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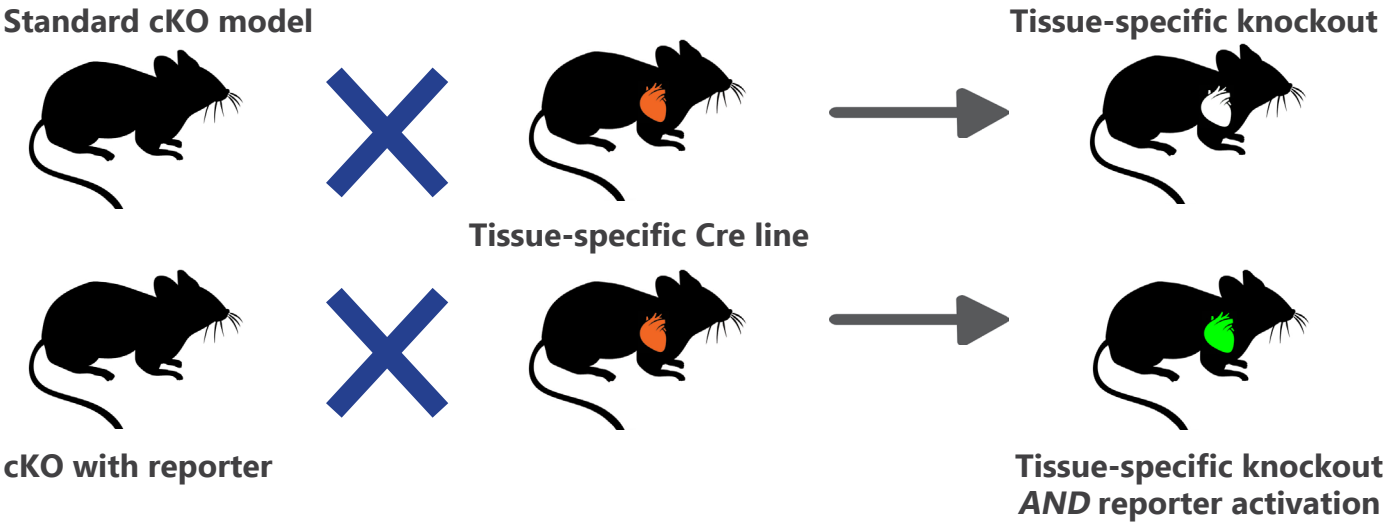
Enhance your conditional knockout model with a reporter

A conditional knockout model with a reporter functions just like a standard cKO, with the added certainty of a label to mark cells where knockout has occurred. The gene of interest will start out with a normal expression level and there is no reporter expression until recombination occurs. When you inactivate your target gene the reporter will turn on to label exactly which tissues are affected. Simplify your experiments by incorporating a reporter into your next conditional knockout model.

Why add a reporter?



Knockout and validate with a single experiment.

Validating a knockout in the tissue of interest is a crucial part of any experiment that uses a conditional knockout line. Validation is especially important if the cKO or Cre lines are new or being used together in a new approach. However, demonstrating that a Cre line and cKO line are interacting in the expected manner can be difficult. Depending on the gene that's the target of the knockout it may be challenging to demonstrate a knockout has occurred, for example if good antibodies aren't available. Adding a reporter solves this - its expression depends on successful recombination in the targeted allele.

Accurately label cells where recombination has occurred.

Newly-made Cre lines are usually crossed with generic reporter lines to demonstrate tissue-specific Cre expression. Generally the reporter line will express high levels of a protein such as β-galactosidase which should label the cells where Cre is active. However, generalizing the results from a generic reporter line is risky because different genomic loci have different susceptibility to recombination. Cre may be more efficient when used in combination with your particular cKO line compared with the generic reporter, leading to knockout in tissues you didn't expect.

Adding a reporter to the cKO design provides an easily detectable readout from the exact genomic locus you're studying. This enables well-validated experiments to be completed more quickly and with fewer expensive reagents. Depending on which reporter strategy is used a knockout can be verified by observing the reporter's expression directly or by simple immunohistochemistry with easily-obtained antibodies.

Make more experiments possible with a single model.

An easily detectable and certain reporter opens up the range of experiments that can be done with a conditional knockout line. In many cases, particularly when using the TruView design from ingenious targeting laboratory, fluorescence will be detectable in living cells. Cell sorting and primary cell culture are only two examples of protocols that can be enhanced when cells of interest are labeled.

