Genetic Engineering Unlocked: Cre-lox & Beyond

Hosted by Charles River, exclusive distributor of JAX® Mice in 27 European Countries

Presented by:
Brian W. Soper, PhD
Technical Information Services
Presentation Overview

- Basic Cre-Lox mechanism
- Strain types and breeding schemes
  - Tissue-specific knockouts
  - General knockouts
  - Inducible knockouts
  - Reporters
- Cre-Lox web resources (finding mice, Cre activity data, and more)
A Revolutionary Genetic Tool

Cre-Lox system

- Natural part of P1 bacteriophage viral life cycle
- Viral DNA injected into bacteria, circularized using Cre-Lox, and replicated for development of new viruses
Cre-Lox Successfully Engineered in Other Organisms

Organisms

- Yeast
- Plants
- Mammalian cell cultures
- Mice
Cre-Lox Successfully Engineered in Other Organisms

Organisms
- Yeast
- Plants
- Mammalian cell cultures
- Mice

Allows
- Alteration & deletion of DNA
- Regulation of location and timing of gene recombination
A Simple, Two Component System

Cre recombinase

- Site-specific enzyme, catalyzes recombination between two LoxP sites

LoxP site

- 34 base pair DNA sequence
- Location and orientation determines recombination result:
  - Deletion
  - Inversion
  - Translocation

Abundant possibilities for genome manipulation!

Mechanism: Cre-Lox Deletion

- **LoxP**
- **GeneX**
- **LoxP**

Floxed target gene

Cre excision

Knockout allele
Mechanism: Cre-Lox Inversion

![Diagram showing Cre-Lox Inversion mechanism]
Mechanism: Cre-Lox Translocation

Reciprocal Translocation (3;6)
Cre-Lox Tissue-Specific Knockout

Homozygous “floxed” mouse

Liver-specific cre transgene
Ex: B6.Cg-Tg(Alb-Cre)21Mgn/J (003574)
Cre-Lox Tissue-Specific Knockout

Homozygous “floxed” mouse

Liver-specific cre transgene
Ex: B6.Cg-Tg(Alb-Cre)21Mgn/J (003574)

Cre-Lox mouse:
Heterozygous for gene X conditional knockout after 1 generation
Cre-Lox Tissue-Specific Knockout (continued)

Hemizygous Alb-cre heterozygous “floxed” gene
Cre-Lox Tissue-Specific Knockout
(continued)

Homozygous “floxed” mouse

Hemizygous Alb-cre heterozygous “floxed” gene
Cre-Lox Tissue-Specific Knockout (continued)

Homozygous “floxed” mouse

Hemizygous Alb-cre heterozygous “floxed” gene

25% homozygous for gene X conditional knockout (2\textsuperscript{nd} generation)
Cre-Lox Tissue-Specific Knockout (continued)

Homozygous “floxed” mouse

Hemizygous Alb-cre heterozygous “floxed” gene

25% homozygous for gene X conditional knockout (2\textsuperscript{nd} generation)
Improving Conditional Knockout Efficiency

12.5% conditional gene X knockouts

Heterozygous null mouse (traditional knockout)

Hemizygous Alb-cre heterozygous “floxed” gene
Improving Conditional Knockout Efficiency

Homozygous “floxed” mouse

Hemizygous Alb-cre heterozygous null mouse

25% conditional gene X knockouts
Cre-Lox Germline Knockouts

Homozygous “floxed” mouse

oocyte-specific cre expression
Ex: C57BL/6-Tg(Zp3-cre)93Knw/J (003651)

2 more generations to produce homozygote null mouse
Cre-Lox Knockout Breeding Scheme

Cre mouse – *cre* transgene (Tg) early, widespread expression promoter B6.Cg-(Sox2-cre)1Amc/J (008454)

Offspring: 50% heterozygous knockout after 1 generation
Offspring 2\textsuperscript{nd} generation: 25\% homozygous knockout

Cre-Lox Knockout Breeding Scheme (continued)
**Cre-Lox Summary**

**Tissue-specific deletion**
- 2 generations of breeding
- Cre *required* to maintain line for future generations
- Genotype of whole mouse: homozygous *flox*; Cre
- Tissue-specific genotype: homozygous *flox*-deleted; Cre

