National Cancer Institute

Complex and inducible construct design for mouse models

by Mark Lewandoski

Mouse 101

NCI, NIH May 29, 2012

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Transgenic mouse resources

Table 1 Web-sites of major organizations and institutions providing public access to databases, computational tools and technology platforms for genome, physiological and anatomical analysis of normal and transgenic mice

Name of database	Address
Genomes	
1000 Genomes Project	http://www.1000genomes.org
Ensembl Project	http://www.ensembl.org
Genome Research	http://www.genome.org
Mouse Genome Informatics (MGI)	http://www.informatics.jax.org
National Institute of Environmental Health Sciences – Center for Rodents Genetics	http://www.niehs.nih.gov/research/resources/collab/crg/
Genetic Diseases	
National Center for Biotechnology Information – Online Mendelian Inheritance in Man Project	www.ncbi.nlm.nih.gov/omim
Single Nucleotide Polymorphisms	
Broad Institute	http://www.broad.mit.edu
European Molecular Biology Laboratory – Mouse SNP Miner	http://bioinfo.embl.it/SnpApplet
International HapMap Project	http://www.hapmap.org/
National Center for Biotechnology Information – Single Nucleotide Polymorphisms Project	http://www.ncbi.nlm.nih.gov/projects/SNP
Genome Wide Association Studies	http://www.genome.gov/gwastudies/
Copy Number Variation	
Database of Genomic Variants	http://projects.tcag.ca/variation/
Open Genomics – Copy Number Variants	www.opengenomics.com/cnv
Quantitative Trait Loci	
Animal Quantitative Trait Locus Database	http://www.animalgenome.org/QTLdb
GeneNetwork	http://www.genenetwork.org/
Knockout Mice Projects	
Knockout Mice Project Respiratory	http://www.komp.org
European Conditional Mouse Mutagenesis Program (EUCOMM)	http://www.eucomm.org/
International Knockout Mouse Consortium (IKMC)	http://www.knockoutmouse.org
Genome Expression database	
European Bioinformatics Institute – Microarray Informatics	http://www.ebi.ac.uk/microarray/
Genomic Institute of Novartis Research Foundation – BioGPS	http://biogps.gnf.org/
National Center for Biotechnology Information – Gene Expression Omnibus	http://www.ncbi.nlm.nih.gov/geo
Mouse Atlas of Gene Expression	http://www.mouseatlas.org/data/mouse/devstages
Gene Expression Database in 4D	http://4dx.embl.de/4DXpress
Edinburgh Mouse Atlas of Gene Expression	http://www.emouseatlas.org/emage/home.php
Sources of genetically engineered mice and ES cells	
International Mouse Strain Resource (IMSR)	http://www.findmice.org
International Gene Trap Consortium (IGTC)	http://www.igtc.org
Cre-X-Mice: A Database of Cre Transgenic Lines	http://nagy.mshri.on.ca/cre
CreZoo: A Databasse of Cre Transgenic lines	http://bioit.fleming.
Center for Animal Resources and Development Database (CARD)	http://cardb.cc.kumamoto-u.ac.jp/transgenic/index.jsp
European Mouse Mutant Archive (EMMA)	http://www.emmanet.org/
RIKEN BioResource Center	http://www2.brc.riken.jp/lab/animal/search.php
Japan Mouse/Rat Strain Resources Database	http://www.shigen.nig.ac.jp/mouse/jmsr/top.jsp
Mutant mouse regional resource centers	http://www.mmrrc.org
Atlases of mouse anatomy, histology, physiology and embryology	
The Mouse Anatomical Dictionary	http://www.informatics.jax.org/mgihome/GXD/GEN/AD/
Edinburgh Mouse Atlas Project (EMAP)	http://genex.hgu.mrc.ac.uk
Allen Institute for Brain Science: Developing Mouse Brain Atlas	http://developingmouse.brain-map.org/atlas/index.html
High resolution mouse brain atlas	http://www.hms.harvard.edu/research/brain/atlas.html#
Histology Atlas – University of Illinois	http://www.med.uiuc.edu/histo/small/atlas/index.html

Table 1 (continued)

Name of database	Address
Duke Center for In Vivo Microscopy	http://www.civm.duhs.duke.edu/devatlas/index.html
Visible Mouse	http://ccm.ucdavis.edu/tvmouse
Caltech MRI Atlas of Mouse Development	http://mouseatlas.caltech.edu/
Centre for Modeling Human Diseases	http://www.cmhd.ca/enu_mutagenesis/physiology_stats/physiology_stats.html
DevTox	http://www.devtox.org/

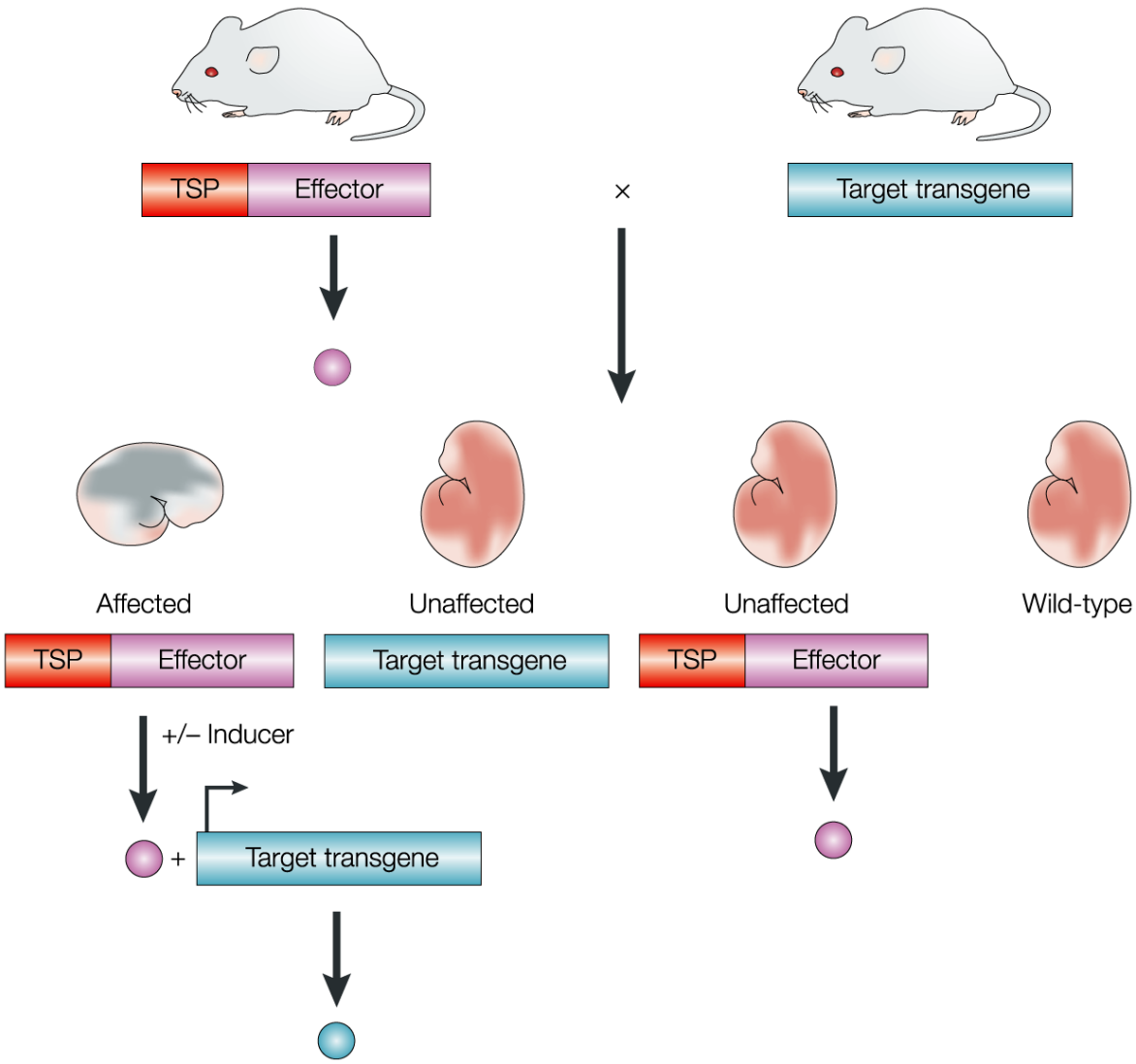
Late 90's: "Don't KO your gene; flox it instead!"

Early 00's: "Don't removed neo; flrt it instead!"

2012: "check out the knockout consortium websites"

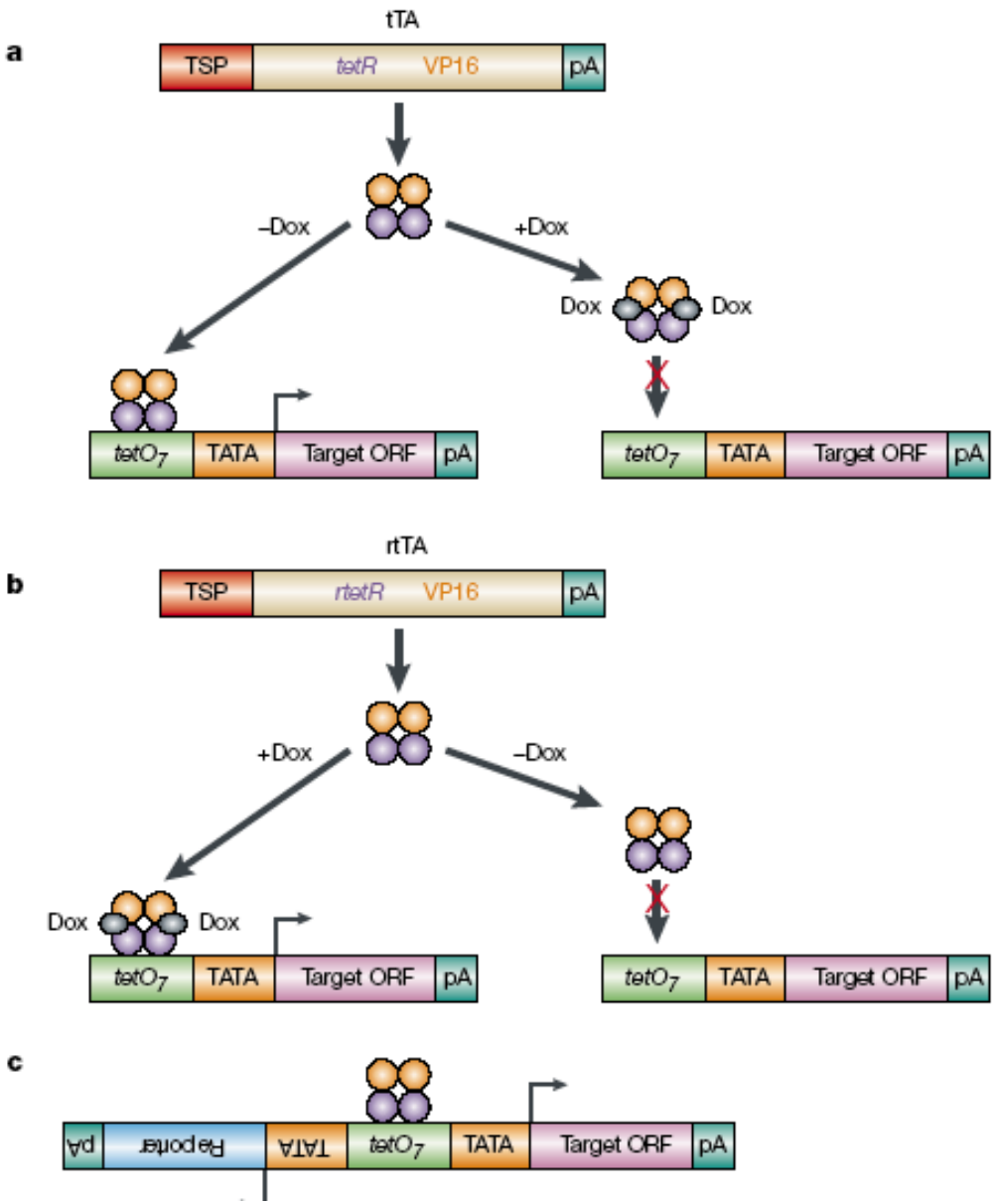
New Routes for transgenesis of the mouse
Belizario et al (2012) J Appl. Genetics May 9. [Epub ahead of print]

Binary transgenics



Lewandoski, M. (2001) **Nature Reviews | Genetics** 2: 743

Binary transgenics based on transcriptional transactivation



Lewandoski, M. (2001) Nature Reviews Genetics 2: 743

Binary transgenics based on transcriptional transactivation

In vivo gene regulation using tetracycline-regulatable systems
2009 Stieger K et al Adv Drug Deliv Rev. Jul 2;61(7-8):527-41