Two Popular Strategies for Adding a Reporter to a Conditional Knockout Model:

Inverted reporter gene strategy

• Reporter gene sequence placed next to target region, in opposite orientation to the target gene's sequence. Inverted reporter sequence is not initially expressed due to inverted orientation.

• A specific arrangement of lox sites is used to create conditional knockout model with inverted reporter. Cre will simultaneously invert the reporter sequence, bringing it into frame, while inactivating the target gene.

• Reporter expression is driven by the promoter of the target gene, labeling cells where that gene would be expressed if it hadn't been inactivated.

TruView Conditional Knockout™ *only from ingenious*

• ingenious' proprietary TruView strategy utilizes a unique split GFP sequence with parts of the reporter gene initially separated.

• Cre deletes the region between the two parts of the GFP sequence, bringing them together to activate expression. Most of the target gene's sequence is included in this region so the deletion also inactivates that gene.

• A strong CAG promoter is part of the split GFP design, so cells where knockout has occurred are labeled by bright GFP fluorescence.

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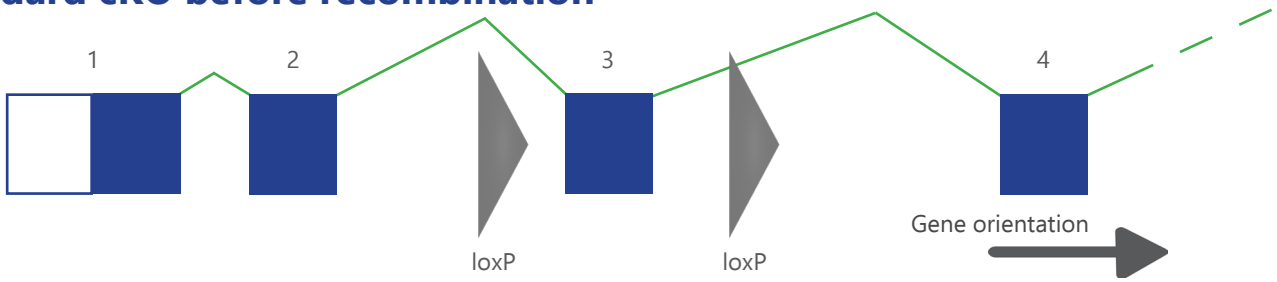
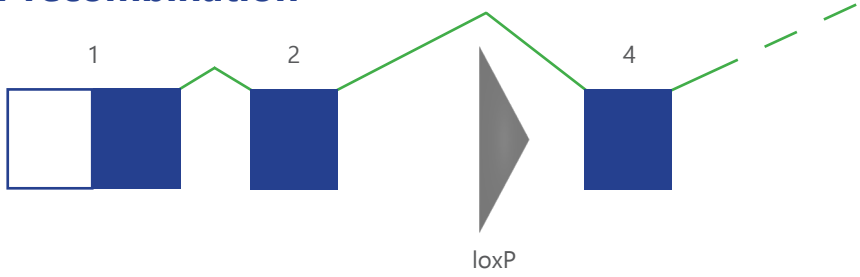
Standard cKO before recombination

