Cre - lox Technology and Breeding Schemes for Research

Emily L. Jocoy, PhD
Technical Information Scientist
The Jackson Laboratory

World renowned non-profit genetics research institute and international training center

Bar Harbor, Maine

Leading provider of genetically defined mice and services including pre-clinical research

Sacramento, California
The Jackson Laboratory

**Research:** investigating genetics and biology of human disease

**Resources:** JAX® Mice & Services, bioinformatics data, technical publications and more...

**Education:** world-class courses, internships and other programs

[www.courses.jax.org](http://www.courses.jax.org)

JAX® Mice

The *Gold Standard* for Biomedical Research

- NIH funded resource
- 5,000 strains and growing
  - 2.7 million mice shipped annually
  - 16,000 investigators globally
- Unsurpassed genetic quality & animal health
- Best characterized & referenced ~100 new pubs/week
- Common inbred strains (C57BL/6J, BALB/cJ, DBA/2J) support development/collection of specialty strains and other valuable community research resources
Online Resources

- JAX® Mice Database
  www.jax.org/jaxmice

- Technical Support Online
  www.jaxmice.jax.org/support

- Mouse Genome Informatics
  www.informatics.jax.org

- Mouse Phenome Database
  www.jax.org/phenome

- And many more unique resources
C57BL/6J – Most Characterized

Available phenotype data for this strain

- appearance and coat color
- behavior – activity and motor function
- behavior – anxiety
- behavior – avoidance
- behavior – exploratory
- behavior – learning and memory
- behavior – social
- behavior – stress reactivity
- behavior – wilderness
- blood chemistry – electrolytes
- blood chemistry – enzymes
- blood chemistry – glucose
- blood chemistry – miscellaneous
- blood chemistry – proteins
- blood chemistry – urea nitrogen
- blood coagulation
- blood hematology – CBC – differentials
- blood hematology – CBC – platelets

By intervention
- APAP
- assisted reproduction
- Cd
- diazepam
- ENU
- EtOH
- high-fat diet
- imipramine
- lamotrigine
- methamphetamine
- NaCl
- ovalbumin i.p.
- pathogen
- saccharin
- sucrose

By misc. grouping
- aainn-related studies

Also for this strain ...

- Photos and appearance-related alleles
- Scan entire mouse genome to see where C57BL/6J differs most vs. other strain(s), based on the dense SNP map
- List SNPs for C57BL/6J and compare vs. other strains

http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rtn=strains/details&stocknum=000664
Strain Surveys

Baseline Blood Pressure
JAX® Services

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• Surgical & preconditioning services
• Use of innovative technologies and state-of-the-art equipment
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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

- 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 \textit{loxP} sites

\textit{loxP} site

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

\textit{Abundant possibilities for genome manipulation!}

Cre - lox Deletion

Floxed target gene

Cre excision

Knockout allele
Cre - lox Inversion

GeneX

Cre

loxP GeneX loxP

loxP GeneX loxP
Cre - lox Translocation

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

homozygous loxP ("floxed") mouse

Liver-specific cre transgene
Ex: B6.Cg-Tg(Alb-Cre)21Mgn/J
(Stock No. 003574)
Cre - lox Tissue-Specific Knockout

homozygous “floxed” mouse

Liver-specific cre transgene
Ex: B6.Cg-Tg(Alb-Cre)21Mgn/J
(Stock No. 003574)

Cre-lox mouse:
hetereozygous for geneX conditional knockout after 1 generation
Cre - lox Tissue-Specific Knockout (cont.)

homozygous “floxed” mouse

hemizygous cre heterozygous “floxed” gene

25% homozygous for geneX conditional knockout after 2 generations
Improving Conditional Knockout Efficiency

heterozygous null mouse (traditional knockout)

hemizygous cre heterozygous “floxed” gene

12.5% conditional GeneX knockouts
Improving Conditional Knockout Efficiency (cont.)