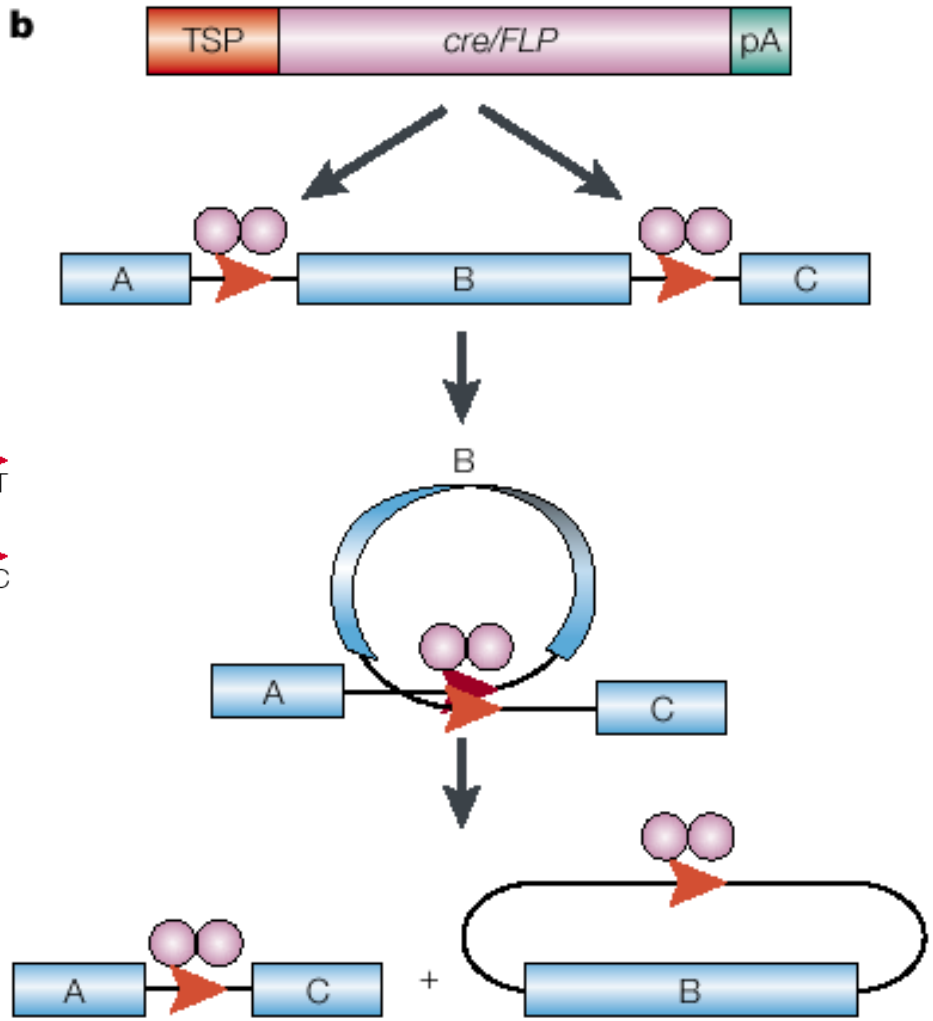
The Power of Reversibility: regulating Gene activities via tatracycline-Controlled Transcription
2010 Schonig Bujard and Gossen Methods Enzymol. 477: 429–453

http://www.zmbh.uni-heidelberg.de/bujard/Homepage.html

Mostly, transcriptional transactivation is used for gain-of-function studies, but see

An Inducible and Reversible Mouse Genetic Rescue System
2008 Zeng et al Plos Genetics 4: e10000069

Binary transgenics can be based on DNA recombination



Lewandoski, M. (2001) Nature Reviews Genetics 2: 743

Table 1 SSRs and some of their derivatives useful for mouse SSR technology

SSR / target site	Properties and application(s)	Reference(s)
A) SSR systems with proven efficiency in cultured mammalian cells as well as in mice		
Cre/loxP	Biological function: DNA excision for dimer reduction of bacteriophage P1 plasmids	Sternberg et al. 1981
	Most efficient and widely used SSR tool *in vitro* and in *vivo*	See text
EGFP-Cre	Fusion with an N-terminal EGFP; facilitates recombinase detection	Le et al. 1999
iCre	Codon-improved version for expression in mammalian cells	Shimshek et al. 2002
Cell-permeable Cre	Fusion with membrane translocation sequences such as the basic HIV-TAT peptide; the efficiency of cell-permeable is not clear Cre proteins in vivo (see the chapter by C. Patsch and F. Edenhofer, this volume)	Jo et al. 2001; Joshi et al. 2002; Peitz et al. 2002
CreLBDs	Various fusions with mutated steroid receptor LBDs; inducible by synthetic ligands of the LBD	See Sect. 4
Flp/*FRT*	Biological function: DNA inversion for amplification of yeast 2-μm plasmid	Vetter et al. 1983
	Removal of selection cassettes and other more specialized transactions	Rodriguez et al. 2000; Schnutgen et al. 2005
Flpe	Mutated version selected in a protein evolution strategy with increased activity	Buchholz et al. 1998; Rodriguez et al. 2000
$FlpeER^{T2}$	Tamoxifen-inducible version of Flpe, might perform similar to CreER fusions in mice (see Sect. 4)	Hunter et al. 2005
B) SSR systems with proven efficiency in cultured mammalian cells and potential utility in mice		
ΦC31/*att*	Biological function: DNA integration and excision of *Streptomyces* phage ΦC31	Thorpe and Smith 1998
	Potentially useful for stable integration of transgenes	Olivares et al. 2002; Belteki et al. 2003
ΦC31-NLS	A version with a C-terminal nuclear localization signal; displays enhanced efficiency	Andreas et al. 2002
β recombinase/*six*	Biological function: Resolution of plasmid oligomers in Gram-positive bacteria	Rojo and Alonso 1994
	Catalyzes exclusively intramolecular recombination like excision and inversion	Diaz et al. 1999
β-EGFP	Fusion with a C-terminal EGFP; facilitates recombinase detection	Servert et al. 2006
β-AR, β-EGFP-AR	Fusion with the androgen receptor LBD; inducible with mibolerone; also functional as a triple fusion with a central EGFP	Servert et al. 2006
Dre/*rox*	Cre-like recombinase encoded by the P1-related bacteriophage D6	Sauer and McDermott 2004

AR, angrogen receptor; EGFP, enhanced green fluorescent protein; ER, estrogen receptor; LBD, ligand-binding domain; NLS, nuclear localization signal

Feil R (2007) Handb Exp Pharmacol 178:3

Multiple new site-specific recombinases for use in manipulating animal genomes

Aljoscha Nern[1], Barret D. Pfeiffer[1], Karel Svoboda, and Gerald M. Rubin[2]

Transcriptional transactivation
vs.
Site-specific DNA recombination

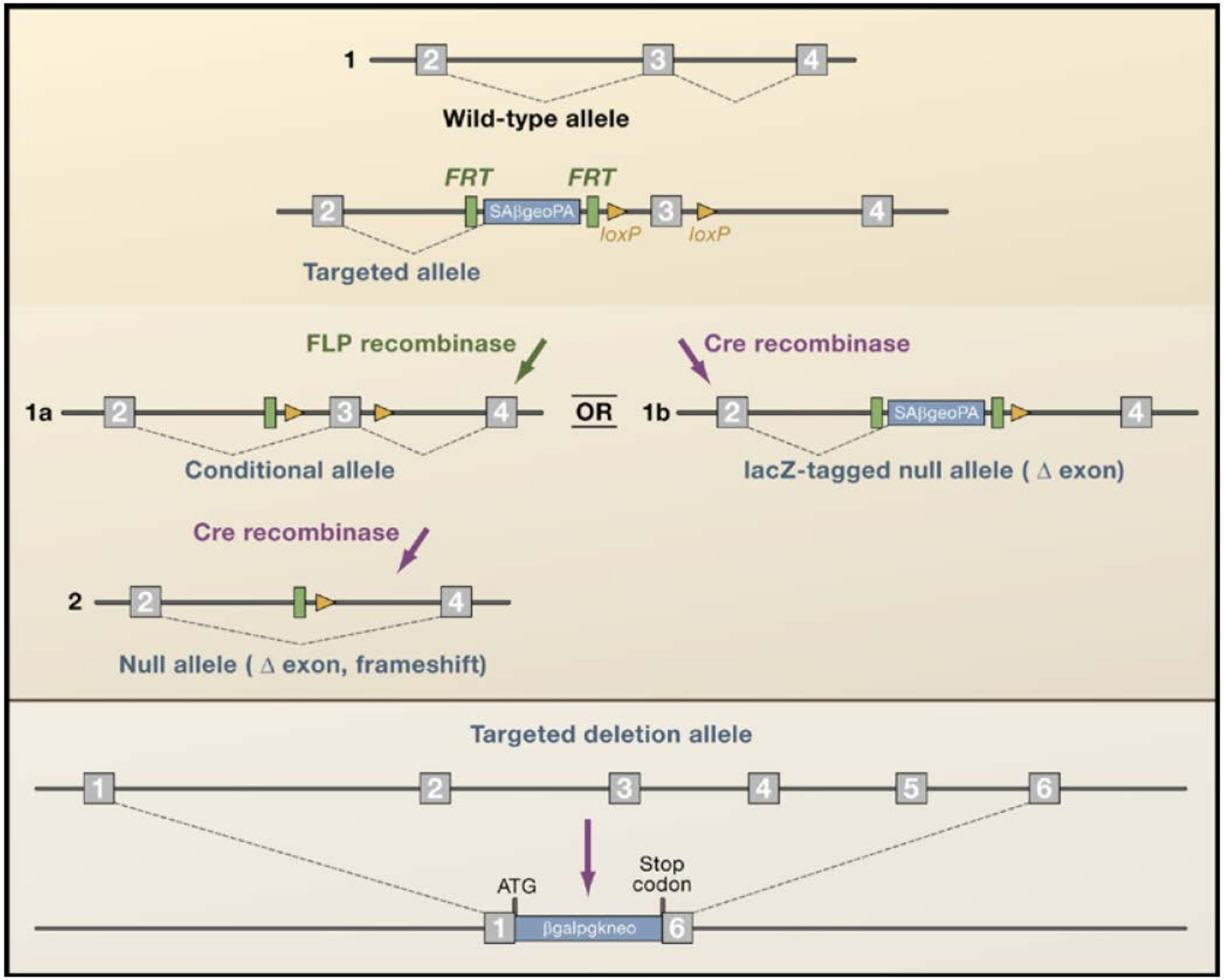
Transcriptional transactivation

1) Can be regulated exogenously with an inducer.
2) Reversibility makes it ideal for activation studies (gain of function).
3) Sensitivity of transactivation levels to inducer concentration.

Site-specific DNA recombination

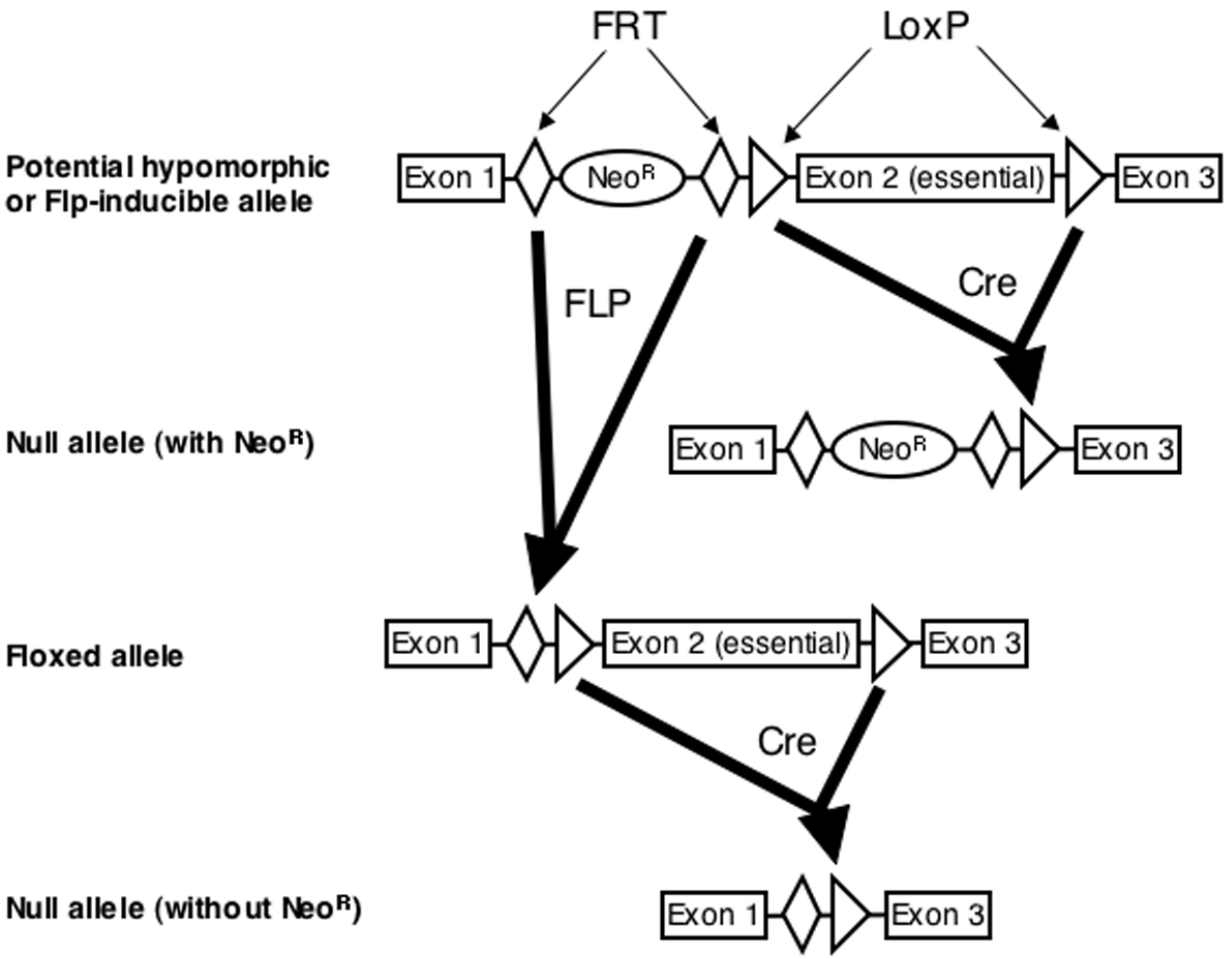
1) Can be regulated exogenously, but many Cre lines do not use such a regulatable Cre variant.
2) Irreversibility makes it ideal for tissue-specific knockouts (loss of function).
3) Irreversibility makes it ideal for cell lineage studies.

