

Transgenic Mice

How to make a Transgenic Mouse

Reporter Genes

Knock-In as Knock-Out

Neural-Specific Promoters

Conditional Transgenics

Optogenetics

(Viral Transfection)

same principles, but only short-term insertion of genes into cells at injection site

1

How to Make A Transgenic Mouse

Pronuclear Injection

Embryonic Stem Cells

2

Oocyte injection

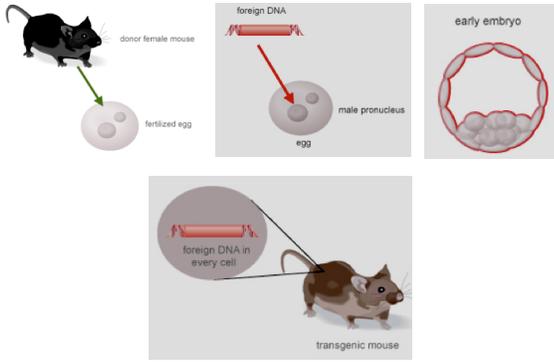


copyright by TCF Naumann

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Pronuclear Microinjection

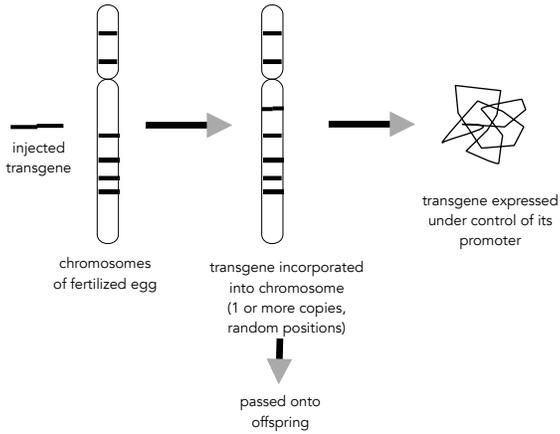
tandem integration of multiple copies continues for 2-3 cell divisions



Wellcome Trust, Chen Journal of Investigative Dermatology Symposium Proceedings (2005) 10, 37-46

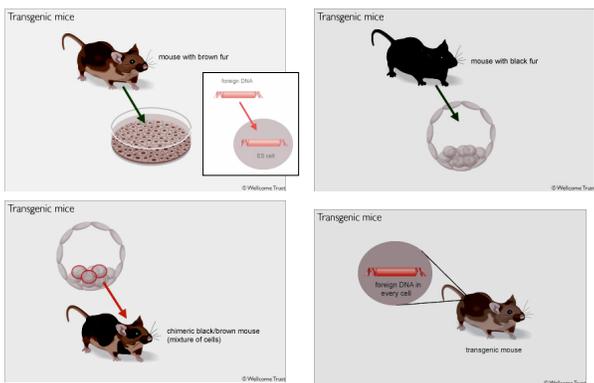
4

Random Integration



5

Embryonic Stem Cells

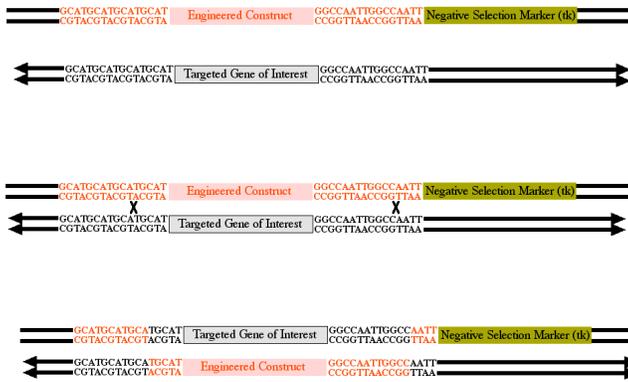


Wellcome Trust

6

Homologous Recombination in ES cells

7



<http://www.bio.davidson.edu/genomics/method/homolrecomb.html>

Transgenic Facilities

8

The screenshot shows the University of Florida Animal Care Services website. The main heading is "Mouse Models Core". Below the heading, it states "Since April 2009 ACS has produced 272* Transgenic Founders." and "* Number as of December 2012". There is a link to "Click on links below to access service request forms and service descriptions". The page lists several services: "Transgenic Mice Creation", "Knockout/Knockin Mice Generation", and "Sperm Cryopreservation".

