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Conditional knockout using Cre-LoxP mediated recombination

Techniques cafe 10.05.15

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What is Cre-LoxP?

- **Homologous recombination technique:**
  - Deletion → Conditional
  - Inversion
  - Translocation
  - Insertion

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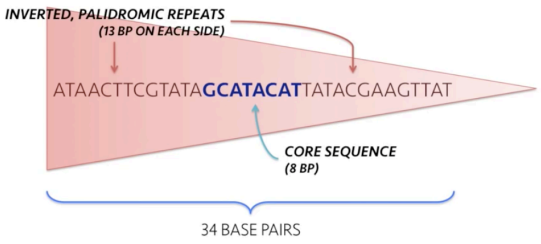
How does it work?

- **Cre**
  - **C**yclization **re**combinase
  - Mediates homologous recombination between LoxP-sites
- **LoxP-sites**
  - LoxP (**l**ocus **o**f **x**-over)



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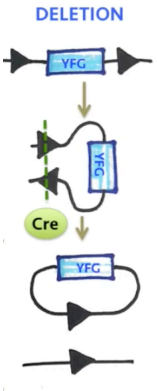
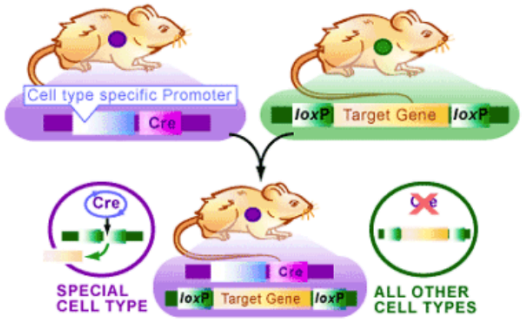
How does it work?



Spatial regulation



(Pechisker, 2006)

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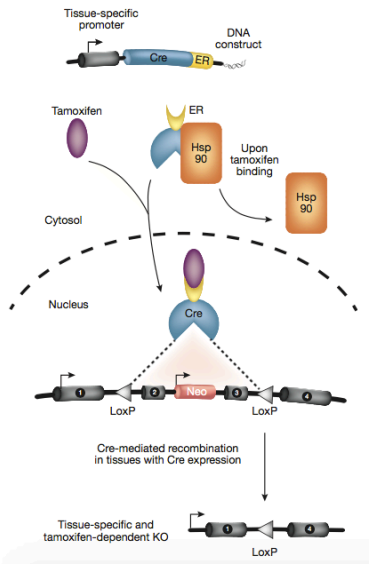
Temporal regulation

- Inducible knockout with tamoxifen



(Günschmann et al, 2014)

Cre-LoxP

1. Names and contributions of group members

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2. Technique chosen:

Conditional gene knockout using Cre-LoxP recombination

3. What does this technique 'do'?

The technique is used to knockout a gene in a specific tissues or at different developmental stages - or both.

4. What applications is this technique employed for?

Cre-LoxP is used to investigate the function of a gene product by observing what happens when the gene is not expressed under specific circumstances.

5. What questions relating to gene regulation and/or development can be addressed using this technique? Provide two examples (peer-reviewed papers) that use this technique.

Conditional knockout using Cre-LoxP mediated recombination allows knockout of a desired gene in a spatial and temporal manner. The technique is particularly useful in the study of genes for which alterations of expression may be lethal in early stages of development. Furthermore the technique is applicable if one wishes to investigate the function of a gene in a specific cell type/tissue/organ or at a specific time.

In a study of the function of the methyltransferase G9a in Th cell differentiation by Lehnertz et al, Cre-LoxP mediated recombination was utilized to specifically knockout G9a in hematopoietic cells (Lehnertz et al, 2010). In the study a transgenic mice strain was generated in which Cre was regulated by a hematopoietic-specific promoter and a part of the G9a gene was flanked by loxP sites to enable cell type specific knockout of G9a. G9a is a gene essential in early development (Tachibana et al, 2002), and by applying Cre-LoxP mediated conditional knockout, the lethality of G9a depletion in early development was circumvented and thereby allowed the study to identify a function of G9a in Th cell differentiation and function.

In a paper by Gunschmann et al, researchers discuss two methods in which Cre-loxP can be induced by adding a molecule; Cre-LoxP induced gene knockout in transgenic mice in the tamoxifen-inducible Cre-LoxP system and tetracycline-inducible Cre-loxP system. Induced gene knock-out allows the researchers to control when they want the knockout to happen without killing the organism. In one of the experiments, the tetracycline/doxycycline-inducible Cre-loxP system would either promote or prevent the binding of tTA protein to the sequence behind the Cre gene. The binding of tTA protein results in the expression of Cre, which subsequently leads to the knockout of a gene at the loxP sites. Meanwhile in the other system, Cre gene is in front of a promoter tissue specific sequence. However, tamoxifen presence is necessary to translocate Cre recombinase to the nucleus, where the loxP sites are located. In this experiment, the investigators were able to test the effects of tamoxifen in tissue-specific cells. In the other system, investigators were able to observe what happened to cells that not only produce the protein tTA but what would happen when tetra/deoxy were present when the cells produced a modified or unmodified form of tTA.

A good example of the tamoxifen induced Cre-LoxP system is found in the study conducted by Denton et al (2008). Researchers discovered initially that the conventional germline deletion of TBRII is lethal in early embryonic development (Denton et al, 2008). Therefore, performing a knockout and observing its effect on the organism postnatally could only be accomplished with tamoxifen-inducible Cre-LoxP system

in transgenic mice. A potent lineage-specific far upstream enhancer regulates the expression of Cre, which is linked to Col1a2 gene, and the loxP sites are at the TBRII gene. The results showed only mice that had had tamoxifen administered developed the null allele of TBRII. This allowed the researchers to study what the effects null-TBRII would have on skin healing without killing the mouse.

6. What critical reagents are required to use this technique?

Transgenic Mice with the Cre-loxP should have:

LoxP sites flanking regions of the gene one wishes to knockout

Expression of Cre

- driven by a tissue/cell type specific promoter or
- inducible Cre-LoxP-system

7. What critical information is required to be able to employ this technique?

To employ Cre-LoxP mediated recombination it is critical to know the sequence of the gene of interest, to enable the insertion of LoxP sites at the desired regions of the gene or the surrounding DNA.

8. References:

B. Lehnertz and others, 'Activating and Inhibitory Functions for the Histone Lysine Methyltransferase G9a in T Helper Cell Differentiation and Function', *Journal of Experimental Medicine*, 207 (2010), 915–22 <http://dx.doi.org/10.1084/jem.20100363>.

Makoto Tachibana and others, 'G9a Histone Methyltransferase Plays a Dominant Role in Euchromatic Histone H3 Lysine 9 Methylation and Is Essential for Early Embryogenesis', *Genes and Development*, 16 (2002), 1779–91 <http://dx.doi.org/10.1101/gad.989402>.

Christopher P Denton and others, 'Inducible Lineage-Specific Deletion of TβRII in Fibroblasts Defines a Pivotal Regulatory Role during Adult Skin Wound Healing', *Journal of Investigative Dermatology*, 129 (2009), 194–204 <http://dx.doi.org/10.1038/jid.2008.171>.

Christian Günschmann and others, 'Transgenic Mouse Technology in Skin Biology: Inducible Gene Knockout in Mice', *Journal of Investigative Dermatology*, 134 (2014), e22 <http://dx.doi.org/10.1038/jid.2014.213>.