International Mouse Knockout Project



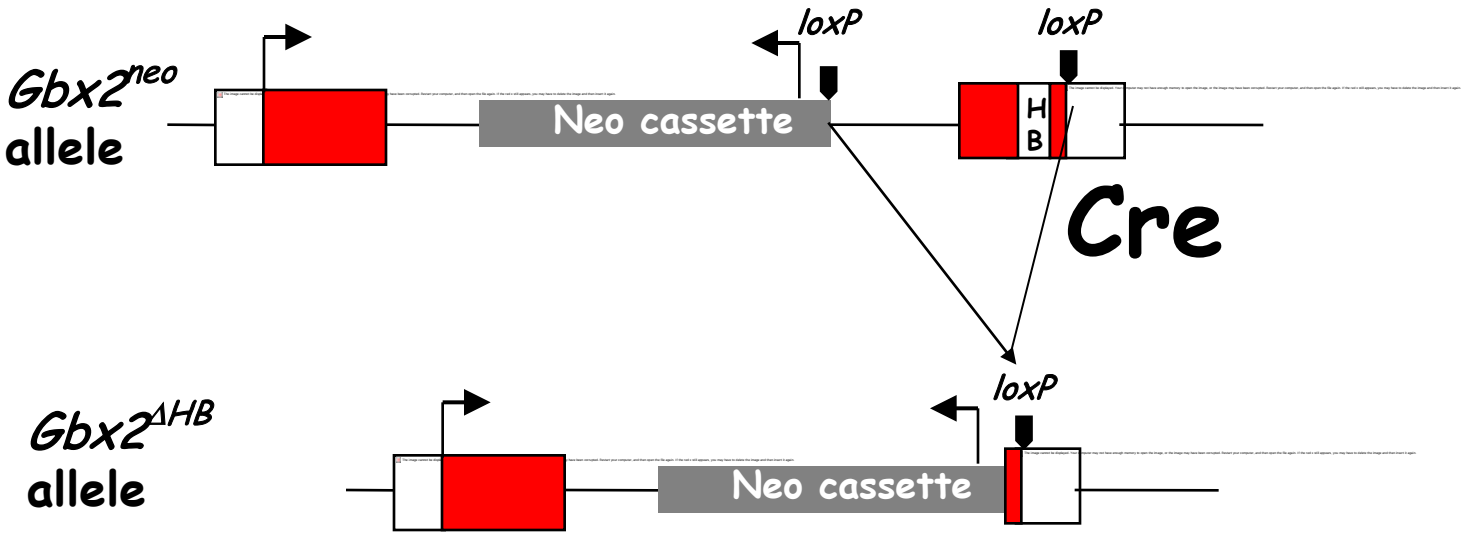
The International Mouse Consortium (2007) Cell 128:9

Allelogenic strategy

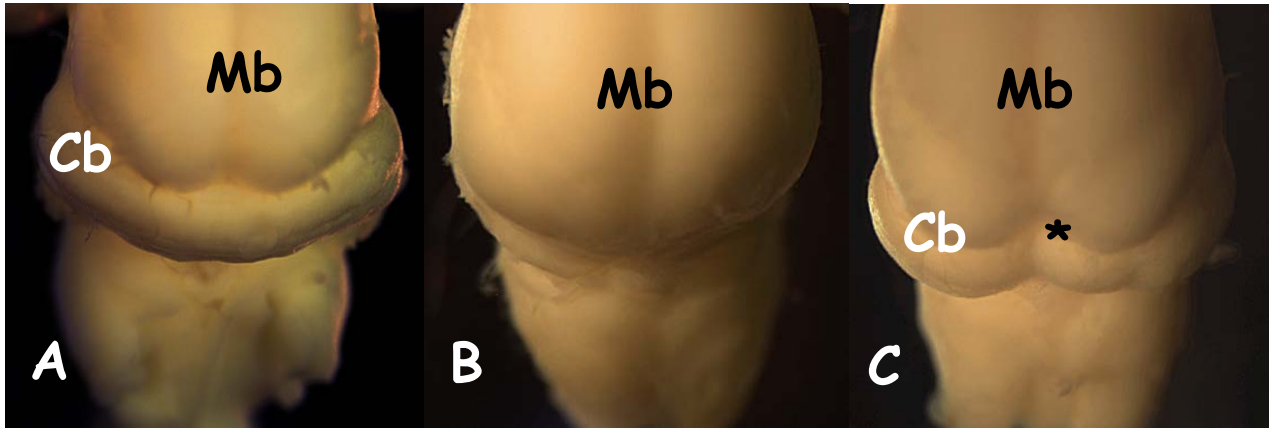


Lewandoski, M. (2007) Handb Exp Pharmacol 178:235

Gbx2^{neo} allele is a hypomorphic allele

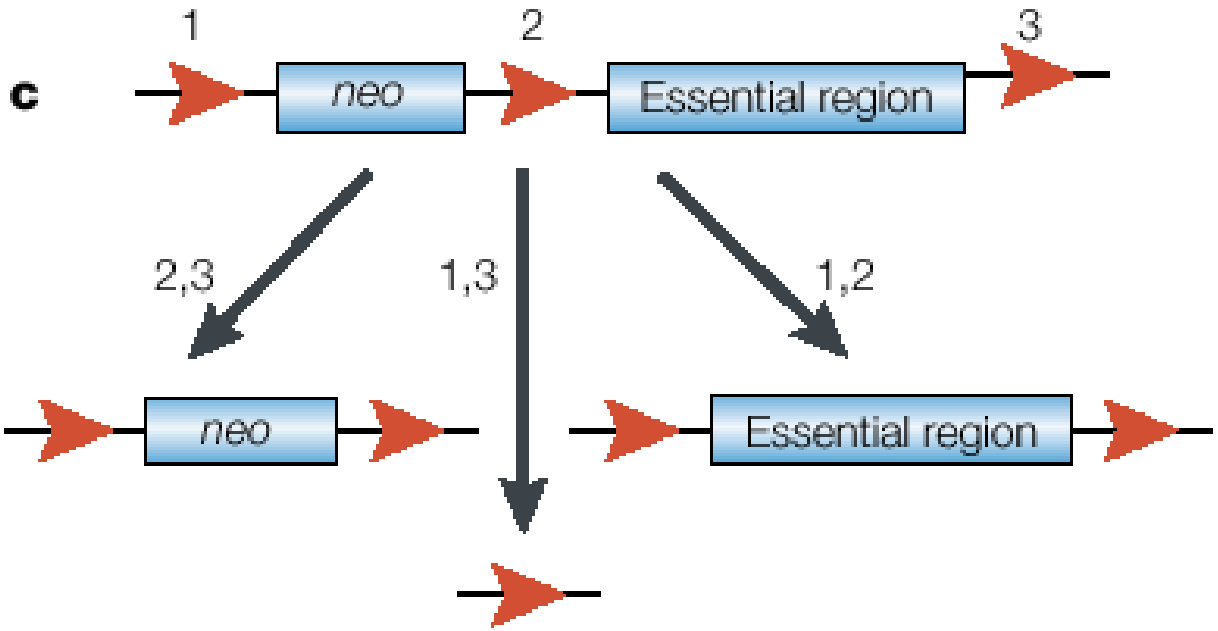


Normal | Gbx2$^{null/null}$ | Gbx2$^{neo/neo}$



Waters and Lewandoski (2006) Development 133:1991

Removal of neo *in vivo* (strategy with 3 loxP sites)



Useful mice and strategy described in
Holzenberger et al (2000) Nucleic Acids Res 28:E92.
Xu et al (2001) Genesis 30:1–6

Lewandoski, M. (2001) Nature Reviews Genetic 2:743

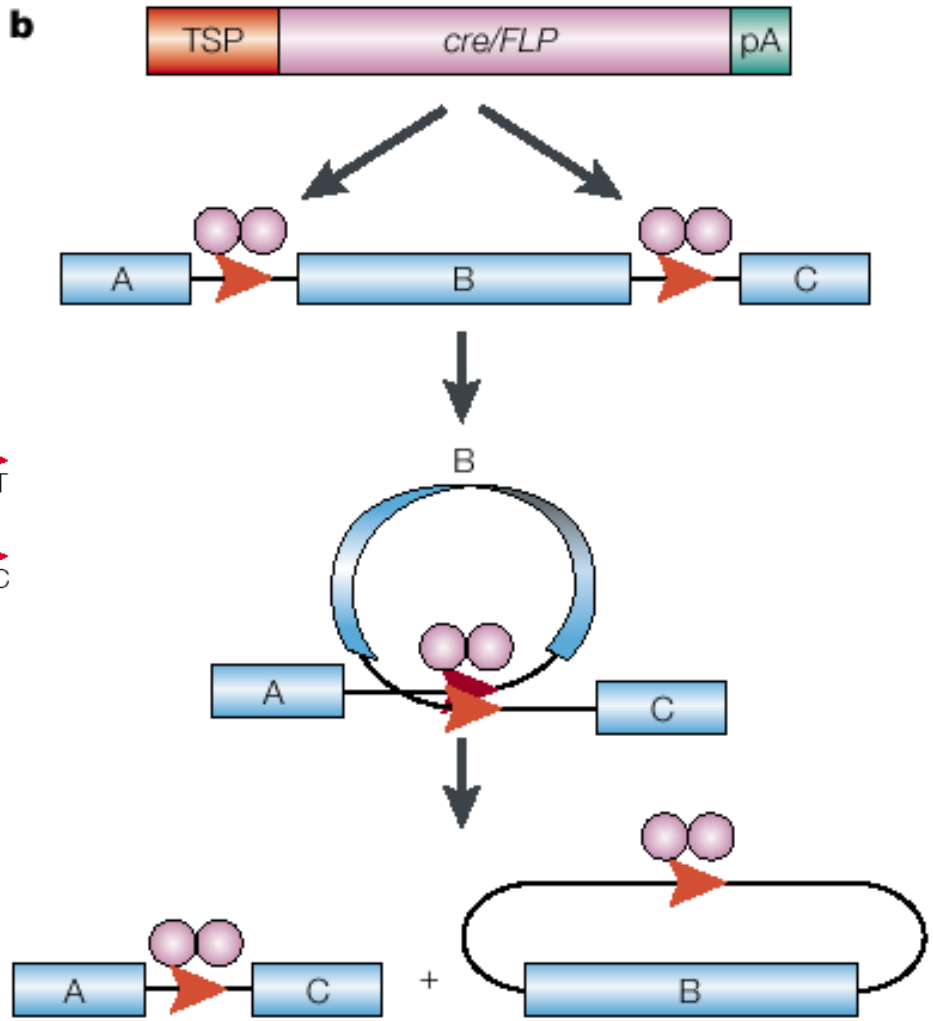
The selection cassette (e.g.neo) may cause a loss-of function allele such as a hypomorph…

And thus may interfere with conditional KOs:

But hypomorhs also call tell you something unique about your system.

So: eat your cake and have it too.

Site-specific recombination: Cre/FLP

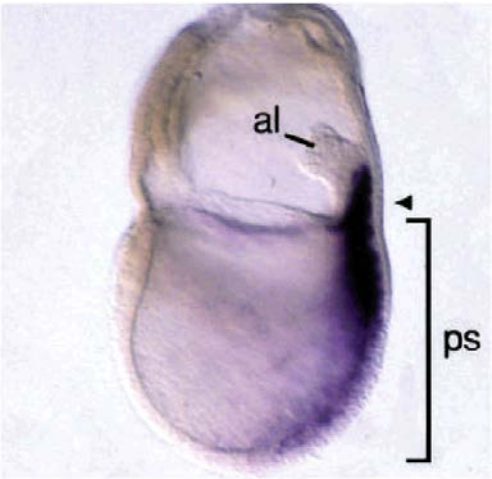


Lewandoski, M. (2001) Nature Reviews Genetic 2:743

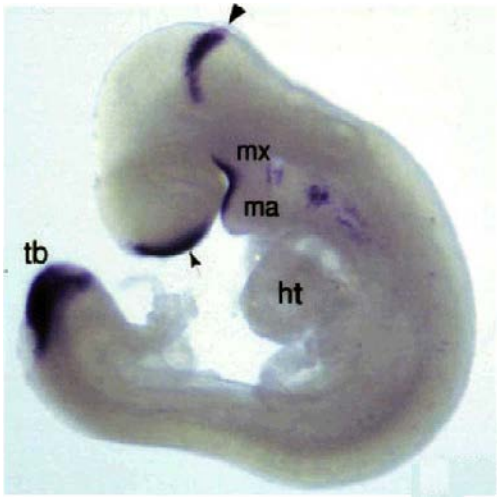
Allelogenic strategy: generating multiple alleles from one targeting event