**Germline/Embryonic deletion**
- 2-3 generations of breeding
- Cre *not* required after germline deletion (can breed it out)
- Genotype of whole mouse, germplasm, organs & tissues: homozygous *flox*-deleted (knockout) for gene of interest
Inducible Cre Mouse Models

Inducible Cre mouse – tamoxifen dependent Cre function
Ex: B6.Cg-Tg(Cre/Esr1)5Amc/J (004682)

Homozygous LoxP mouse

2 Generations

+ Tamoxifen

Induce homozygous knockout of gene X with tamoxifen
Cre Considerations

Mosaicism

- Some target cells may not express Cre, or *LoxP* sites may not recombine
- May be integration site specific; evaluate multiple cre transgenic founders

Expression in unexpected tissues (including germline)

- Use Cre-positive males for breeding
- Test expression using reporter

*LoxP* site recombination efficiency

- Affected by position and distance


Cre mosaicism in mouse mammary gland epithelial cells
Cre Considerations
(continued)

Breeding efficiency

- If possible, have cre transgene on a different chromosome than the floxed allele
- Often good idea to breed out cre transgene after germline deletion

Cre may produce a phenotype by itself

- Insertion site effects; changes in gene expression
- “Cre toxicity”

Target gene may be expressed prior to Cre recombination

Consider that genetic background may affect phenotype


The FLP-FRT System

- Analogous to the Cre-Lox system
- Derived from the yeast *Saccharomyces cerevisiae*
- Becoming more popular in mouse-based research
- Flipase recombinase (FLP) recognizes a pair of target sequences (FRT) that flank a genomic region of interest
Using FLP-FRT with Cre-Lox
JAX Cre Reporter Strains

Cre Reporter Strains

- Used to assess Cre activity in tissue(s) of interest
- Only one generation of breeding needed
- Ex: B6.129S4Gt(ROSA)26Sor\(^{tm1Sor}\)/J (003474)
Cre Reporter Data

LacZ expression following widespread Cre recombination

B6.129S4Gt(ROSA)26Sor^{tm1Sor}/J (003474)

Cre Reporter Breeding Scheme

Ex: heart-specific cre transgenic
**Could be any Cre recombinase strain

LacZ stain confirms Cre activity in expected tissues
Other Cre Reporter Variations

Reporter switching

Ex. STOCK Tg(ACTB-Bgeo/GFP)21Lbe/J (003920)
Cre-Lox Disease Model

B6.129S4-\textit{Kras}^{tm4Tyj/J} (008179)

Cre-Lox Disease Model

B6.129S4-\(Kras^{tm4Tyj}/J\) (008179)

Mice develop mammary neoplasms

Only one round of breeding needed
Other Cre-Lox Variations

Conditional Transgene with Reporter
Other Cre-Lox Variations

Conditional Transgene with Fusion Protein Reporter

![Diagram of Cre-Lox Variations]

Promotor LoxP STOP LoxP TransgeneX GFP

Promotor LoxP TransgeneX GFP

35
Other Cre-Lox Variations
 Conditional transgene with reporter

Promoter

LoxP  STOP  LoxP

TransgeneX  IRES  GFP

Promoter

LoxP

TransgeneX  IRES  GFP

THE JACKSON LABORATORY
Other Cre reporter Variations: “Brainbow” Mice

- Multiple fluorescent protein sequences
- Pairs of incompatible LoxP sites
- LoxP sites alternated to create mutually-exclusive recombination events
- Following cre excision, one of 3 outcomes (colors) possible in cre expressing cells/tissues

“Brainbow” Mice

- Tamoxifen-inducible CAG-Cre transgenic
- Cell autonomous expression of RFP, YFP & CFP
- Neurons & some astrocytes of the dentate gyrus in the hippocampus

“Brainbow” Mice  

**Multiple copies & multiple integrations of the Brainbow transgene →**

- **B6.Cg-Tg(Thy1-Brainbow1.0)HLich/J (007901)**
  - 8 transgene copies & 90 color variations

