ingenious targeting laboratory

Conditional+Reversible Mouse Models: Advantages and Applications for Life Science Research

If you've wished in the past that you could switch a gene's expression multiple times then you should consider the conditional+reversible design. This model adds another layer of control over gene expression to meet your research needs.

New options beyond the mouse models you know

Targeted transgenic knockin mouse models support a myriad of projects. This broad category of mouse model designs includes the versatile cDNA knockin approach which is typically employed to express a specific transcript in place of a targeted mouse gene. cDNA knockin allows expression of a new sequence using most regulatory features of an endogenous gene: the upstream promoter, the 5' UTR and the chromosomal environment. This strategy allows expression of human sequence in place of mouse, tissue-specific expression of Cre or a reporter gene, and more. Beyond these standard options are a wide variety of strategies that may be exactly what your research plans require.

Take your models two steps further

Taking the static cDNA knockin one step further, ingenious is able to create models that allow conditional (tissue-specific and/or temporal) activation of your cDNA at your locus of choice. The cDNA could encode a marker gene, a mutant form of the targeted gene's sequence or more. For example this could allow you to study your gene first as WT and then induce the activation of your cDNA. However, once the cDNA is activated you can no longer study your gene as WT because the switch is permanent. The future of mouse models is to bring conditional activation even further with the ability to reverse the activation of the cDNA, all within one mouse model.

By taking advantage of multiple recombinase enzymes to switch the cDNA a second time, a conditional+reversible model gives you additional flexibility to carry out more complex experiments.

Standard cDNA Knockin Model		Always expresses knockin sequence
Conditional cDNA Knockin	Can switch the expressed sequence one time e.g. WT to mutant	
Conditional+ Reversible cDNA Knockin	Switch expressed sequence one time e.g. WT to mutant	Then switch expression again e.g. Mutant to WT

Figure 1

A strategy with broad applications

The flexibility of the conditional+reversible design can be applied across a wide range of disciplines because almost any sequences can be used in the expression construct. For example the target gene could be modified to initially express a particular reporter gene. In a sub-population of cells that expression could be switched so the first reporter is shut off and a mutant disease-causing sequence is expressed. Later the gene is switched back to expressing its wild-type sequence so the effects of temporarily expressing the mutant sequence can be assessed. The first expression switch is controlled by Cre recombinase and hundreds of Cre expression lines exist already with different tissue-specific expression patterns. The second switch is activated by giving the mice the drug tamoxifen so timing can be controlled precisely. The two expression switches can be triggered days or months apart.

Conditional+Reversible Control Knockin Example

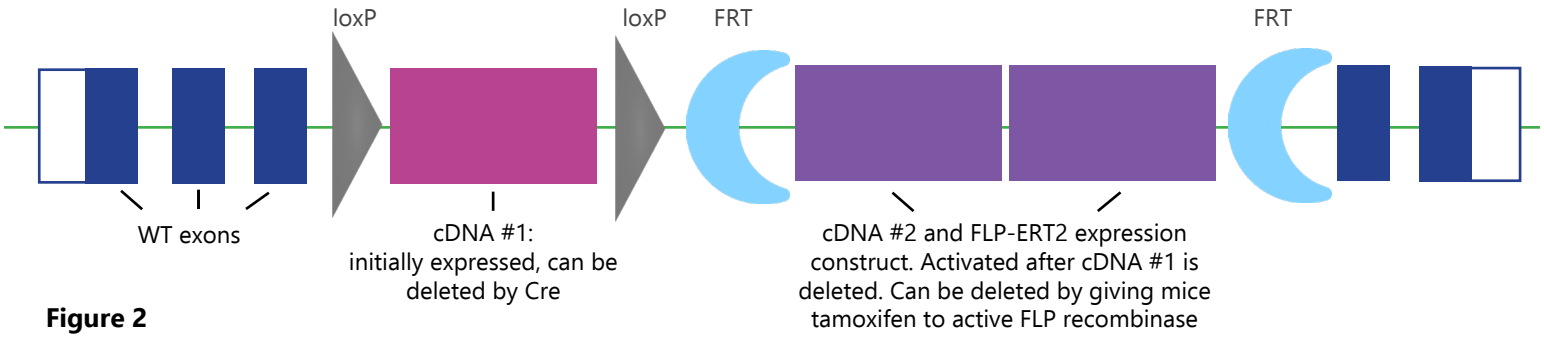

Figure 2

Details about this design

The conditional+reversible mouse model design is made possible by a large targeted sequence knockin which is precisely placed into your gene of interest. Two new separate coding sequences are inserted with each one flanked by recognition sequences for a different recombinase enzyme. As shown in figure 2, the sequence labeled cDNA #1 is initially expressed in place of the target gene's wild-type sequence. cDNA #1 is flanked by loxP sites so in any cell where Cre is active this sequence will be deleted, leading to expression of cDNA #2. An additional coding sequence is placed next to cDNA #2 encoding the recombinase FLP-ERT2. This enzyme can be activated by giving the mice the drug tamoxifen, which will allow FLP to recognize the FRT sequences in the construct and delete cDNA #2. With both cDNA #1 and cDNA #2 deleted the gene reverts to expressing its wild-type sequence. Such a design allows for expression of cDNA #1, then conditional expression of cDNA #2 in cells where Cre is active, and finally reversion to expression of the gene's wild-type sequence.

Additional strategies are available. Contact us today to discuss a custom mouse, rat, or rabbit model.