Fgf8 is expressed in a dynamic pattern during embryogenesis

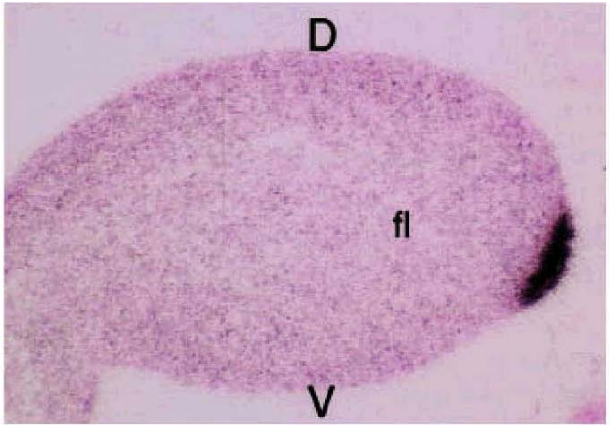
gastrulation

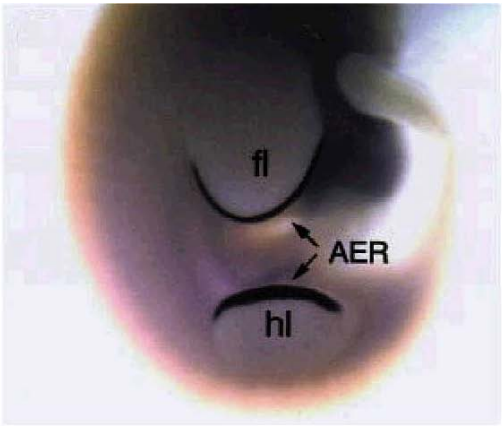


brain

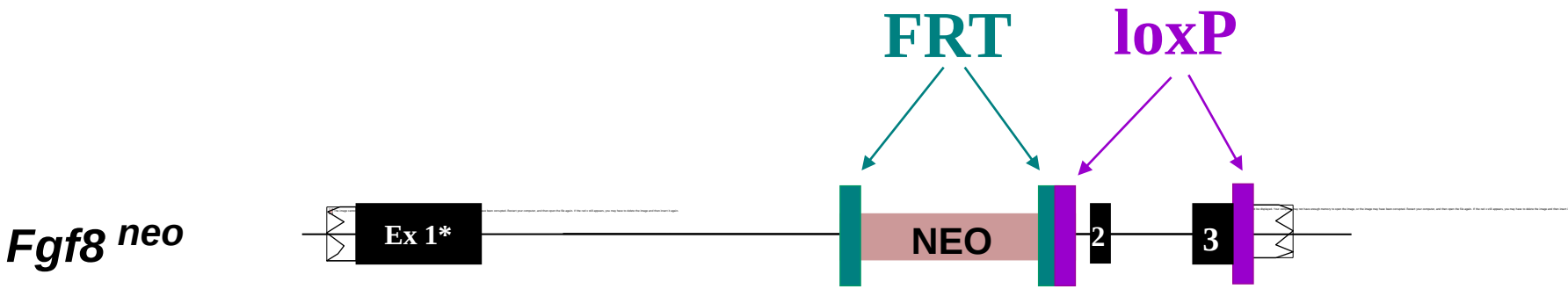


limb

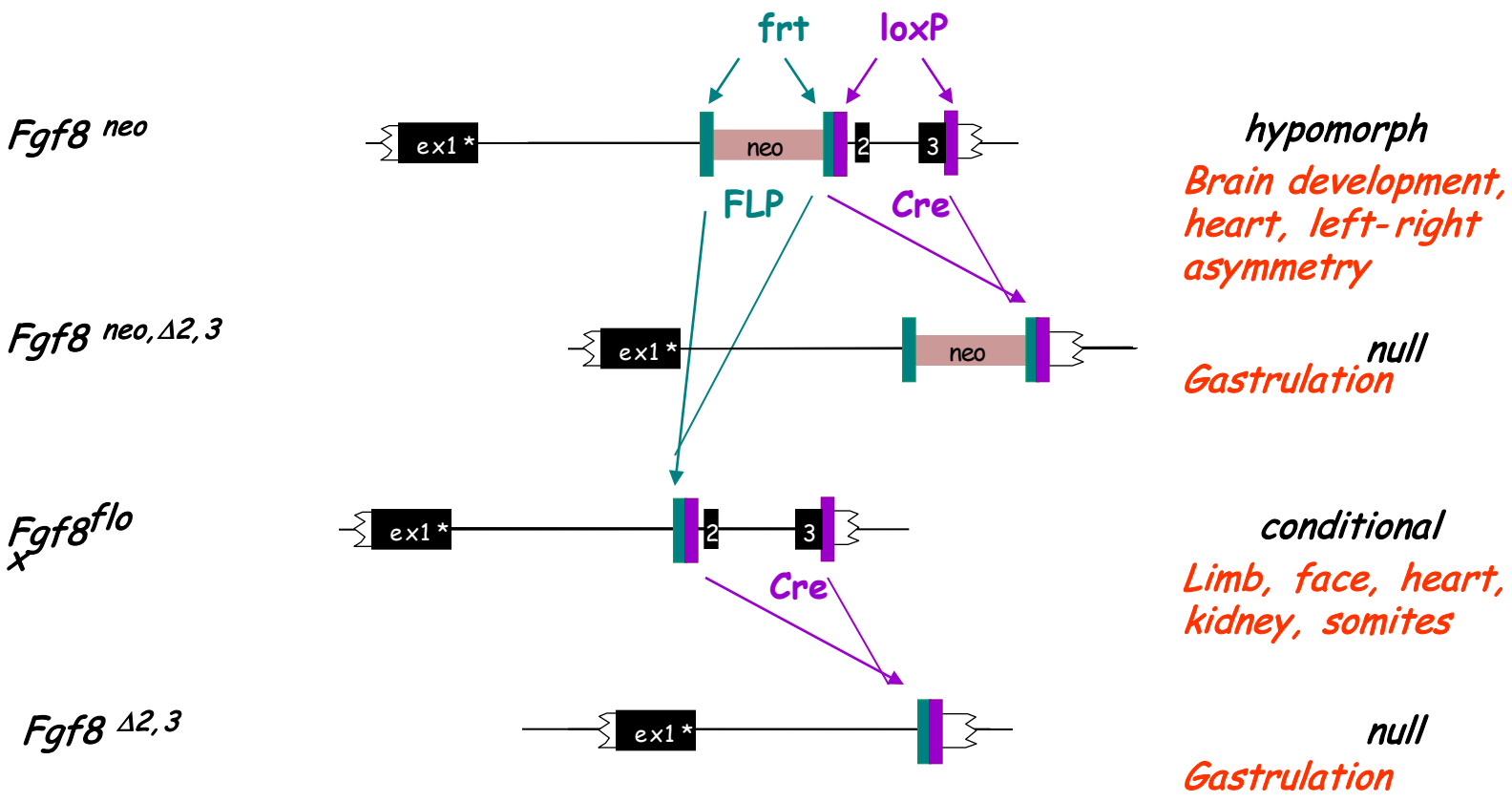




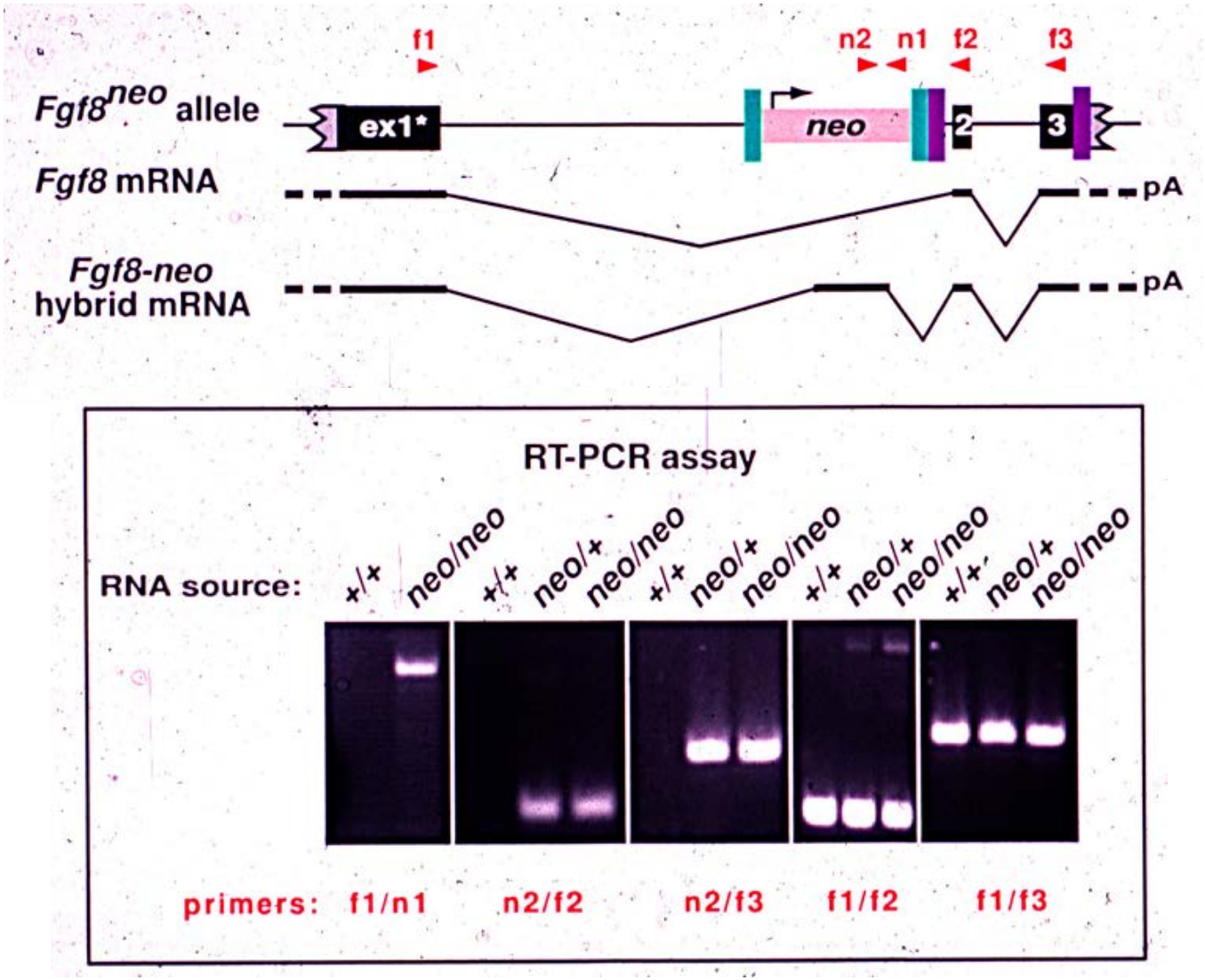
Flox an essential region of Fgf8 (exons 2 and 3) and “Flrt” neo



Allelogenic Strategy:
An allelic series generated from one targeted mouse line



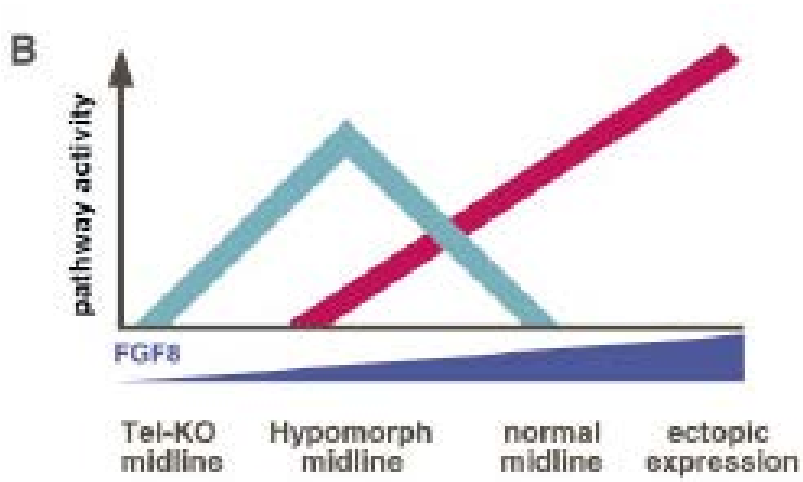
The neo cassette interferes with splicing in the *Fgf8* hypomorph allele



Meyers et al (1998) Nature Genetics 18:136

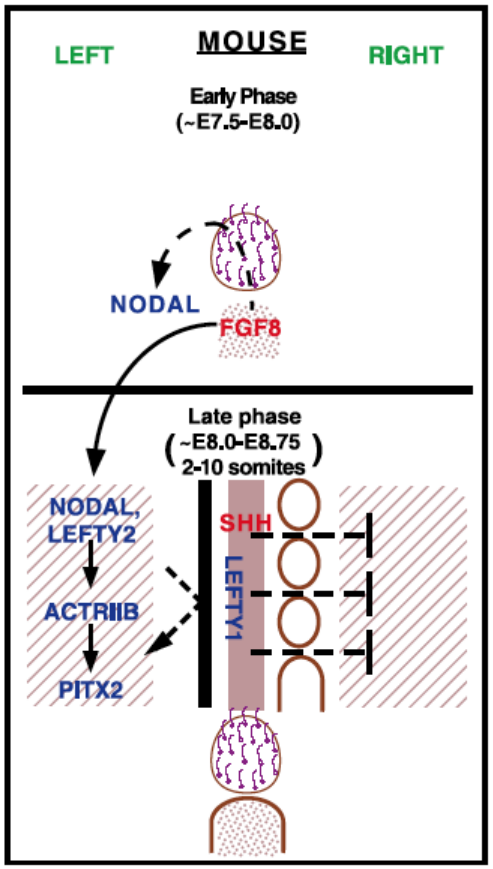
Fgf8 hypomorhic allele has been important in studies of…

…brain development



Storm et al (2003) Dosage of Fgf8 determines whether cell survival is positively or negatively regulated in the developing forebrain PNAS. 100:1757.

…left/right axis determination



Meyers EN, Martin GR (1999) Differences in left-right axis pathways in mouse and chick: functions of FGF8 and SHH. Science. 285:403-6.

The neo casette can interfere with expression in any orientation or location

Table 3 | **Loss of target-gene function due to *neo*^{R} cassette**

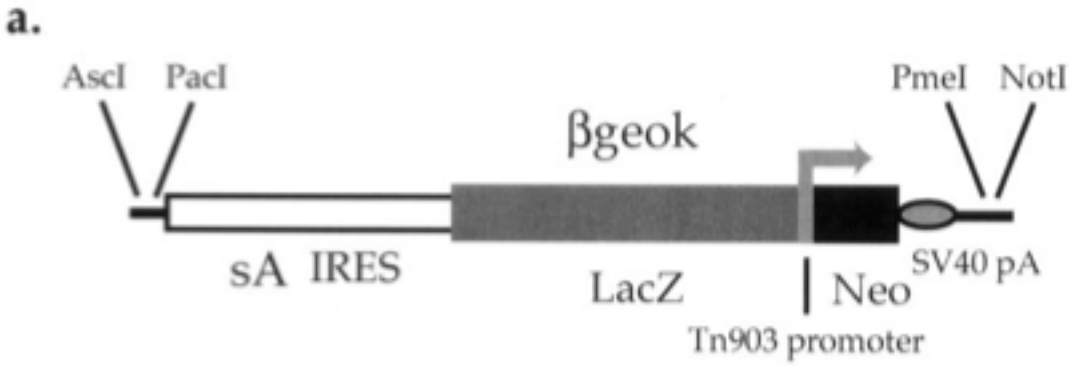
Floxed gene	Location and orientation* of *neo*^{R}	Reference
Fgf8	Intron; same orientation	57
N-Myc	Intron; same orientation	58
Fgfr1	Intron; same orientation	61
Gck	Intron; same orientation	55
Pitx2	Intron; same orientation	62
Pck1	Intron; same orientation	147
Krt1-14	Intron; same orientation	90
Krt1-10	Intron; same orientation	89
Fgfr3	Intron; opposite orientation	148
Hnf4α	Intron; opposite orientation	149
Brca1	Intron; opposite orientation	59
Slc2a4	3' UTR	150
Fgf8	3' UTR	67
Nodal	3' UTR	151
Scap	5' UTR	152
Bcl2l	5' promoter region	153

*Orientation of *neo*^{R} cassette relative to transcription of floxed gene. (*Bcl2l*, B-cell lymphoma; *Brca1*, breast cancer gene 1; *Fgf8*, fibroblast growth factor 8; *Fgfr3*, fibroblast growth factor receptor 3; *Gck*, glucokinase; *Hnf4α*, hepatocyte nuclear factor 4, alpha; *Krt1-x*, keratin complex 1, gene x; *Pck1*, phosphoenolpyruvate carboxykinase; *N-Myc*, neuroblastoma myc-related oncogene; *Pitx2*, pituitary homeobox 2; *ScapP*, Srebp cleavage-activating protein; *Slc2a4*, solute carrier family 2, member 4 (also called *Glut4*); Srebp, sterol regulatory element-binding protein; UTR, untranslated region.)