- **B6;CBA-Tg(Thy1-Brainbow1.0)LLich/J (007910)**
  - >8 transgene copies & 166 color variations

Like pixels on a TV screen—combinations of fluorophores produce expanded color palettes

Improved Fluorescent Cre Reporters

Ai3  B6.Cg-Gt(ROSA)26Sor^{tm3(CAG-EYFP)}Hze/J  (007903)

Ai2  B6.Cg-Gt(ROSA)26Sor^{tm2(CAG-EYFP)}Hze/J  (007920)

Ai9  B6;129S6-Gt(ROSA)26Sor^{tm9(CAG-tdTomato)}Hze/J  (007905)

Ai9  B6.Cg-Gt(ROSA)26Sor^{tm9(CAG-tdTomato)}Hze/J  (007909)

Ai14 B6.Cg-Gt(ROSA)26Sor^{tm14(CAG-tdTomato)}Hze/J  (007914)

Ai6  B6.Cg-Gt(ROSA)26Sor^{tm6(CAG-ZsGreen1)}Hze/J  (007906)

Dr. Hongkui Zeng, Allen Institute for Brain Science
http://transgenicmouse.alleninstitute.org/
Allen Institute for Brain Science

New cre reporters × brain-specific cre transgenics

B6.Cg-Gt(ROSA)26Sor^{tm9(CAG-tdTomato)Hze/J} (007909) × B6.Cg-Tg(Camk2a-cre)T29-1Stl/J (005359)

http://transgenicmouse.alleninstitute.org/
B6.Cg-Gt(ROSA)26Sor^tm6(CAG-ZsGreen1)Hze/J (007906) x FVB-Tg(GFAP-cre)25Mes/J (004600)

http://transgenicmouse.alleninstitute.org/
JAX Cre Repository

Largest collection of Cre-Lox strains

- 330+ cre-expressing
- 130+ inducible Cre strains
- 480+ floxed genes
- 90+ floxed Stop Cre reporters

Importing over 200 new neuronal specific Cre strains
http://cre.jax.org/NeuroCres.html

http://cre.jax.org
Locating JAX Cre Strains

www.jaxmice.jax.org/query
JAX Cre Repository
http://cre.jax.org

The Jackson Laboratory Cre Repository
The JAX Cre Repository's aim is to provide the scientific community with a centralized, comprehensive set of well-characterized Cre Driver lines and related information resources.

Available strains include Cre expressing strains, inducible Cre strains, Cre reporter strains, and loxp-flanked (floxed) strains.

Lists of available JAX Cre strains
You can also go to www.creportal.org to search for Cre-expressing strains by promoter or site of activity, view detailed characterization of Cre activity including images, and get links to strains available in repositories.

The Cre Repository was funded by the National Center for Research Resources (grant numbers RR032556, RR025117 and RR001183) and is currently supported by the Office of Research Infrastructure Programs/OD (grant numbers OD011190-02 and OD010972-35) of the National Institutes of Health (NIH). Additional support comes from the National Institute of Dental and Craniofacial Research (NIDCR, grant number DE020052), the NIH Neuroscience Blueprint and an anonymous foundation. Please reference these grants in citations acknowledging use of the resource.

Funding support
The Cre Repository is supported by grants from the Office of Research Infrastructure Programs of the National Institutes of Health (NIH).

News
- Combating the challenge of off-target Cre effects in research

Related publication
JAX Cre Repository
Locating Strains by Tissue Expression Site/Promoter

Search by keyword within browser; type “ctrl + F”

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Strain Name</th>
<th>Gene</th>
<th>Tissue Expression</th>
<th>Repository Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>016532</td>
<td>B6.FVB(129)-Tg(Alb1-cre)1Dlr/J</td>
<td>Alb1, Albumin (Mouse)</td>
<td>Repository- Live</td>
<td></td>
</tr>
<tr>
<td>01833</td>
<td>FVB(Cg)-Tg(Alb1-cre)1Dlr/J</td>
<td>Alb1, Albumin (Mouse)</td>
<td>Repository- Live</td>
<td></td>
</tr>
<tr>
<td>018569</td>
<td>129-Alpltm1(cre)Nagy/J</td>
<td>Alpl, alkaline phosphatase, liver/bone/kidney (mouse)</td>
<td>Repository - Live</td>
<td></td>
</tr>
<tr>
<td>007915</td>
<td>129S.FVB-Tg(Amh-cre)8815Reb/J</td>
<td>Amh, anti-Mullerian hormone (mouse)</td>
<td>Repository - Live</td>
<td></td>
</tr>
</tbody>
</table>

