/recognition sequence pairs are also used such as FLP/FRT and Dre/rox.

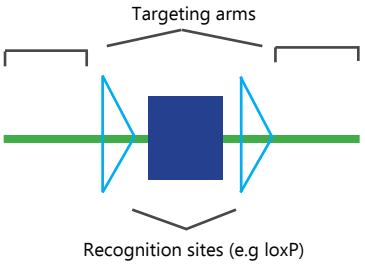
When to use a conditional knockout model?

- Knockout of your gene is lethal during embryonic development.
- You wish to study the effects of knockout in a particular tissue.

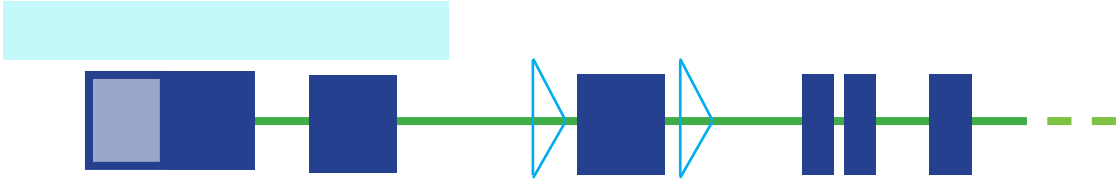
How a conditional knockout model is made

1. **Analyze gene of interest,** including exons (dark blue), introns (green), and known promoter regions (light blue). Identify which exon to flox ( ).

2. **Create targeting material.** The key component is a new DNA molecule that matches the targeted region and has recongniton sites added to flank the targeted exon. Targeting arms help integrate the altered sequence into the correct location.



3. **Integrate the new sequence** into the target location in the genome. Careful screening is required to verify that insertions occurred in the correct location and no unwanted mutations were introduced.

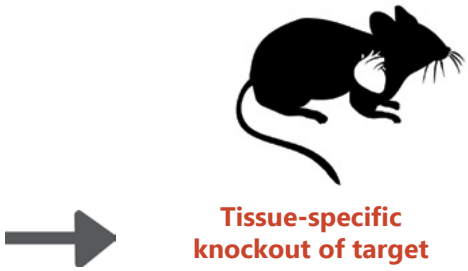


How is a conditional knockout line used?

Conditional knockout mouse line. Gene initially expressed in wild-type pattern.

x

Tissue-specific recombinase line (e.g. Cre recombinase)



Tissue-specific knockout of target gene

General Strategy Considerations

Careful analysis of the target gene is essential when creating a new conditional knockout model. An ideal strategy will cause no change in the expression of the gene before recombination, and loss of gene function after recombination. To create a successful design:

• Avoid modifications that can disrupt promoters or splice sites

• Target exons such that deletion will introduce a frame shift. Look at the starting and ending frame for each exon to find the ones where the starting and ending frames are different.

• Target the earliest possible exon, so the frame shift introduces early stop codons as early as possible. This can trigger a mechanism called nonsense-mediated decay that degrades mRNA before translation starts. It also ensures that if protein is made from the transcript it terminates quickly and may be degraded.

• If no exon can be targeted to create a frame shift, flox a key domain, or the promoter and transcription start site, or even the whole gene.

cKO vs KO

Conditional Knockout (cKO)	Traditional/Constitutive Knockout (KO)
Before recombination the gene functions as normal.	Gene disrupted at all times.
Control where and when knockout occurs.	Gene is knocked out in all cells.
Must be crossed with recombinase-expressing line for knockout.	Crossing with other lines not required for knockout.
Negative effects on embryonic development can be avoided.	Embryonic development may be affected by knockout.

Note: a conditional knockout line can be used to generate a constitutive knockout line.
Cross your cKO line with a line that expresses the appropriate recombinase ubiquitously, or specifically in germ cells. The knockout allele will be passed on to the next generation.