Lewandoski, M. (2001) Nat Rev Genetics 2:743

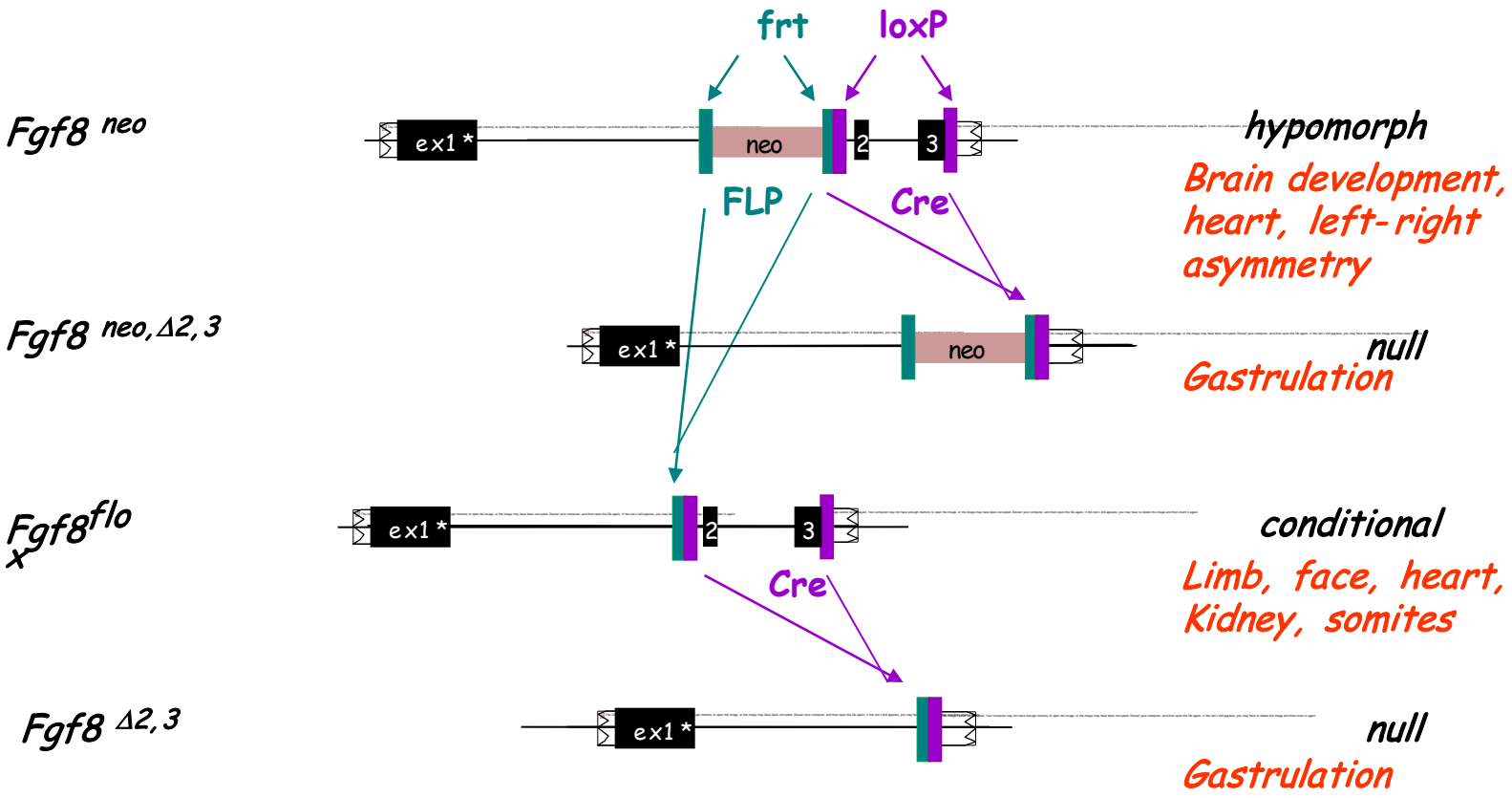
Will the selection cassette (e.g. neo) always interfere with gene expression?

No…but cassettes exist to ensure that it will:

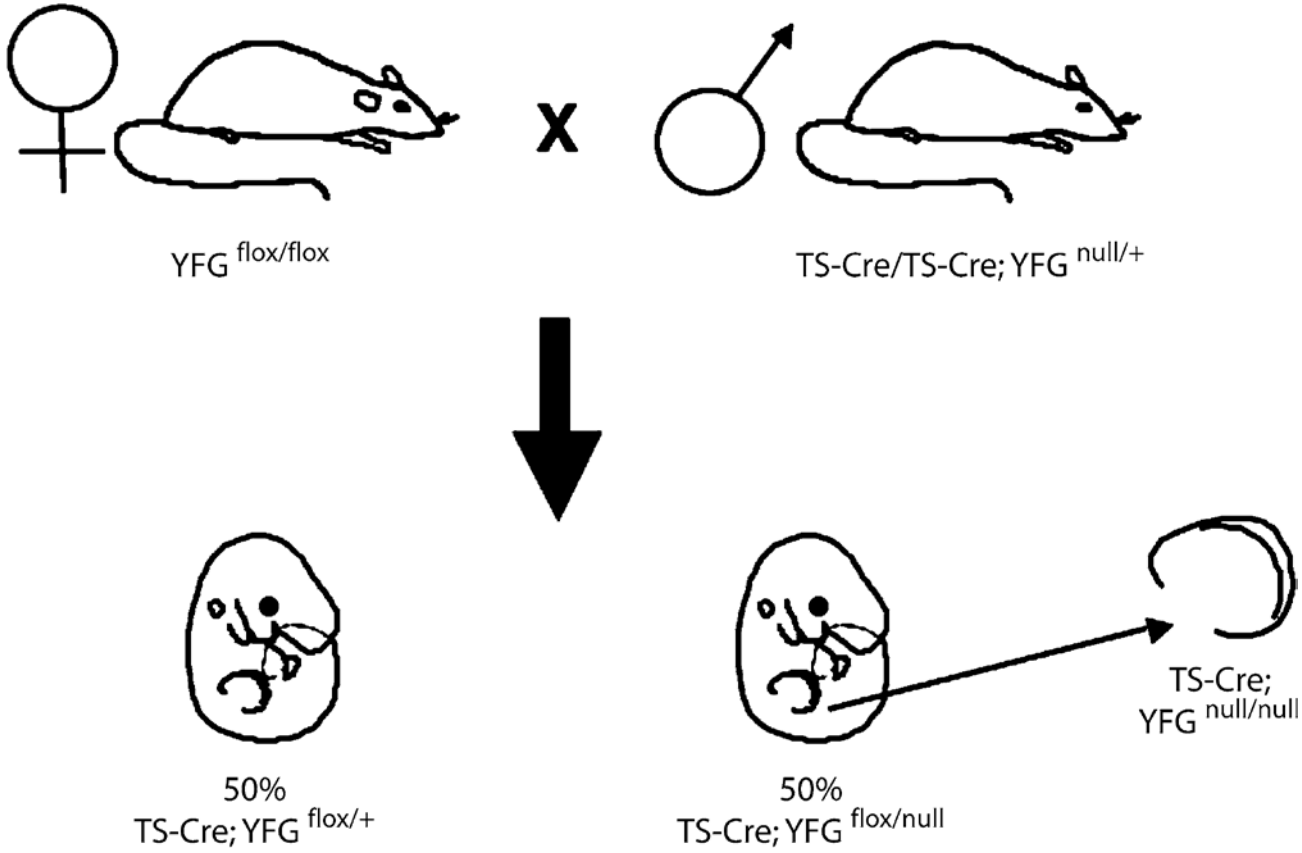


A Reliable lacZ Expression Reporter Cassette for Multipurpose, Knockout-First Allele (2004) Testa et al Genesis 38: 151

Allelogenic Strategy:
An allelic series generated from one targeted mouse line



Best cross for generation of conditional mutants

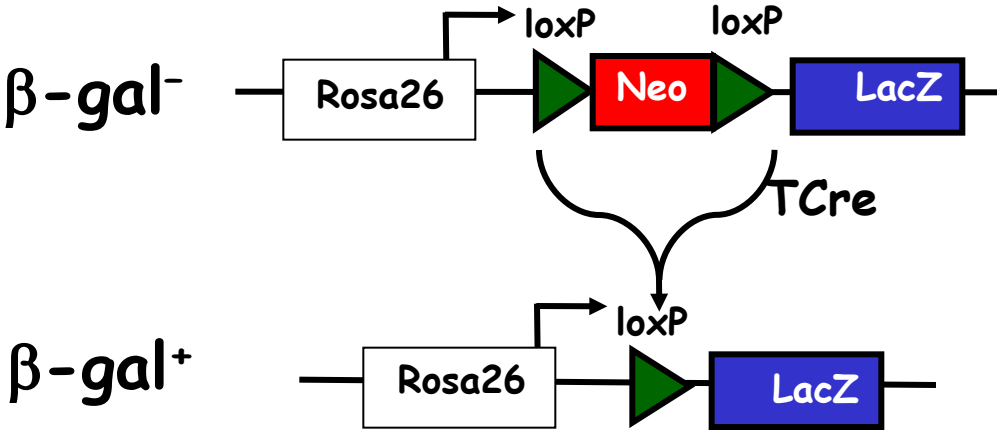


Lewandoski, M. (2007) Handb Exp Pharmacol 178:235

1) Simplest mouse husbandry with 50% mutants and controls.
2) Cre parent is male; avoids complications due to potential germline activity in the Cre+ female (more on this later).

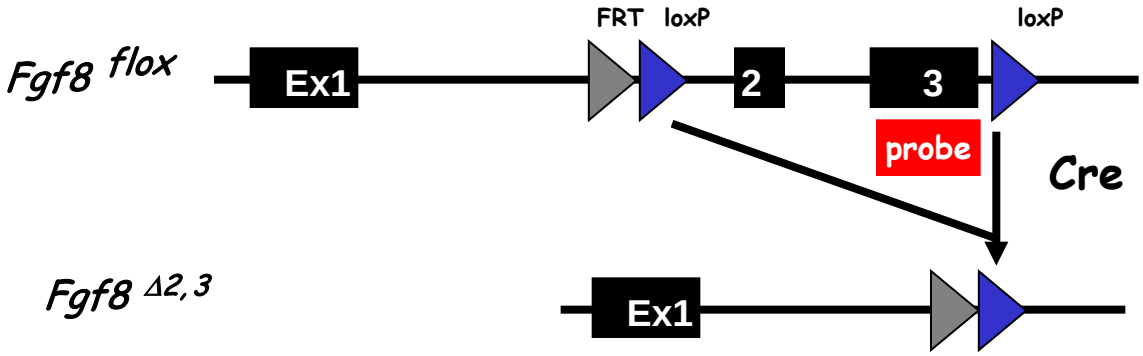
"TS" = Tissue-specific; "YGF" = Your favorite gene.

TCre recombination occurs in the emerging early mesoderm



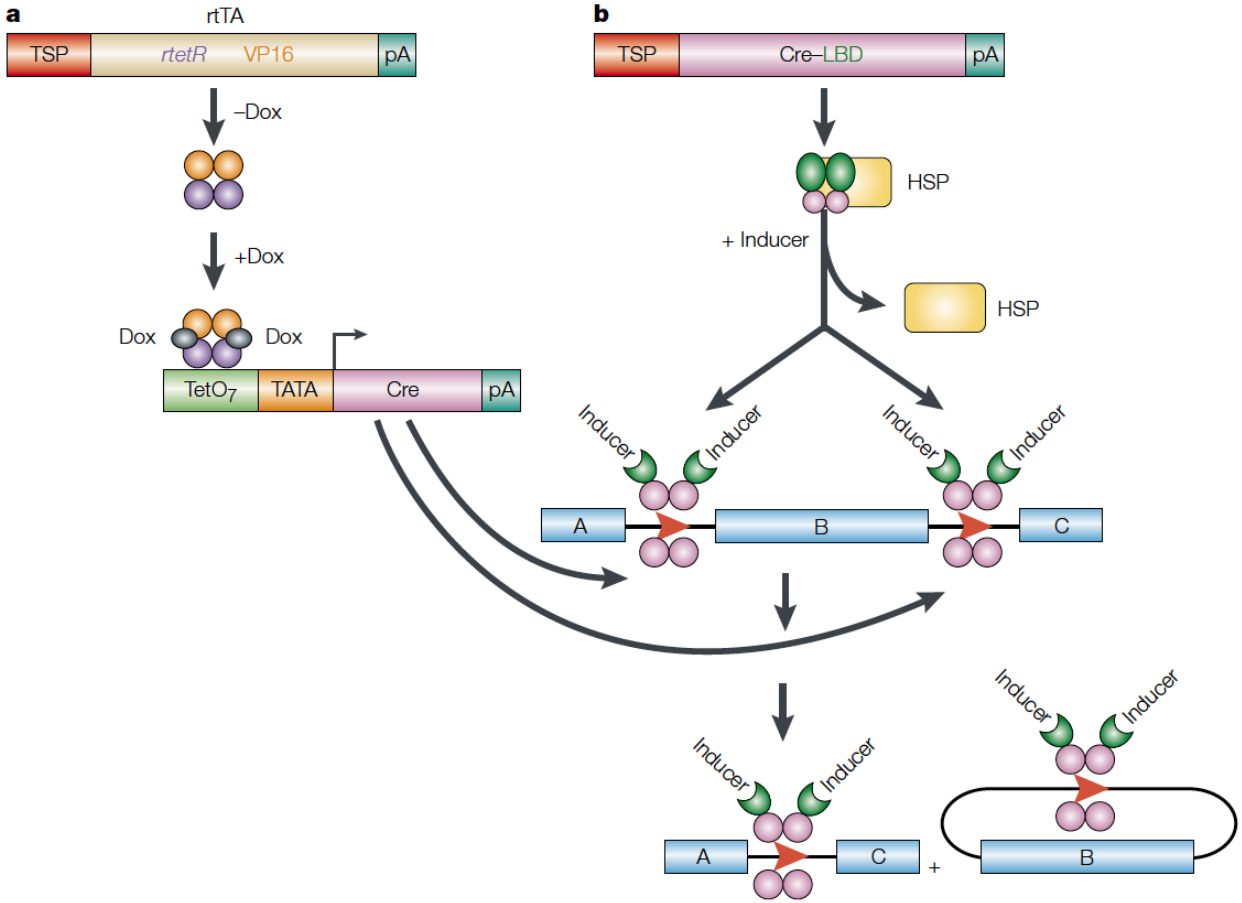
Perantoni et al Development (2005)