homozygous “floxed” mouse

hemizygous cre heterozygous null mouse

25% conditional GeneX knockouts
Cre-lox Germline Knockouts

- homozygous “floxed” mouse
- oocyte-specific cre expression
  Ex: C57BL/6-Tg(Zp3-cre)93Knw/J (Stock No. 003651)

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

Cre mouse – *cre* transgene (Tg) early, widespread expression promoter
FVB/N-Tg(EIIa-Cre)C5379Lmgd/J (Stock No. 003314)

Offspring: 50% heterozygous knockout after 1 generation
Cre - lox Knockout Breeding Scheme

Offspring 2nd generation: 25% homozygous knockout
**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 (Stock No. 004682)

Induce homozygous knockout of GeneX with tamoxifen

homozygous loxP mouse
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
- loxP site recombination efficiency affected by position
- Cre could be active in ectopic locations (including germline)
- Cre may produce a phenotype by itself
  - Insertion site effects
  - “Cre toxicity”

Consider using the Cre transgenic line itself as a control

Cre Considerations (cont.)

- Target gene may be expressed prior to Cre recombination
- If possible, have *cre* transgene on a different chromosome than the floxed allele
- Often good idea to breed out *cre* transgene after germline deletion
- Consider that genetic background may affect phenotype
Cre Reporter Strains

- **Green** (GFP or ZsGreen)
- **Yellow** (YFP)
- **Blue** (LacZ)
- **Red** (RFP or tdTomato)
- **Cyan** (CFP)
Cre Reporter Strains

- Used to assess Cre activity in tissue(s) of interest
- Only one generation of breeding needed
- Ex: B6.129S4Gt(ROSA)26Sor<sup>tm1Sor</sup>/J (Stock No. 003474)
Cre Reporter Data
B6.129S4-Gt(ROSA)26Sor\textsuperscript{tm1Sor}/J (Stock No. 003474)

LacZ expression following widespread Cre recombination

**Cre Reporter Breeding Scheme**

B6.129S4-Gt(ROSA)26Sor^tm1Sor/J (003474)

**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

STOCK Tg(ACTB-Bgeo/GFP)21Lbe/J (Stock No. 003920)
Cre - lox Disease Model

B6.129S4-Kras<sup>tm4Tyj</sup>/J (Stock No. 008179)

Cre - lox Disease Models

**B6.129S4-Kras^{tm4Tyj}/J (Stock No. 008179)**

**STOCK Tg(MMTV-cre)4Mam/J (Stock No. 003553)**

Only one round of breeding needed

Other Cre - lox Variations
Conditional Transgene with Reporter

Promotor → loxP → LacZ → STOP → loxP → TransgeneX
Other Cre - lox Variations

Conditional Transgene with Fusion Protein Reporter

Promotor  \(\text{loxP} \)  STOP  \(\text{loxP} \)  TransgeneX  GFP

Promotor  \(\text{loxP} \)  TransgeneX  GFP
Other Cre - lox Variations
Conditional transgene with reporter
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 →

3 transgene copies & 10 color variations
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

B6.Cg-Gt(Rosa)26Sor^{tm3(CAG-EYFP)Hze}/J (007903)
B6.Cg-Gt(Rosa)26Sor^{tm2(CAG-EYFP)Hze}/J (007920)
B6;129S6-Gt(Rosa)26Sor^{tm9(CAG-tdTomato)Hze}/J (007905)
B6.Cg-Gt(Rosa)26Sor^{tm9(CAG-tdTomato)Hze}/J (007909)
B6.Cg-Gt(Rosa)26Sor^{tm14(CAG-tdTomato)Hze}/J (007914)
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 x brain-specific cre transgenics

B6.Cg-Gt(ROSA)26Sor<sup>tm9(CAG-tdTomato)Hze</sup>/J (007909) x B6.Cg-Tg(Camk2a-cre)T29-1Stl/J (005359)

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

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

http://cre.jax.org

Largest collection of Cre – lox strains
• 250+ cre-expressing
• 80+ inducible Cre strains
• 310+ floxed genes
• 60+ floxed Stop Cre reporters
• Importing over 200 new neuronal specific Cre strains

http://cre.jax.org/NeuroCres.html
JAX Cre Repository

The goal of the JAX Cre Repository is to provide the scientific community with a centralized, comprehensive set of well-characterized Cre Driver lines and related information resources. To enhance the value of this collection, we are conducting a comprehensive analysis of Cre line excision function in both target and non-target tissues using Cre reporter lines and presenting the annotated data in this website.