For example:

- Cre is expressed specifically in testis Sertoli cells.
- STOCK Mttp<sup>tm2Sgy</sup> Ldlr<sup>tm1Her</sup> Apob<sup>tm2Sgy</sup> Tg(Mx1-cre)1Cgn/J

Find: Liver

[THE JACKSON LABORATORY](#)
The Jackson Laboratory Cre Repository

The JAX Cre Repository's aim is to provide the scientific community with a centralized, comprehensive set of well-characterized Cre driver lines and related information resources.

Available strains include Cre expressing strains, inducible Cre strains, Cre reporter strains, and loxp-flanked (floxed) strains.

Related topics

Donate a strain
Submit your novel mouse strain to The Jackson Laboratory.
Learn more
Cre Expression Data

Cre lines characterized by the JAX Cre Resource
The Jackson Laboratory Cre Repository uses the following LacZ Staining protocol:

<table>
<thead>
<tr>
<th>Stock number (links to data sheet)</th>
<th>Strain name (links to expression data)</th>
<th>Promoter (species)</th>
<th>Site of expression</th>
<th>Expression data thumbnail</th>
</tr>
</thead>
<tbody>
<tr>
<td>003574</td>
<td>B6.Cg-Tg(Alb-cre)21Hgn/J</td>
<td>Alb, albumin (rat)</td>
<td>Cre recombinase expression is expected in the Liver</td>
<td><img src="image1" alt="Liver expression" /></td>
</tr>
<tr>
<td>005359</td>
<td>B6.Cg-Tg(Camk2a-cre)T29-1st/J</td>
<td>Camk2a, calcium/calmodulin-dependent protein kinase II alpha (mouse)</td>
<td>Cre recombinase expression is expected in the forebrain, specifically the CA1 pyramidal cell layer in the hippocampus</td>
<td><img src="image2" alt="Forebrain expression" /></td>
</tr>
<tr>
<td>004126</td>
<td>C57-B6.Cd19&lt;tim1(cre)Cgn&gt;1ghb/J</td>
<td>Cd19</td>
<td>Cre recombinase expression is expected in B cells</td>
<td><img src="image3" alt="B cells expression" /></td>
</tr>
<tr>
<td>006137</td>
<td>B6.Cg-Tg(Cdh5-cre)7Mia/J</td>
<td>Cdh5, cadherin 5</td>
<td>Embryonic and adult cre recombinase expression in endothelium of developing and quiescent vessels of all organs examined, as well as within a subset of hematopoietic cells.</td>
<td><img src="image4" alt="Endothelial expression" /></td>
</tr>
</tbody>
</table>

http://cre.jax.org/data
Cre Expression Data

Cre lines characterized by the JAX Cre Resource

The Jackson Laboratory Cre Repository uses the following LacZ Staining protocols:

### JAX Cre Repository LacZ Staining

<table>
<thead>
<tr>
<th>Stock number/links to data sheet</th>
<th>Strain name (links to expression data)</th>
<th>Promoter (species)</th>
<th>Site of expression</th>
<th>Expression data thumbnail</th>
</tr>
</thead>
<tbody>
<tr>
<td>003574</td>
<td>B6.Cg-Tg(Alb-cre)21Mgn/J</td>
<td>Alb, albumin (rat)</td>
<td>Cre recombinase expression is expected in the Liver</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stock number/links to data sheet</th>
<th>Strain name (links to expression data)</th>
<th>Promoter (species)</th>
<th>Site of expression</th>
<th>Expression data thumbnail</th>
</tr>
</thead>
<tbody>
<tr>
<td>005359</td>
<td>B6.Cg-Tg(Camk2a-cre)T29-15U/J</td>
<td>Camk2a, calcium/calmodulin-dependent protein kinase II alpha (mouse)</td>
<td>Cre recombinase expression is expected in the forebrain, specifically the CA1 pyramidal cell layer in the hippocampus</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stock number/links to data sheet</th>
<th>Strain name (links to expression data)</th>
<th>Promoter (species)</th>
<th>Site of expression</th>
<th>Expression data thumbnail</th>
</tr>
</thead>
<tbody>
<tr>
<td>004126</td>
<td>C57.Gt&lt;cm1(tet)_Gnap1&gt; Tg&lt;ghB&gt;1/1</td>
<td>Gd19</td>
<td>Cre recombinase expression is expected in B cells</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stock number/links to data sheet</th>
<th>Strain name (links to expression data)</th>
<th>Promoter (species)</th>
<th>Site of expression</th>
<th>Expression data thumbnail</th>
</tr>
</thead>
<tbody>
<tr>
<td>006137</td>
<td>B6.Cg-Tg(Cdh5-cre)7Mia/J</td>
<td>Cdh5, cadherin 5</td>
<td>Embryonic and adult cre recombinase expression in endothelium of developing and quiescent vessels of all organs examined, as well as within a subset of hematopoietic cells.</td>
<td></td>
</tr>
</tbody>
</table>

http://cre.jax.org/data
JAX Repository:
Cre Expression Data

- B6.129S4-\textit{Gt}(ROSA)26Sor^tm1Sor/J (003474)
- Bred to LacZ reporter Strain

20X Lung

10X Heart

20X Skin
JAX Repository: Cre Expression Data

- Evaluated @ four time points: E10.5, E15.5, P7 and P56 (adult)
  - whole-mount or fresh frozen sections
  - full necropsy (P7 & P56) – 11 organ systems, 30 organs/structures, 89 substructures
- NanoZoomer slide scanner to capture data at 20X
- Data shared with Creportal database
Cre Portal @ MGI

Recombinase (cre) Activity

MGI collects and annotates expression and activity data for recombinase-containing transgenes and knock-in alleles.

Access Data

Find recombinase-carrying alleles
Search for alleles assayed for specificity/activity in an anatomical structure.

Recombinase activity in: [Go]

Search for alleles by promoter/driver activity.

Recombinase driven by: [choose one] [Go]

Retrieve all alleles
Retrieve a list of all recombinase-containing transgenes and knock-in alleles.

MGI Recombinase Allele Report
Tab-delimited version

Related searches
Search for recombinase-containing transgene or knock-in alleles, or floxed or frt alleles using MGI's Allele Query Form.

FAQs

How do I...
.. find existing recombinase-expressing transgenes and knock-ins that have a given promoter (driver)? FAQ
.. find the promoter (driver) that I'm looking for if it is not on the selection list? FAQ
.. find a recombinase-containing transgene or knock-in that is expressed in a specific tissue? FAQ
.. get a full list of all transgenes and knock-ins that express recombinase? FAQ
.. order mice with a desired recombinase construct? FAQ

More FAQs
Cre Portal Tutorial

Recombinase Allele Data Include

560 Recombinase-containing knock-in alleles
1,411 Recombinase-containing transgenes
1,991 Total recombinase transgenes and alleles
553 Drivers in recombinase transgenes
383 Drivers in recombinase knock-in alleles
2,963 Tissues in recombinase specificity assays

More...