TCre-mediated inactivation of *Fgf8*



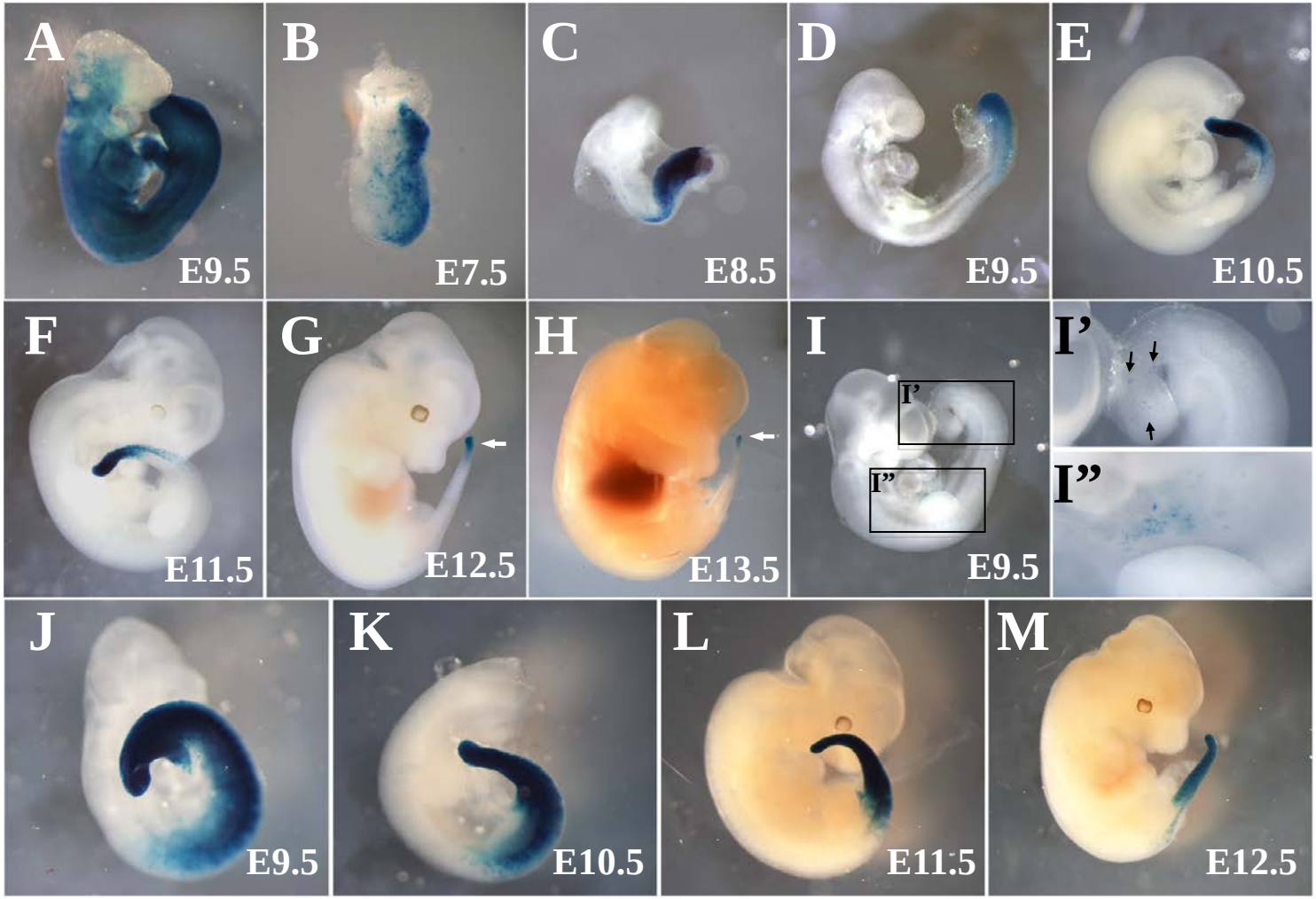
Perantoni et al., Development (2005)

Inducible Cre Systems



Lewandoski, M. (2001) Nature Reviews Genetics 2: 743

A TAM-inducible TCre line



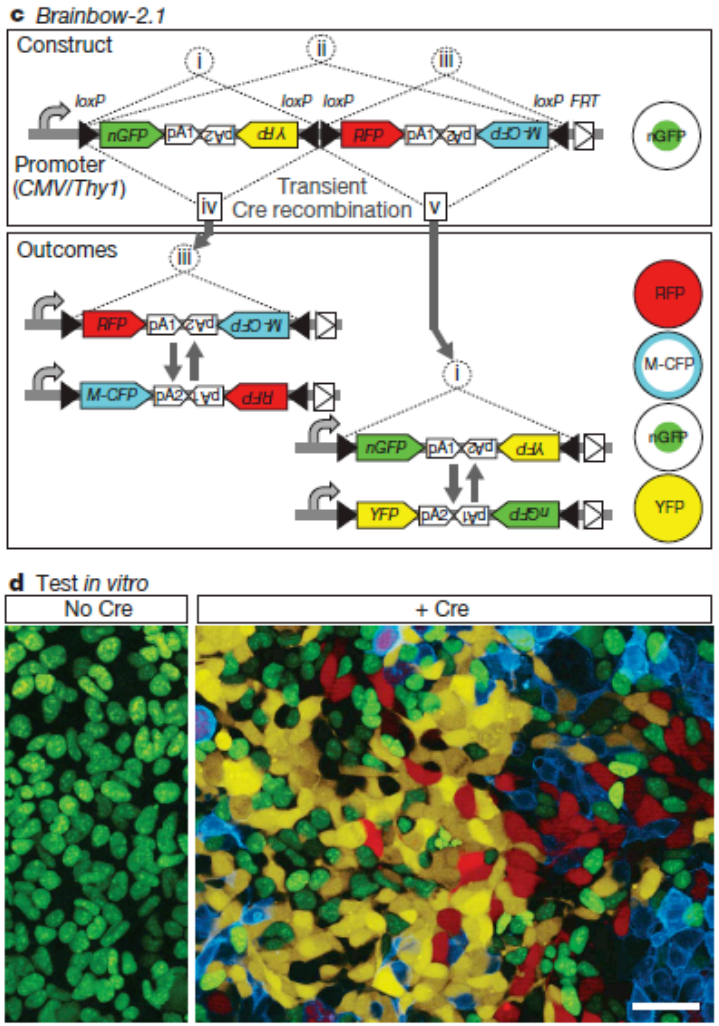
Other available Cre reporters

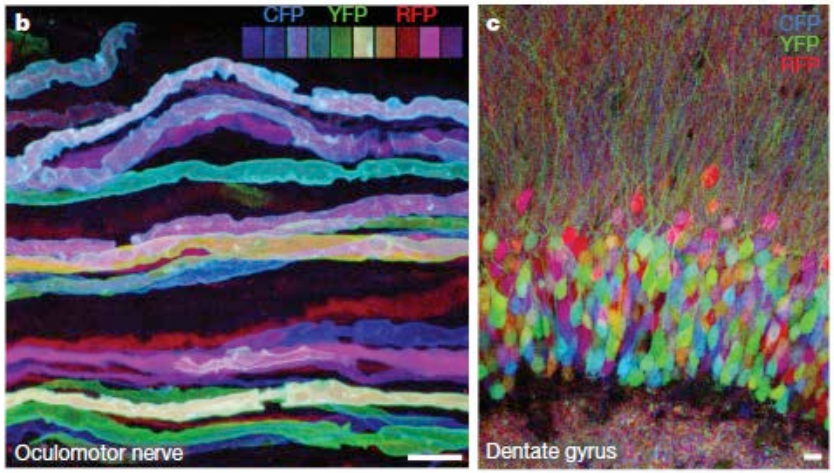
Alkaline phosphatase (Z/AP)
β-galactosidase (R26R)
Enhanced green fluorescent protein
Enhanced yellow fluorescent protein
Enhanced cyan fluorescent protein
luciferase

For details see

Lewandoski, M. (2001) Nat Rev Genetics 2:743
Lewandoski, M. (2007) Handb Exp Pharmacol 178:235
Muzumdar et al (2007) genesis 45:593
Woolfenden, S (2009) Genesis 47: 659
Dymecki SM, et al (2010) Methods Enzymol 477:183

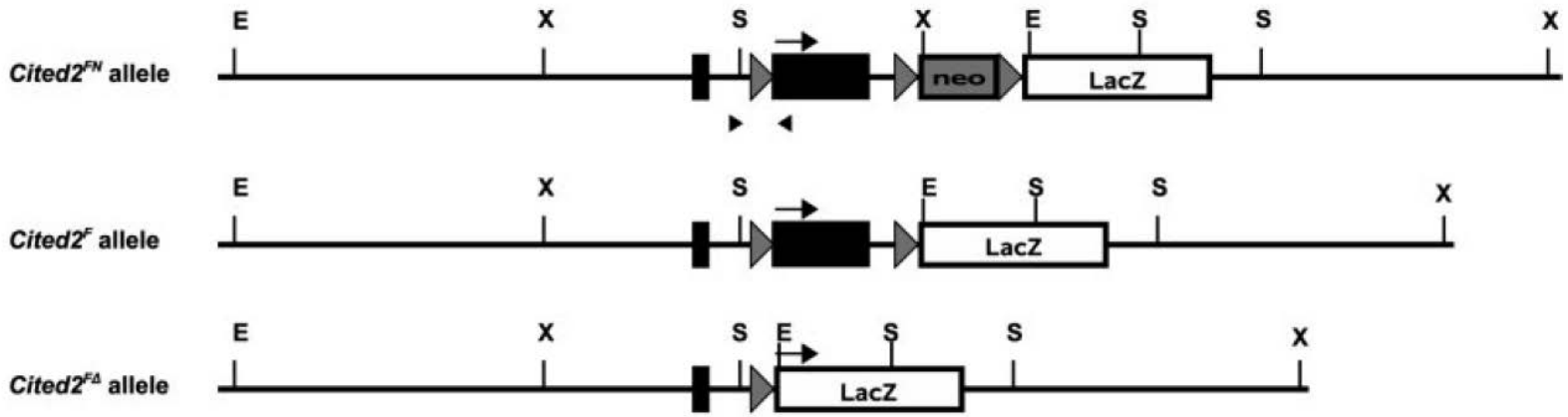
“Brainbow”

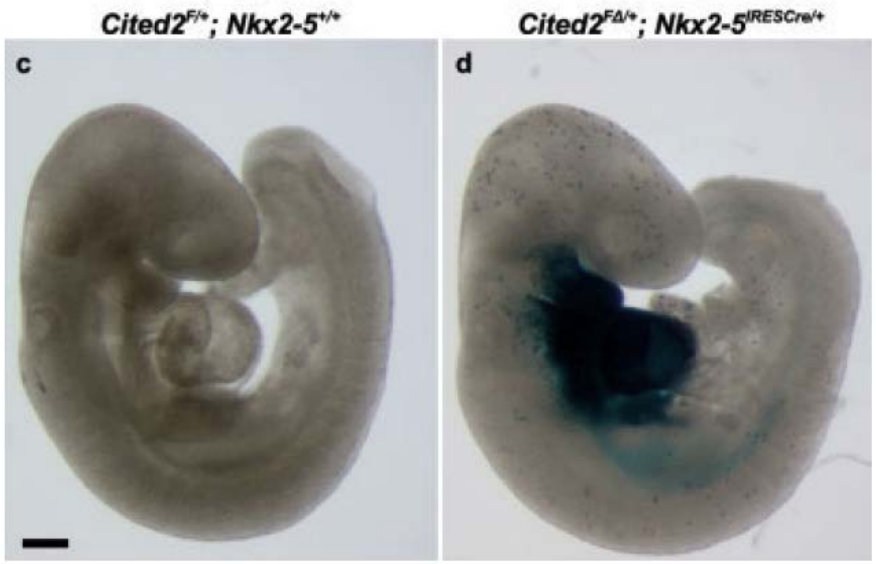




Transgenic strategies for combinational expression of fluorescent proteins in the nervous system. Livet et al (2007) Nature 450:56

One could design the target allele so recombination BOTH knocks out the gene & activates a reporter... … but the reporter may generate a hypomorph.





Preis et al (2006) Genesis 44: 579

Some advice about making mice carrying Cre and floxed alleles

Some limitations of Cre/loxP approaches

1) Not all floxed regions in the genome are equally recombinable

Vooijs, M.et al. A highly efficient ligand-regulated Cre recombinase mouse line shows that LoxP recombination is position dependent. EMBO Rep. 2 :292 (2001).

2) High Cre activity can be detrimental

Schmidt, E. E. et al. Illegitimate Cre-dependent chromosome rearrangements in transgenic mouse spermatids. Proc. Natl Acad. Sci. USA 97, 13702 (2000).

Loonstra, A. et al. Growth inhibition and DNA damage induced by Cre recombinase in mammalian cells. Proc.Natl Acad. Sci. USA 98, 9209–9214 (2001).