Mouse Models Core

Since April 2009 ACS has produced 272* Transgenic Founders.
* Number as of December 2012

Click on links below to access service request forms and service descriptions

Transgenic Mice Creation
We will inject your transgene expression construct DNA into zygote pronuclei to produce Transgenic Mice by gene transfer.
[Guarantee: 30 live births per transgene construct]

Knockout/Knockin Mice Generation
We will inject your targeted embryonic stem cells into blastocysts to produce Knockout or Knockin Mice.
[Guarantee: 40 blastocyst injections per ES cell line]

Sperm Cryopreservation
We will cryopreserve sperm collected from two of your transgenic and/or KO males. Session fee includes one quality

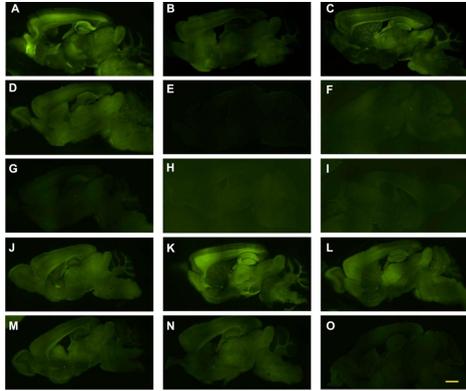
Integration

9

- Copy number
- Chromosomal position
 - Activation by nearby enhancers
 - Silencing by chromatin inactivation
- Assessment of Lines
 - (e.g. thy1-R18 expression)

thy1-R18 expression lines

10



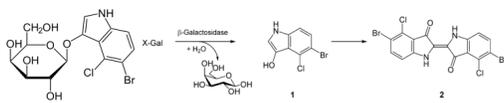
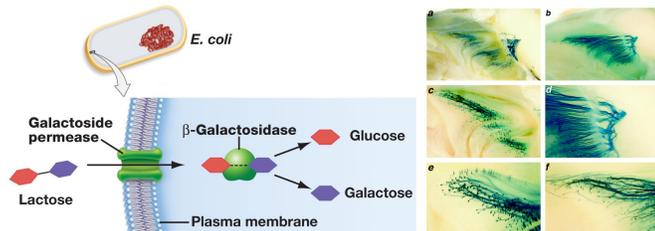
Reporter Genes

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B-galactosidase (lacZ) & X-Gal
Jelly Fish GFP et al.
BrainBow
Genetically Engineered Calcium Indicators

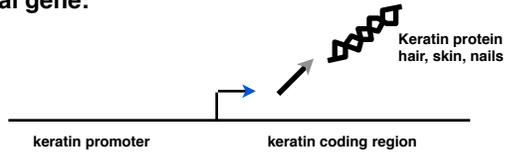
lacZ: beta-galactosidase

12

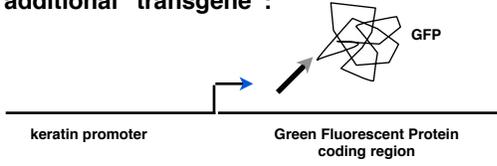


Converts X-Gal to blue pigment

Normal gene:



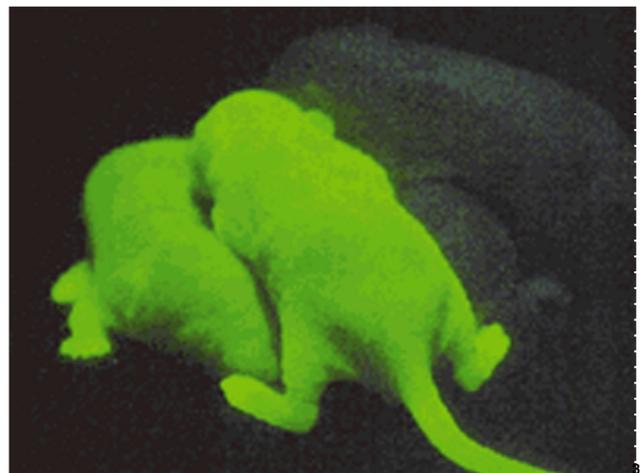
Inject additional "transgene":



13



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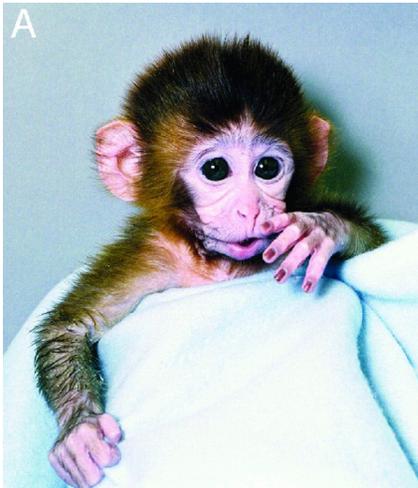
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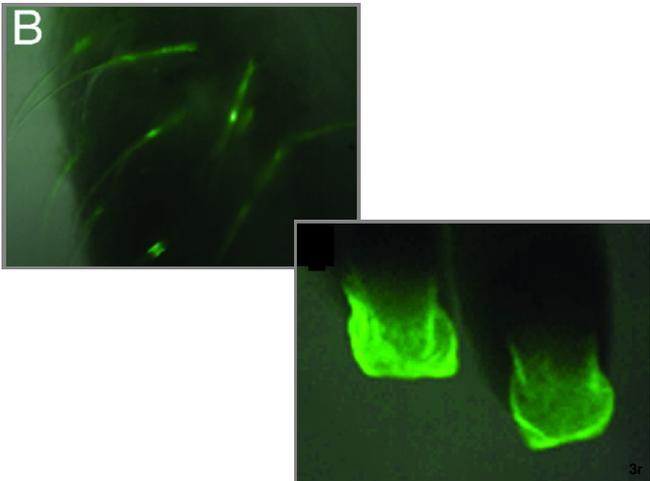
http://www.nichd.nih.gov/news/releases/images/green_brown_mice.jpg