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.

- List of strains expressing Cre
- List of Cre reporter strains
- List of strains with floxed alleles

The Cre Repository is supported by the NCRR, the NIH Neuroscience Blueprint and an anonymous Foundation.

http://cre.jax.org
### Cre Recombinase Expression

<table>
<thead>
<tr>
<th>Stock No</th>
<th>Strain Name</th>
<th>Promoter (Species)</th>
<th>Standard Supply</th>
</tr>
</thead>
<tbody>
<tr>
<td>003574</td>
<td>B6.Cg-Tg(Alb-cre)21Nga/J</td>
<td>Alb, albumin (rat)</td>
<td>Repository - Live</td>
</tr>
<tr>
<td></td>
<td></td>
<td>liver</td>
<td></td>
</tr>
<tr>
<td>005359</td>
<td>B6.Cg-Tg(Camk2a-cre)T29-1StI/J</td>
<td>Camk2a, calcium/calmodulin-dependent protein kinase II alpha (mouse)</td>
<td>Repository - Live</td>
</tr>
<tr>
<td></td>
<td></td>
<td>forebrain; pyramidal cell layer</td>
<td></td>
</tr>
<tr>
<td>004126</td>
<td>C.129P2-Cd19tm1(cre)Cgn/J</td>
<td>Cd19, CD19 antigen (mouse)</td>
<td>Repository - Live</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B cells</td>
<td></td>
</tr>
<tr>
<td>004426</td>
<td>B6;S3L-Tg(Cga-cre)3Sac/J</td>
<td>Cga, glycoprotein hormones, alpha subunit (mouse)</td>
<td>Repository - Cryopreserved</td>
</tr>
<tr>
<td></td>
<td></td>
<td>anterior and intermediate lobes of the pituitary gland, as well as in cardiac and skeletal muscle; low to no level of expression is detected in the posterior pituitary, lungs, kidneys, brain, adrenal gland and gonads</td>
<td></td>
</tr>
<tr>
<td>006410</td>
<td>B6;129S6-Chattm1(cre)tlou/J</td>
<td>Chat, choline acetyltransferase (mouse)</td>
<td>Repository - Live</td>
</tr>
</tbody>
</table>

Search by keyword within browser
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/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>Liver</td>
<td></td>
</tr>
<tr>
<td>005359</td>
<td>B6.Cg-Tg(Camk2a-cre)T29-1Stl/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>![Expression data thumbnail]</td>
</tr>
<tr>
<td>006410</td>
<td>B6;129S6-Chat&lt;sup&gt;tm1(cre)Lowl&lt;/sup&gt;/J</td>
<td>Chat, choline acetyltransferase (mouse)</td>
<td>Cre recombinase activity is reported in all cholinergic neurons.</td>
<td>![Expression data thumbnail]</td>
</tr>
</tbody>
</table>

http://cre.jax.org/data
Cre Portal @ MGI

Recombinase (cre) Specificity

MGI collects and annotates expression and transgenes and

- cavities & their linings
- endocrine system
- head
- hemolymphoid system
- integumental system
- limbs
- liver and biliary system
- mesenchyme
- muscle
- nervous system
- renal and urinary system
- reproductive system
- respiratory system
- sensory organs
- skeletal system
- tail
- early embryo
- extraembryonic component
- embryo-other
- postnatal-other

Image reproduced with permission of the Journal of Cell Science.

Access Data

FIND RECOMBINASE-CARRYING ALLELES

Search for alleles assayed for specificity.

Recombinase specificity in: (choose one)

Search for alleles by promoter/driver specificity.