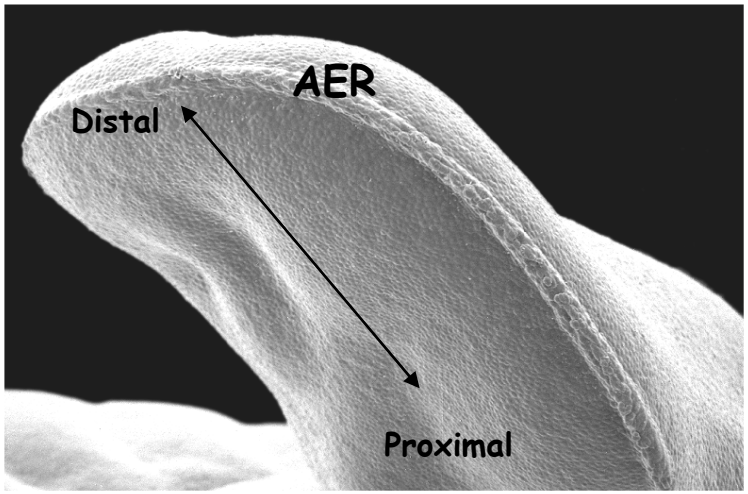
Silver, D. P. & Livingston, D. M. Self-excising retroviral vectors encoding the Cre recombinase overcome the Cre-mediated cellular toxicity. Molecular Cell 8, 233-243 (2001).

Pfieffer A. et al. Delivery of the Cre recombinase by a self-deleting lentiviral vector: efficient gene targeting *in vivo* Proc.Natl Acad. Sci. USA 98, 11450–11455 (2001).

Naiche, L.A. & Papaioannou. V.E. Cre activity causes widespread apoptosis and lethal anemia during embryonic development Genesis 45, 768 (2007)

Carefully characterize a new Cre line…

… because different Cre lines with similar but non-identical activity may be useful in quickly changing biological contexts



Earlier AER-specific inactivation of Bmpr1a:
"BMPs required for AER formation"

Ahn et al (2001□□) Development 128: 4419

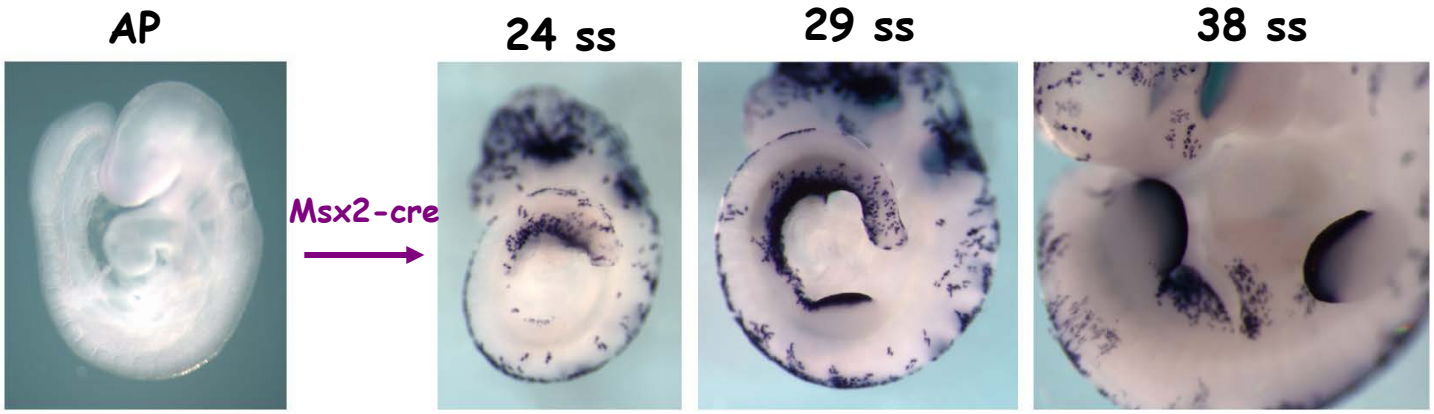
Latter AER-specific inactivation of Bmpr1a:
"BMPs required for AER loss"

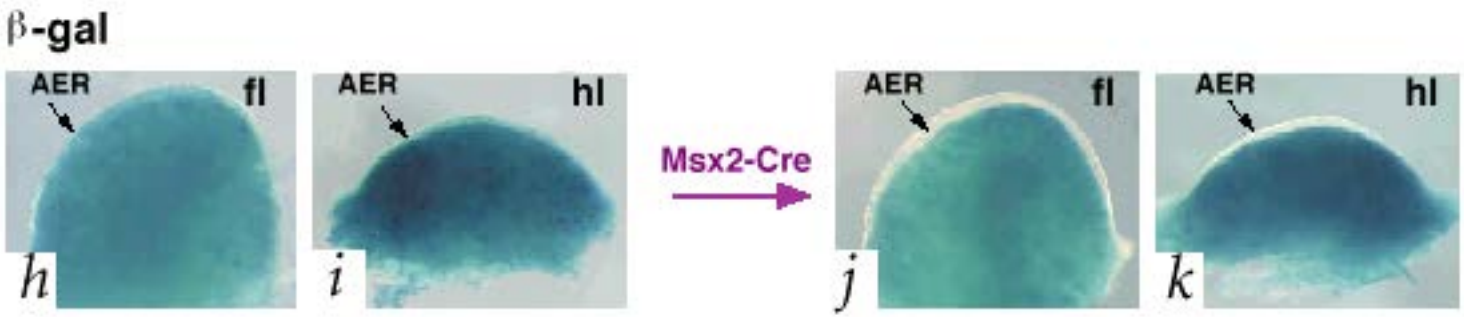
Pajni-Underwood et al (2007) Development 134: 2359

Carefully characterize a new Cre line…

… because unexpected activity may open doors to collaborations

Msx2-cre is one of 13 published lines expressed in the limb bud, not all intentionally generated for that purpose.

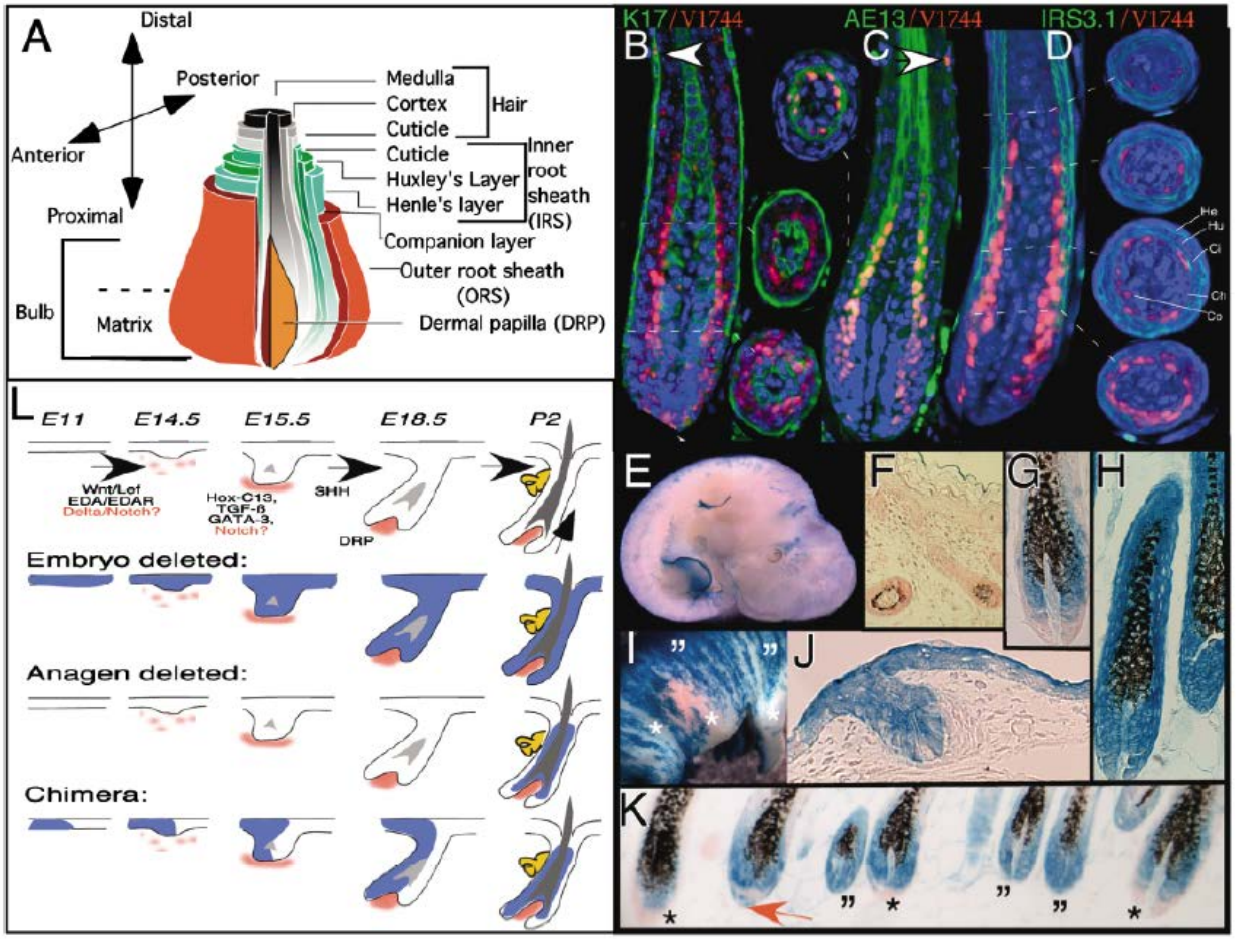




Carefully characterize a new Cre line…

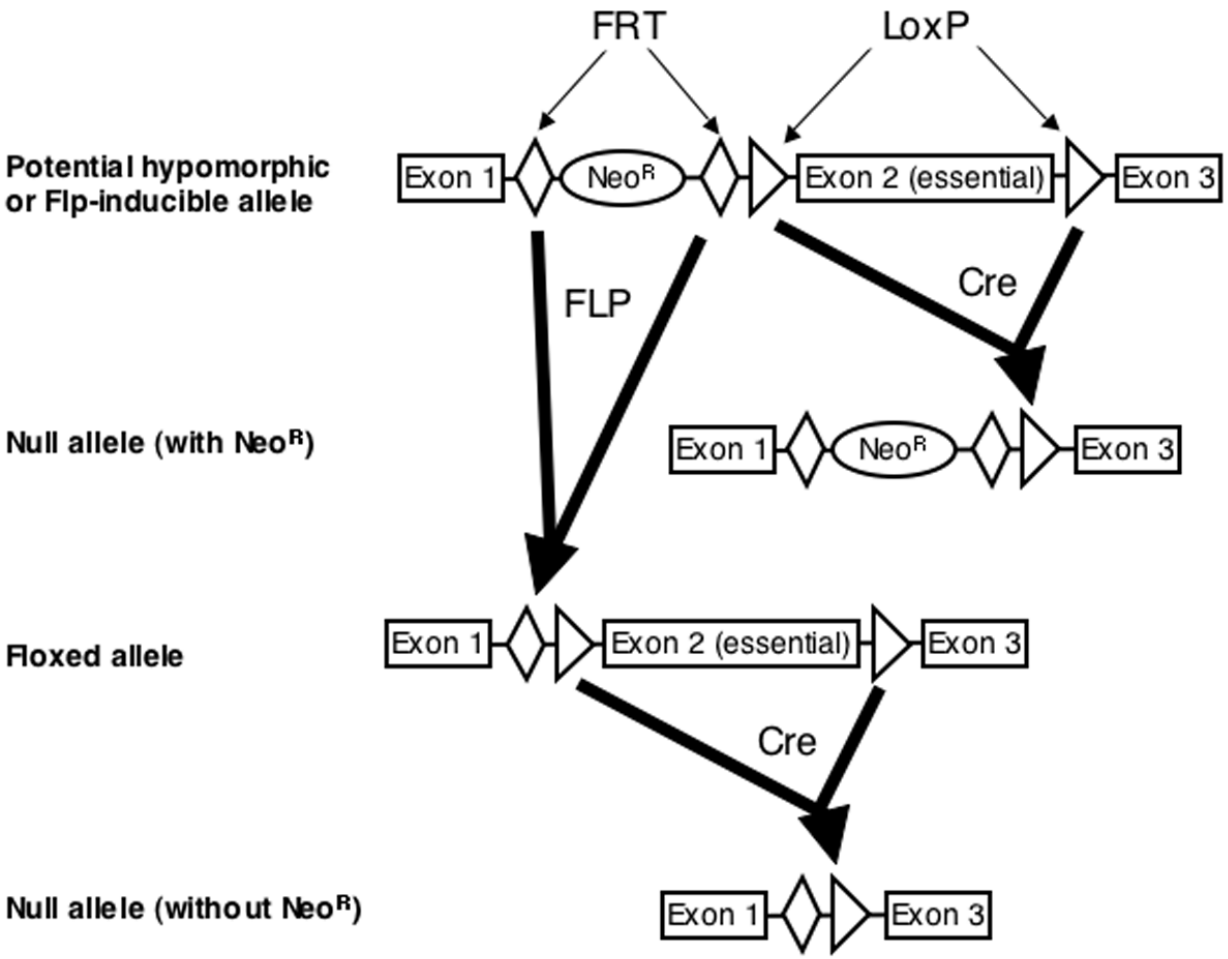
… because unexpected activity may open doors to collaborations

Msx2-cre is active in the hair follicle.