Search by site of expression or Promoter

www.creportal.org
## Cre Portal Search Results

**Link to Phenotypic Data, Images, & References**

### You searched for...

**Anatomical System** equals *muscle*

*Click column headings to sort table data. Drag headings to rearrange columns.*

<table>
<thead>
<tr>
<th>Driver</th>
<th>Allele Symbol</th>
<th>Gene; Allele Name</th>
<th>Recombinase Data</th>
<th>Muscle</th>
<th>Allele Synonym</th>
<th>Find Mice (IMSR)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTA1</td>
<td>Tg(ACTA1-cre)1Mil</td>
<td>transgene insertion 1, Ulrich Muller (phenotype data)</td>
<td>Detected in 6 systems.</td>
<td>Detected</td>
<td>HSA-cre</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>ACTA1</td>
<td>Tg(ACTA1-cre)79me</td>
<td>transgene insertion 79, Judith Melki (phenotype data)</td>
<td>Not detected in 3 systems.</td>
<td>Detected</td>
<td>HSA-Cre, HSA-Cre79, HSA-cre</td>
<td>2 19</td>
<td></td>
</tr>
<tr>
<td>Cga</td>
<td>Tg(Cga-cre)3Sac</td>
<td>transgene insertion 3, Sally A Camper (phenotype data)</td>
<td>Detected in 8 systems.</td>
<td>Detected</td>
<td>alphaGSU-cre, S3, Tg(Cga-cre)53Sac</td>
<td>1 10</td>
<td></td>
</tr>
<tr>
<td>Chat</td>
<td>Chatm1(cre)Low</td>
<td>choline acetyltransferase targeted mutation 1, Bradford B Lowell (phenotype data)</td>
<td>Not detected in 7 systems.</td>
<td>Not Detected</td>
<td>ChAT-IRE6-Cre</td>
<td>1 5</td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>Tg(CMV-cre)1Cgn</td>
<td>transgene insertion 1, University of Cologne (phenotype data)</td>
<td>Detected in 11 systems.</td>
<td>Detected</td>
<td>CMV-Cre, CMV:Cre, Cre deleter, deleter, TgN(CMV-Cre)#Cgn, ubi-cre</td>
<td>4 241</td>
<td></td>
</tr>
<tr>
<td>Csf1r</td>
<td>Tg(Csf1r-cre)1wp</td>
<td>transgene insertion 1, Jeffrey W Pollard (phenotype data)</td>
<td>Detected in 6 systems.</td>
<td>Detected</td>
<td>Tg(Csf1r-cre)jnp</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>E11a</td>
<td>Tg(E11a-cre)C5779Lmgd</td>
<td>transgene insertion C5779, Laboratory of Mammalian Genes and Development, Heiner Westphal (phenotype data)</td>
<td>Detected in 14 systems.</td>
<td>Detected</td>
<td>E11a-Cre, E11a-cre, E11aCre50, E11a-cre, E11aCre deleter, E11a-Cre, TgN(E11a-Cre)C5779Lmgd</td>
<td>2 338</td>
<td></td>
</tr>
<tr>
<td>Fabp4</td>
<td>Tg(Fabp4-cre)1Rey</td>
<td>transgene insertion 1, Ronald N Evans (phenotype data)</td>
<td>Detected in 11 systems.</td>
<td>Detected</td>
<td>eP2-CRE</td>
<td>1 19</td>
<td></td>
</tr>
</tbody>
</table>
## Tissue Specificity of Recombinase

### Tg(ACTA1-cre)79Jme

**Transgene Detail**

| Nomenclature | Symbol: Tg(ACTA1-cre)79Jme  
| Transgene insertion 79, Judith Melki  
| MGI ID: MGI:2447635  
| Synonyms: HSA-Cre, HSA-Cre79, HSA::cre  
| Transgene: Tg(ACTA1-cre)79Jme | Location: unknown |

| Transgene origin | Strain of Origin: (C57BL/6J x SJL)F1 |

| Transgene description | Transgenic (Cre/Flo)  
| Mutation: Insertion  
| This transgene expresses Cre recombinase under the control of a human alpha-skeletal actin promoter, active in striated muscle, heart, and skeletal muscle. (J:67906) |

| Find Mice (IMSR) | Mouse strains and cell lines available from the International Mouse Strain Resource (IMSR) |

| Recombinase specificity | Specificity in: cardiovascular system, embryo-other, limbs, mesenchyme, muscle, nervous system  
| Not detected in: hemolymphoid system, liver & biliary system, renal & urinary system |
Charles River is the Exclusive Distributor of JAX® Mice in 27 European Countries*

Contact Charles River: askcharlesriver@crl.com

*Albania, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Luxembourg, Macedonia, Montenegro, Netherlands, Norway, Poland, Portugal, Serbia, Slovenia, Spain, Switzerland, Sweden, and the United Kingdom