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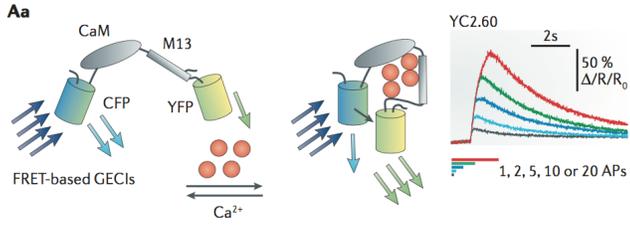


3r

18

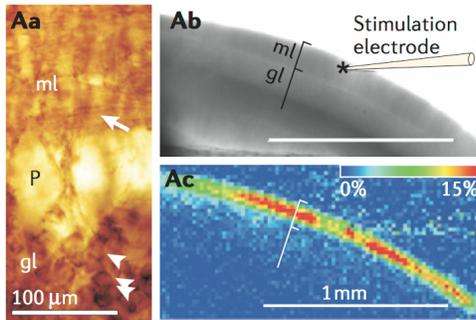


GECI: Genetically Encoded Calcium Indicators



Excitation of CFP with 440 nm light (blue arrows) results in cyan (cyan arrows) and yellow (green arrows) FRET fluorescence. Binding of Ca^{2+} (red circles) to a calcium-binding domain (such as the calmodulin (CaM)-M13 complex shown here) increases the efficacy of FRET between CFP and YFP^{51,52,54} and therefore increases the fluorescence ratio (R) between these two fluorescent proteins. Traces on the right show, for a representative of this indicator class (YC2.60), the change of R (ΔR , normalized to baseline, R_0) when a pyramidal neuron generates 1 (black), 2 (cyan), 5 (blue), 10 (green) or 20 (red) action potentials (APs). The horizontal bars of corresponding colours indicate the time of AP firing.

Nature Review Neurosci.13 (2012) 687



A | Cerebellar granule cells. Aa | This shows immunohistochemical staining against the genetically encoded calcium indicator GCaMP2. GCaMP2 expression (brown) in the granule cells of the cerebellar cortex of mice is under the control of the regulatory sequences of the gene *KCNK1*. The image shows the molecular layer (ml), Purkinje cell layer (P) and the granule cell layer (gl); arrowheads point at granule cell bodies and arrow points at their axons (the parallel fibres). Ab, c | This shows a transmission image of a transverse cerebellar slice (Ab) and change in GCaMP2 (Ac) fluorescence ($\Delta F/F$, indicating a transient elevation of parallel fibre calcium concentration) induced by stimulation of the molecular layer (10 stimuli delivered at 100 Hz). Asterisk indicates tip of stimulation electrode.

Knock-in as Knock-out

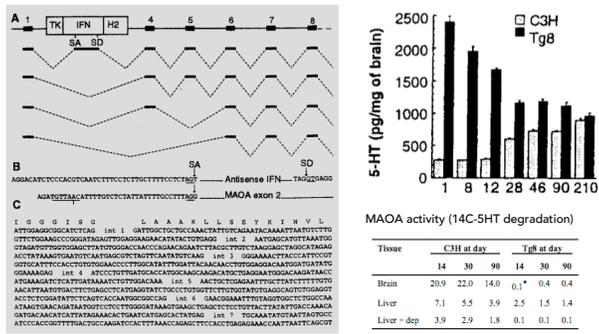
Knock-In Antisense gene

IL-1 & MAOA knockout

thy1-R18 & 14-3-3

Inadvertent landing of transgene in MAOA gene

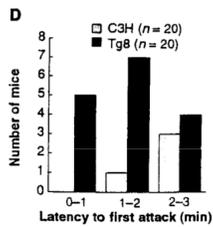
25



MAOA RNA of Tg8 mice (A) Representation of the Tg8 gene encoding MAOA between exons 1 and 8 (the gene for MAOA probably has 15 exons) and structure of the four species of MAOA RNA detected by RT-PCR. Cases, Science 268 (1995)1763