Recombinase driven by: (choose one)

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 knock-in that is expressed in a specific tissue? FAQ
- get a full list of all transgenes and knock-ins that express recombinase? FAQ

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>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)1Mill transgene insertion 1, Ulrich Muller (phenotype data)</td>
<td>detected in 3 systems.</td>
<td>detected</td>
<td>HSA-cre</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>ACTA1</td>
<td>Tg(ACTA1-cre)79)me transgene insertion 3, Sally A Camper (phenotype data)</td>
<td>detected in 6 systems. not detected in 3 systems.</td>
<td>detected</td>
<td>HSA-Cre, HSA-Cre79, HSA::cre</td>
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<tr>
<td>Cga</td>
<td>Tg(Cga-cre)3Sac transgene insertion 3, Sally A Camper (phenotype data)</td>
<td>detected in 6 systems.</td>
<td>detected</td>
<td>alphaSSU-cre, S3, Tgn(Cga-cre)S3Sac</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chat</td>
<td>Chattm1(cre)Low choline acetyltransferase; targeted mutation 1, Bradford B Lowell (phenotype data)</td>
<td>detected in 7 systems. not detected in 6 systems.</td>
<td>not detected</td>
<td>ChAT-IREs-Cre</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>Tg(CMV-cre)1Cgn 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</td>
<td>241</td>
</tr>
<tr>
<td>Csf1r</td>
<td>Tg(Csf1r-cre)1wp transgene insertion 1, Jeffrey W Pollard (phenotype data)</td>
<td>detected in 6 systems.</td>
<td>detected</td>
<td>Tg(Csf1r-cre)1wp</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>EIIa</td>
<td>Tg(EIIa-cre)5379Lmgd transgene insertion 5379, Laboratory of Mammalian Genes and Development, Heiner Westphal (phenotype data)</td>
<td>detected in 14 systems.</td>
<td>detected</td>
<td>E2a-cre, EII-cre, EIIa CreEIIa Cre, EIIaCre, EIIaCre deleter, EII-cre, Tgn(EIIa-cre)C5379Lmgd</td>
<td>2</td>
<td>338</td>
</tr>
<tr>
<td>Fabp4</td>
<td>Tg(Fabp4-cre)1Rev transgene insertion 1, Ronald M Evans (phenotype data)</td>
<td>detected in 11 systems.</td>
<td>detected</td>
<td>aP2-CRE</td>
<td>1</td>
<td>19</td>
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<tr>
<td>Grik4</td>
<td>Tg(Grik4-cre)1G32-4St transgene insertion G32-4, Susumu Tonegawa (phenotype data)</td>
<td>detected in 11 systems.</td>
<td>detected</td>
<td>G32-4 Cre, Grik4-Cre, KA1-Cre</td>
<td>1</td>
<td>9</td>
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<tr>
<td>Hspa2</td>
<td>Tg(Hspa2-cre)1Eddy transgene insertion 1, Edward M Eddy (phenotype data)</td>
<td>detected in 3 systems. not detected in 4 systems.</td>
<td>not detected</td>
<td>Hspa2-cre, Hspa2-cre line 54</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ins2</td>
<td>Tg(Ins2-cre)25Mgn transgene insertion 25, Mark A Magnuson (phenotype data)</td>
<td>detected in 7 systems. not detected in 5 systems.</td>
<td>not detected</td>
<td>[RIP]-Cre, Ins-Cre, Ins2cre, RIP Cre, Rip-cre, RIP2-cre</td>
<td>1</td>
<td>78</td>
</tr>
</tbody>
</table>
**Tg(ACTA1-cre)79Jme Strain Details**

| Nomenclature | Symbol: Tg(ACTA1-cre)79Jme  
Name: 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/Flp)  
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)  
Carrying this Mutation: Mouse Strains: 2 strains available  
Cell Lines: 0 lines available |
| Recombinase specificity | Specificity in: cardiovascular system, embryo-other, limbs, mesenchyme, muscle, nervous system  
Not detected in: hemolymphoid system, liver & biliary system, renal & urinary system |
Thank you!

Would you like to create multiple tissue-specific knockouts but lack the vivarium space to do more than one cross at a time? We can help. Contact us today.

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