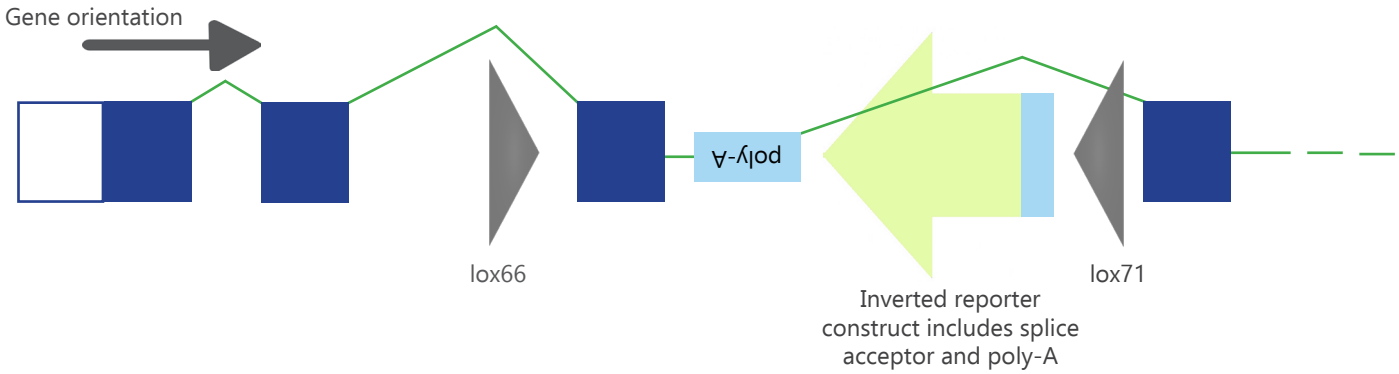


After recombination

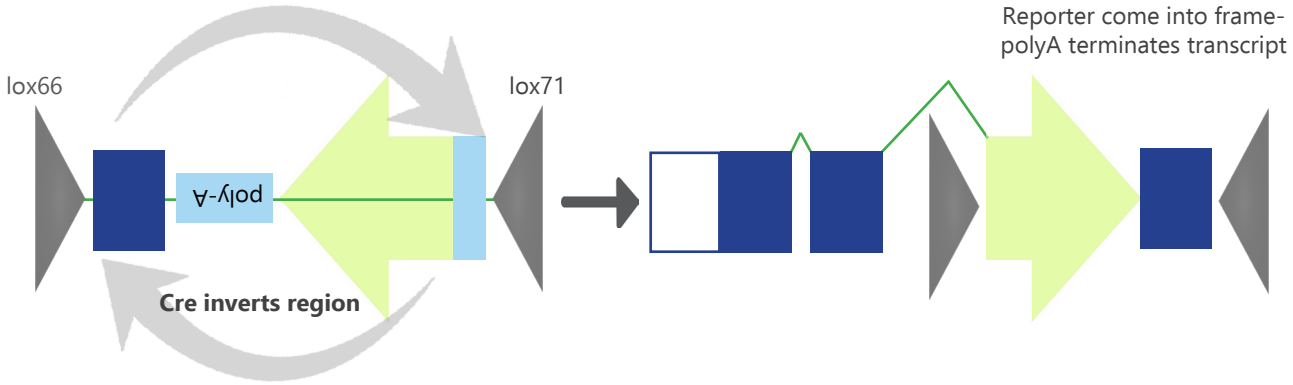


Inverted reporter strategy details

A general scheme of a gene targeted for conditional knockout with an inverted reporter:



Cre takes target exon out of frame by inverting it, and brings reporter into frame. Gene is disrupted and reporter expression activated simultaneously:



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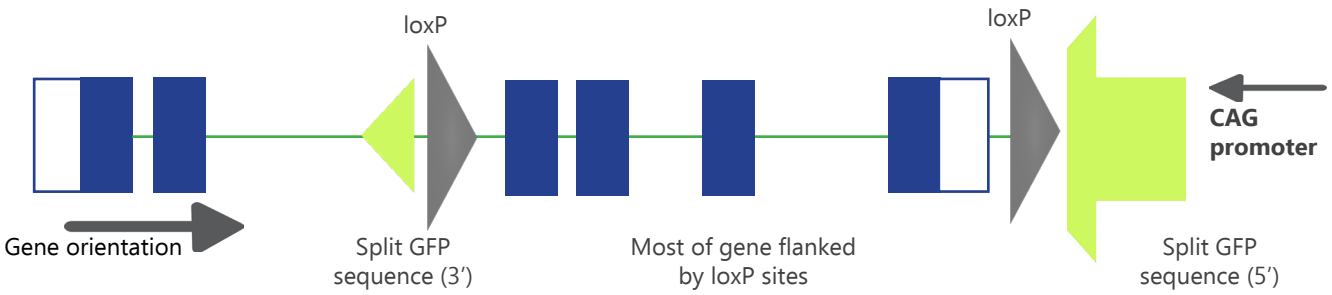
TruView strategy details

A large portion of the target gene is flanked by loxP sites. A CAG promoter is placed downstream of the gene along with a partial GFP sequence:



After recombination most of the gene is deleted. The two partial GFP sequences are brought together and strong reporter expression is driven by the CAG promoter:

	Standard Conditional Knockout	cKO With Reporter	TruView
Proven, Reliable, Cre-Lox Conditional KO	✓	✓	✓
Visualize KO - Internal Control - Individual Cell Labeling - Eliminate Guesswork		✓	✓
Strongly Label All Cells Where Cre Is Active			✓
Only At ingenious			✓

Additional strategies are available. Contacts us today about a custom mouse, rat, or rabbit model.

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