Lack of MAOA -> Aggression

26

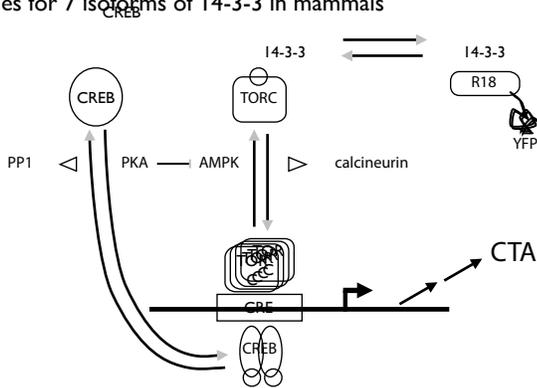


Latency to first biting attack in resident intruder test.

"recently a family has been described in which a point mutation in the gene encoding MAOA abolishes MAOA catalytic activity and is associated with impulsive aggression (Brunner Science 262 (1993)578)."

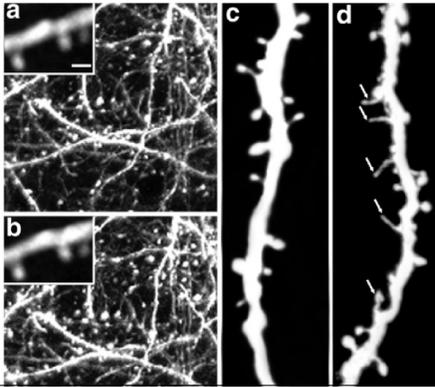
R18 Peptide as 14-3-3 antagonist

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thy1-YFP

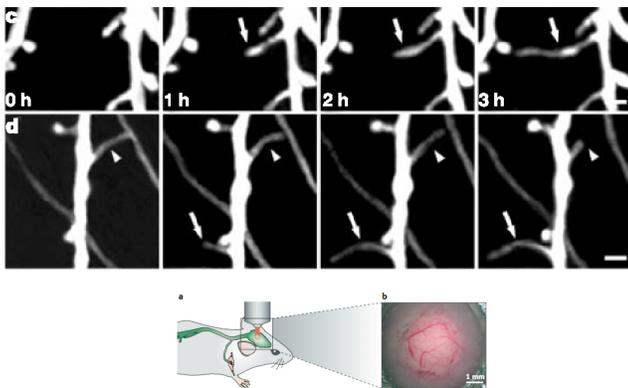
expressed in only ~50% of neurons



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thy1-YFP

visualizing cortical dendritic spines in vivo



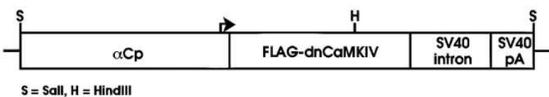
32

Kang, Cell 106 (2001) 771-783

An Important Role of Neural Activity-Dependent CaMKIV Signaling in the Consolidation of Long-Term Memory

α Cp-FLAG-dnCaMKIV-SV40

α CaMKII promoter should limit expression to forebrain
dominant negative CaMKIV should inhibit endogenous CaMKIV activity
FLAG provides a detectable protein tag
SV40 provides detectable mRNA tag



α Cp-FLAG-dnCaMKIV-SV40 intron-SV40 pA, where α Cp is the 8.5 kb DNA fragment derived from the 5' flanking region of the α CaMKII gene and FLAG-dnCaMKIV encodes an epitope tag fused to the amino terminus of dnCaMKIV.

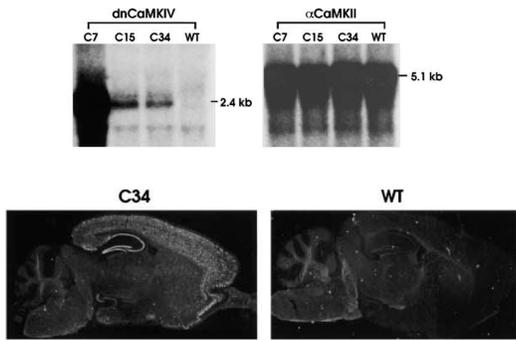
We produced ten transgenic founders (C57BL/6) and chose three lines, C7, C15, and C34, carrying approximately 15, 2, and 1 copies of the transgene, respectively.

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mRNA Verification of Transgene

Northern Blot: SV40 mRNA detected in (3) transgenic lines

In Situ Hybridization: SV40 mRNA expression in line C34 limited to forebrain (mostly hippocampus and cortex)

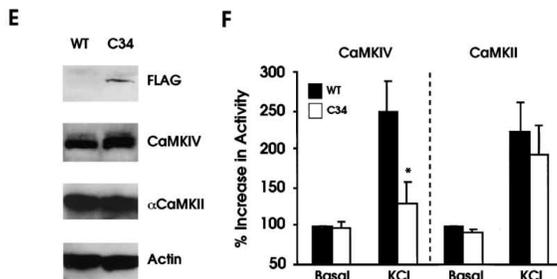


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Protein Verification of Transgene

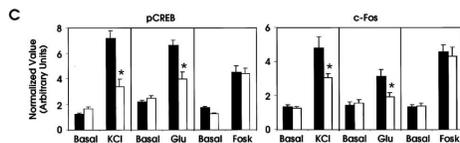
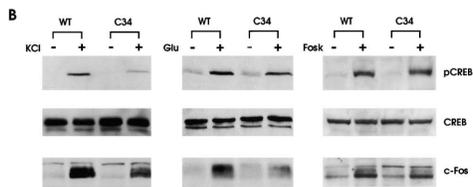
Western Blot: dnCaMKIV flag & protein (slightly larger band) detected

Enzyme Activity: dnCaMKIV expression in line C34 inhibits endogenous CaMKIV activity (induced by KCl depolarization); CaMKII unaffected.



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Effect of Transgene on Signaling

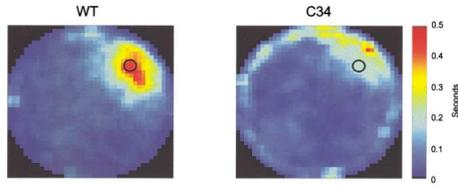


(B) Immunoblot analysis of hippocampal extracts from wild-type and C34 transgenic slices stimulated with KCl, glutamate (Glu), or forskolin (Fosc). The blots were probed with anti-pCREB, anti-CREB, or anti-c-Fos antibodies, respectively. (C) Quantification of average pCREB and c-Fos immunoreactivity obtained from three independent experiments. *p < 0.05 for C34 versus control group.

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Effect of Transgene on Behavior

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(E) The activity histogram representing the total occupancy by wild-type and transgenic mice during the last probe test. Each pixel represents 4 x 4 cm² space. The wild-type mice are more accurate than the transgenic mice in searching the previous platform location (black circle).

Conditional Transgenics

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Tetracycline

Tamoxifen

Ecdysone insect steroid hormone

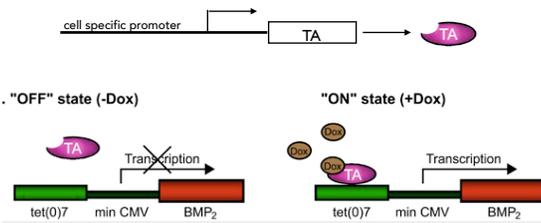
CRE-Lox

(Viral Transfection)

TetOn and TetOff

Tetracycline-controlled transcriptional activation

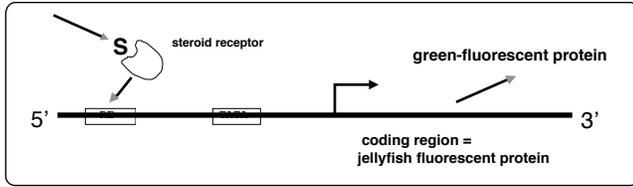
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Doxycycline (tet analog) in drinking water activates transgene in cells expressing Tet Activator protein

Steroid regulated transgene expression

40



Use non-endogenous steroids, such as tamoxifen or ecdysone (insect) hormone

CRE-LOX: Cell-specific gene deletion

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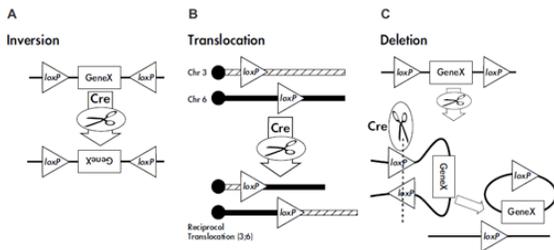
Cre (Cyclization recombination) recombinase enzyme cuts out DNA that falls between two Lox sites.

Cause deletion of a target gene in specific cells:

Put Lox sites around target gene
(present in all copies of the gene in every cell in the body.)

Insert Cre gene under control of a cell-specific promoter
(transgene will be present in all cells of the body,
but Cre protein will only be expressed in specific cells).

Expression of Cre in specific cells will cause target gene to be cut out of genome, but only in the specific cells.



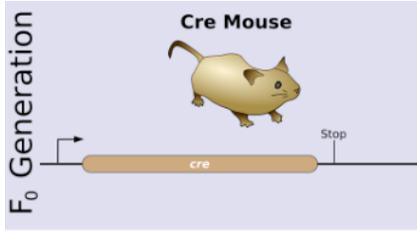
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Figure 2. The outcome of a Cre-lox recombination is determined by the orientation and location of flanking loxP sites. (A) If the loxP sites are oriented in opposite directions, Cre recombinase mediates the inversion of the floxed segment. (B) If the loxP sites are located on different chromosomes (trans arrangement), Cre recombinase mediates a chromosomal translocation. (C) If the loxP sites are oriented in the same direction on a chromosome segment (cis arrangement), Cre recombinase mediates a deletion of the floxed segment.

<http://cre.jax.org/introduction.html>

Cre Expression Strain

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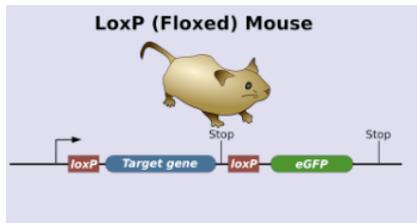


Cre gene codes for Cre recombinase enzyme.
Placed under control of a specific gene promoter, e.g. TH
Expressed in cells that express that gene, e.g. catecholamine cells.
Cre enzyme has no effect on its own.

Matthias Zepper, Wikipedia

Floxed Target Gene Strain

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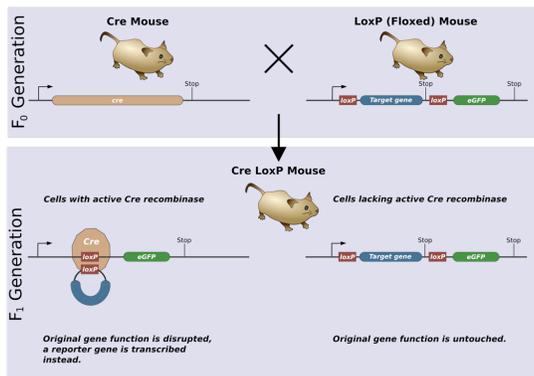


Lox sites inserted next to target gene (e.g. MAPK).
Lox sites have no effect on their own.
Normal expression of target gene.

Matthias Zepper, Wikipedia

Cre x Floxed Mouse

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Only cells expressing Cre have target gene deleted

Jax Cre / Floxed Mice

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Available Cre strains - The Jackson Laboratory

Genetics and your health | Courses and education | Research and resources | JAX® Mice and Services

Available JAX Cre strains

The Jackson Laboratory offers over 300 Cre tool strains.

Listed by promoter, including site of expression:

Research Tools: Cre-lox System

- Cre Recombinase Expression
- Cre-Recombinase Expression: Germline/Embryonic Expression
- Cre-Recombinase Expression: Inducible
- loxP-flanked Sequences
- loxP-flanked Sequences: Test/Reporter

Related technology: FLP-FRT system

- FLP Recombinase Expression
- FLP Recombinase Expression: Germline/Embryonic Expression
- FLP Recombinase Expression: Inducible
- FRT-flanked Sequences
- FRT-flanked Sequences: Test/Reporter

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Leading the search for tomorrow's cures

Cre Lines

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JAX Mice Database - EXPRESSION

cre expression is directed to calretinin interneurons in the brain and cortex by the endogenous *Calb2* promoter/enhancer elements.

013730	B6(Cg)- <i>Calb2^{cre}/creERT2^{flp}/+</i>	<i>Calb2</i> , calbindin 2 (mouse)	Cryopreserved - Ready for recovery
005359	B6.Cg-Tg(<i>Camk2a-cre</i>)T29-15H1/3	<i>Camk2a</i> , calcium/calmodulin-dependent protein kinase II alpha (mouse)	Repository - Live
012362	B6.129S6-Tg(<i>Camk2a-cre/ERT2</i>)1A1ba/3	<i>Camk2a</i> , calcium/calmodulin-dependent protein kinase II alpha (mouse)	Repository - Live
018966	B6N.Cg-Tg(<i>Camk2a-cre</i>)T29-15H1/3	<i>Camk2a</i> , calcium/calmodulin-dependent protein kinase II alpha (mouse)	Under Development - Now Accepting Orders
016097	C57BL/6-Tg(<i>Car1-cre</i>)5F11/3	<i>Car1</i> , carbonic anhydrase 1 (mouse)	Cryopreserved - Ready for recovery
009615	STOCK Tg(<i>Cartpt-cre</i>)1A1ba/3	<i>Cartpt</i> , CART prepropeptide (mouse)	Repository - Live
011086	C57BL/6-Tg(<i>Cck-cre</i>)3C6ve/3	<i>Cck</i> , cholecystokinin (mouse)	Cryopreserved - Ready for recovery

Floxed Lines

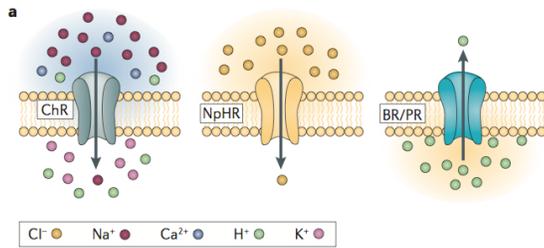
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JAX Mice Database - EXPRESSION

<i>Foxn1</i> , forkhead box N1 (mouse)	012941	B6.129(BL1)- <i>Foxn1^{tm1.120mhu}/+</i>	Cryopreserved - Ready for recovery
<i>Ftn1</i> , ferritin heavy chain 1 (mouse)	018063	B6.129- <i>Ftn1^{tm1.11cl}/+</i>	Repository - Live
<i>Fzd4</i> , Frizzled homolog 4 (Drosophila) (mouse)	011078	B6.129- <i>Fzd4^{tm2.1Nhl}/+</i>	Cryopreserved - Ready for recovery
<i>Fzd5</i> , Frizzled homolog 5 (Drosophila) (mouse)	008620	B6.129- <i>Fzd5^{tm2Nst}/+</i>	Cryopreserved - Ready for recovery
<i>Gabra1</i> , gamma-aminobutyric acid (GABA) A receptor, subunit alpha 1 (mouse)	004318	B6.129(PVB)- <i>Gabra1^{tm16nb}/+</i>	Cryopreserved - Ready for recovery
<i>Gabra4</i> , gamma-aminobutyric acid (GABA) A receptor, subunit alpha 4 (mouse)	006874	B6.129- <i>Gabra4^{tm1.26nb}/+</i>	Cryopreserved - Ready for recovery
<i>Gabra3</i> , gamma-aminobutyric acid (GABA) A receptor, subunit beta 3 (mouse)	008310	B6.129- <i>Gabra3^{tm2.16nb}/+</i>	Cryopreserved - Ready for recovery
<i>Gabrg2</i> , gamma-aminobutyric acid (GABA) A receptor, subunit gamma 2 (mouse)	016830	STOCK <i>Gabrg2^{tm2Linc}/+</i>	Repository - Live
<i>GalnT1</i> , UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylglucosaminyltransferase 1 (mouse)	006895	B6.129- <i>GalnT1^{tm13mm}/+</i>	Cryopreserved - Ready for recovery
<i>GalnT13</i> , UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylglucosaminyltransferase 13 (mouse)	006896	B6.129- <i>GalnT13^{tm13mm}/+</i>	Cryopreserved - Ready for recovery

Channelrhodopsins

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Major classes of single-component optogenetic tools include cation-permeable channels for membrane depolarization (such as channelrhodopsins (ChRs)), chloride pumps (for example, halorhodopsin (NpHR)) and proton pumps (such as bacteriorhodopsin or proteorhodopsin (BR/PR)) for membrane hyperpolarization.

Tye Nature Reviews Neuroscience 13 (2012) 251

Fiber optics to activate channelrhodopsins in vivo

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Optogenetics Starter Kits

- ▶ Complete Fiber Optics Kit for Optogenetics
- ▶ 470 nm LED Light Source
- ▶ Ø1.25 mm or Ø2.5 mm Ferrule Fiber Optic Cannulae and Cables
- ▶ Patch Cable with Optional Rotary Joint



Halassa, Cell 158 (2014) 808-821 State-Dependent Architecture of Thalamic Reticular Subnetworks

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VGAT (vesicular GABA transporter) expressed in thalamic reticular nucleus (TRN), but not other thalamic neurons.

AAV-VGAT-CRE

(Adeno-Associated Virus with VGAT promoter-CRE recombinase) injected into TRN cell bodies, for expression of CRE recombinase in VGAT-positive neurons (but doesn't do anything on its own).

RG-LV-ChR2

(Retrogradely-transported Lentivirus with floxed channelrhodopsin 2) chimeric with other viruses for retrograde transport; injected into visual thalamus targets of TRN neurons, transported back along axon to cell body in TRN;

Result:

ChR2 only expressed in VGAT-CRE containing neurons which project to visual thalamus.

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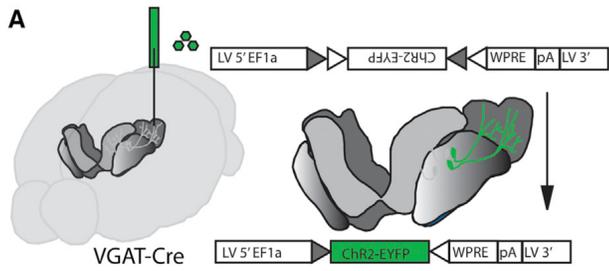
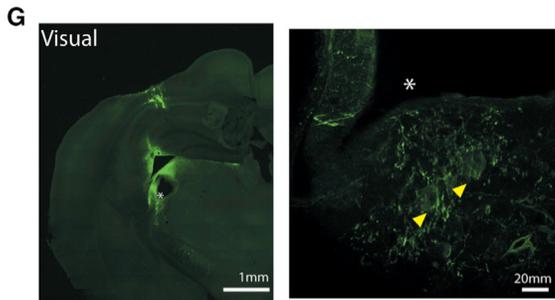


Figure 4. Optogenetic Tagging of TRN Neurons Based on Their Thalamic Targets
(A) Cartoon depiction of optogenetic tagging of visually connected TRN neurons in mice. A RG-LV containing a Cre-dependent Chr2-EYFP is injected into the visual thalamus of a VGAT-Cre mouse. Two to 4 weeks later, Chr2 is robustly expressed in visually connected TRN.

Halassa, Cell 158 (2014) 808-821

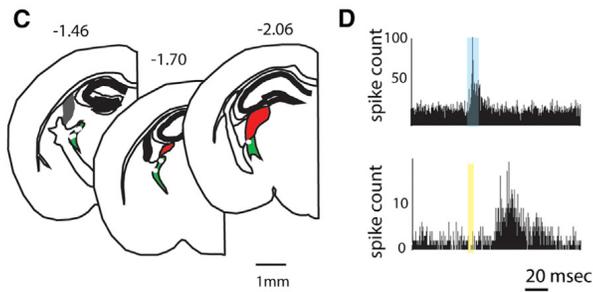
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(G) Example brain sections showing electrolytic lesions of electrode tips for visually connected TRN preparation. Confocal image on the right shows electrode tips (white asterisk) near neurons expressing Chr2-EYFP (yellow arrowheads).

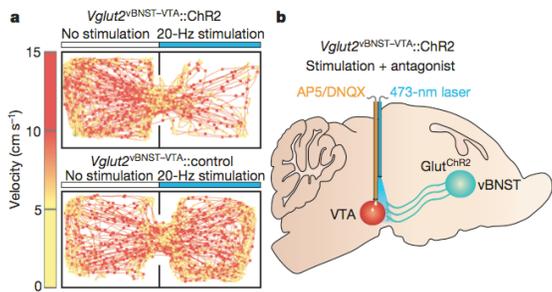
Halassa, Cell 158 (2014) 808-821

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(D) Peri-stimulus time histograms (PSTHs) from two visual-tagged TRN neurons, showing optogenetic drive with short-latency responses (top) and visual drive with longer-latency responses (bottom).

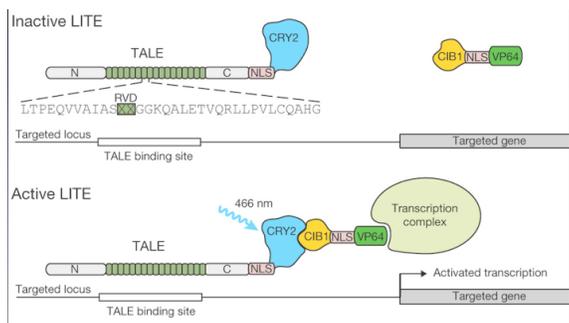
Real-time activation of Chr2 induces aversion



Photostimulation of *Vglut2*^{vBNST-VTA::Chr2} mice resulted in a significant avoidance of a stimulation-paired chamber

Jennings Nature 496 (2013) 224

LITE: Light Inducible Transcriptional Effector System

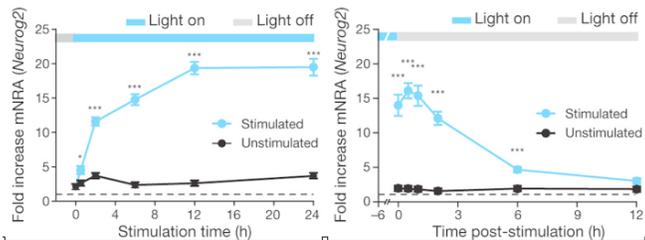


TALE: transcription-activator-like effector proteins, contain repetitive amino-acid sequences that recognize single DNA nucleotides or nucleotide triplets

CRY2: light-sensitive photoreceptor cryptochrome 2

Konermann, Nature 500 (2013) 472
N&V, Nature 500 (2013) 406-8

LITE: Light Inducible Transcriptional Effector System



Konermann, Nature 500 (2013) 472
N&V, Nature 500 (2013) 406-8

Profile of Transgenic Mouse

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What is promoter, expressed transgene, and reporter?

What is predicted spatial expression pattern?

What is actual expression pattern?

Is expression limited to a specific cell-type?

What are the controls (endogenous or exogenous) of transgene expression?

What is temporal pattern of expression? Is expression conditional or constitutive?

What is effect of background strain?

Caveats of Transgenic Models

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