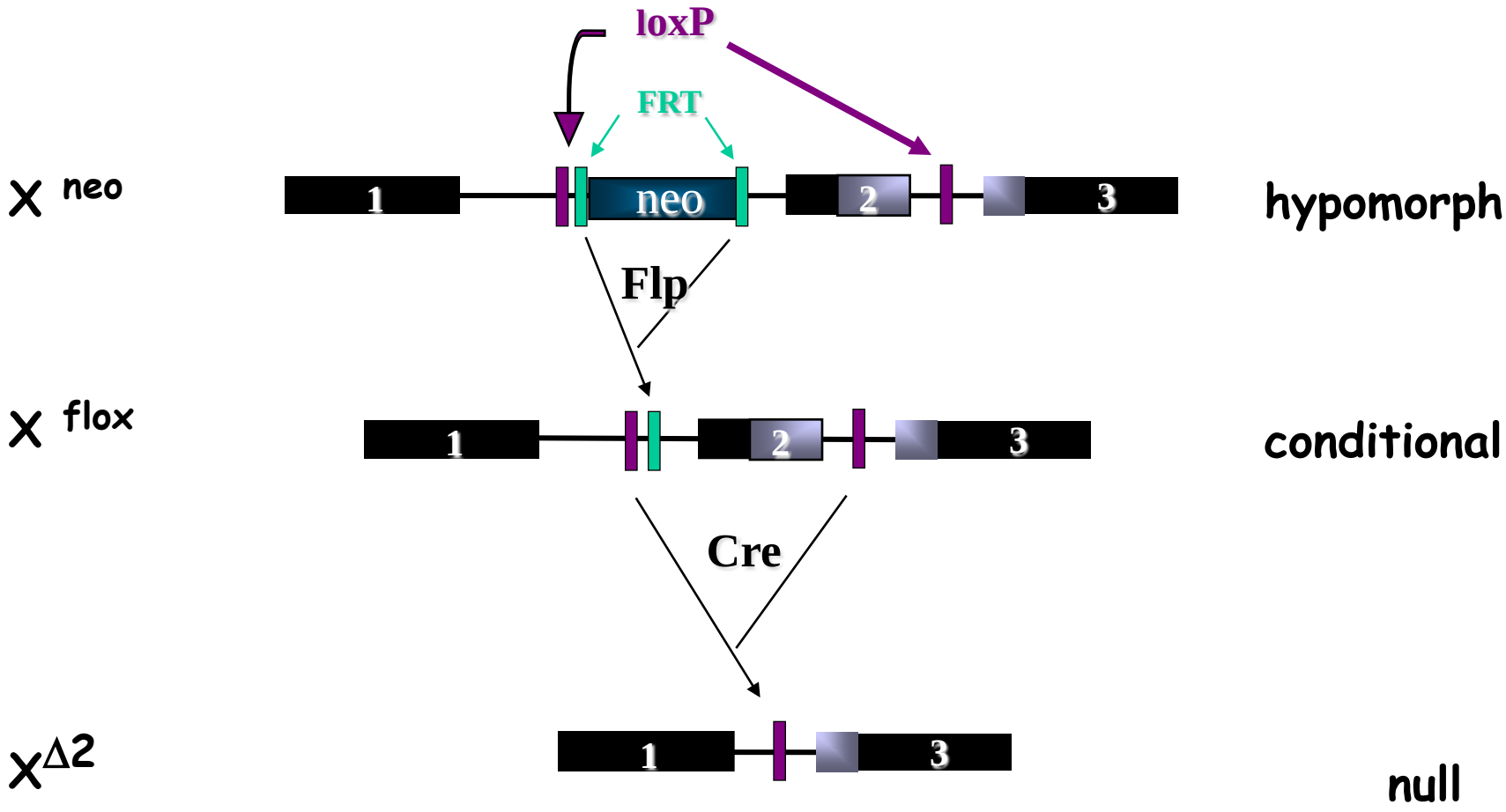
Pan et al (2004□□) Dev Cell 7: 731

Allelogenic strategy



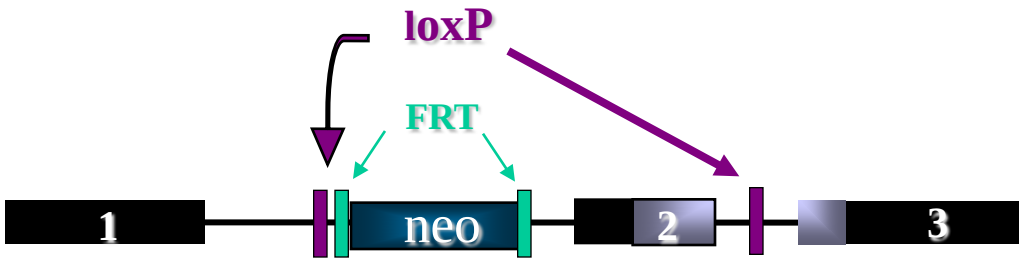
Lewandoski, M. (2007) Handb Exp Pharmacol 178:235

Placement of flrted neo between loxP sites limits your options

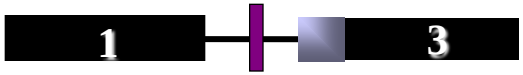


Placement of flrted neo between loxP sites limits your options

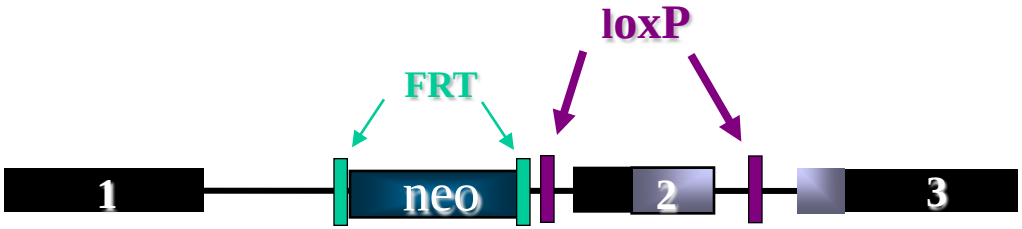
Not preferred



Null allele



Preferred



Null (neo) allele

Null allele

x

BAC(wt); $YFG^{null/+}$ $YFG^{null/+}$ or $YFG^{nullneo/+}$

Genotype progeny with normal phenotype

BAC(wt); $YFG^{null/null}$

$YFG^{+/+}$

$YFG^{null/+}$

No (non-quantitative) PCR can distinguish between these three genotypes

BAC(wt); $YFG^{nullneo/null}$

$YFG^{+/+}$

$YFG^{null/+}$

$YFG^{nullneo/+}$

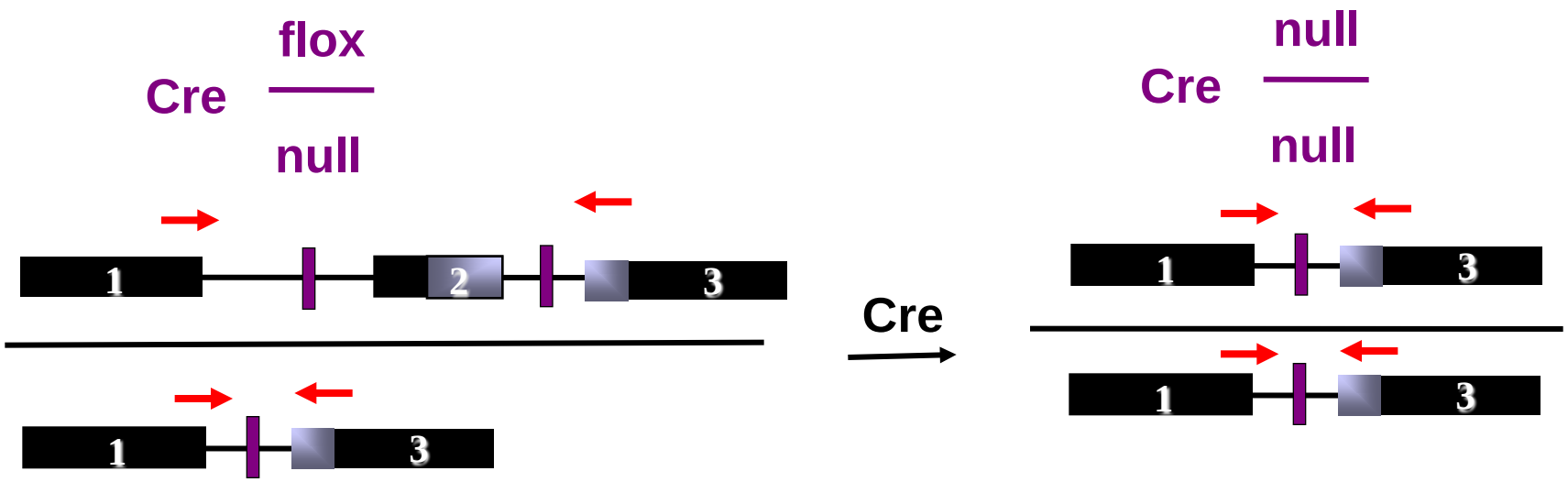
PCR can easily distinguish between these four genotypes

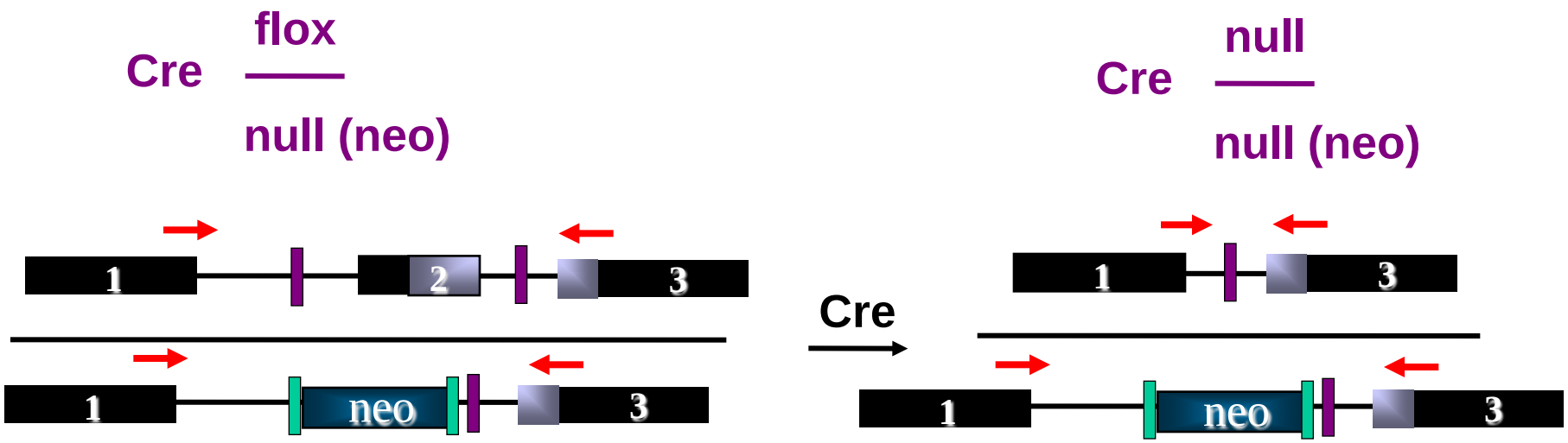
You may need a PCR assay to monitor Cre-mediated recombination

TSPCre; YFG$^{flox/null}$ → TSPCre; YFG$^{null/null}$

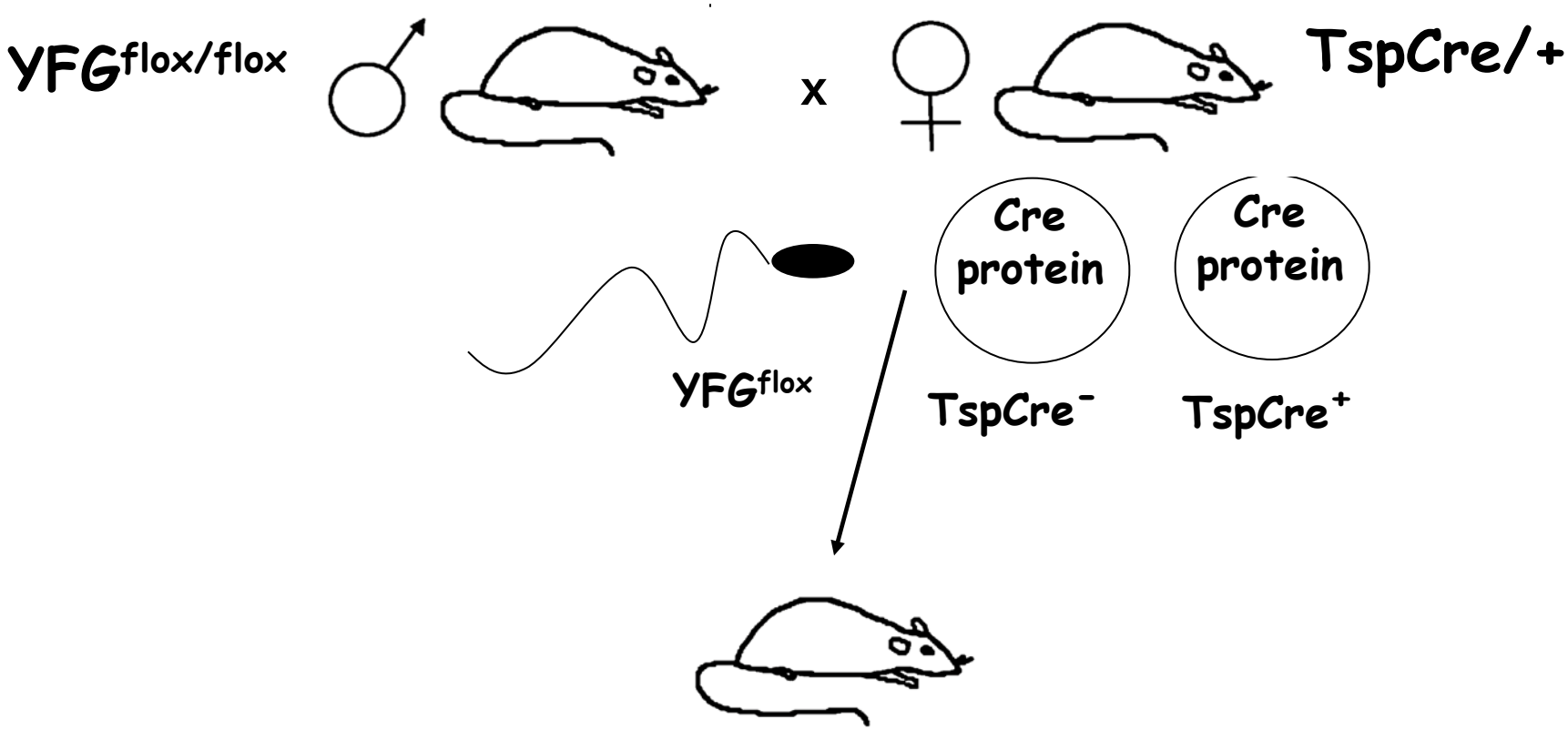
TSPCre; YFG$^{flox/nullneo}$ → TSPCre; YFG$^{null/nullneo}$

Placement of flrted neo between loxP sites limits your options



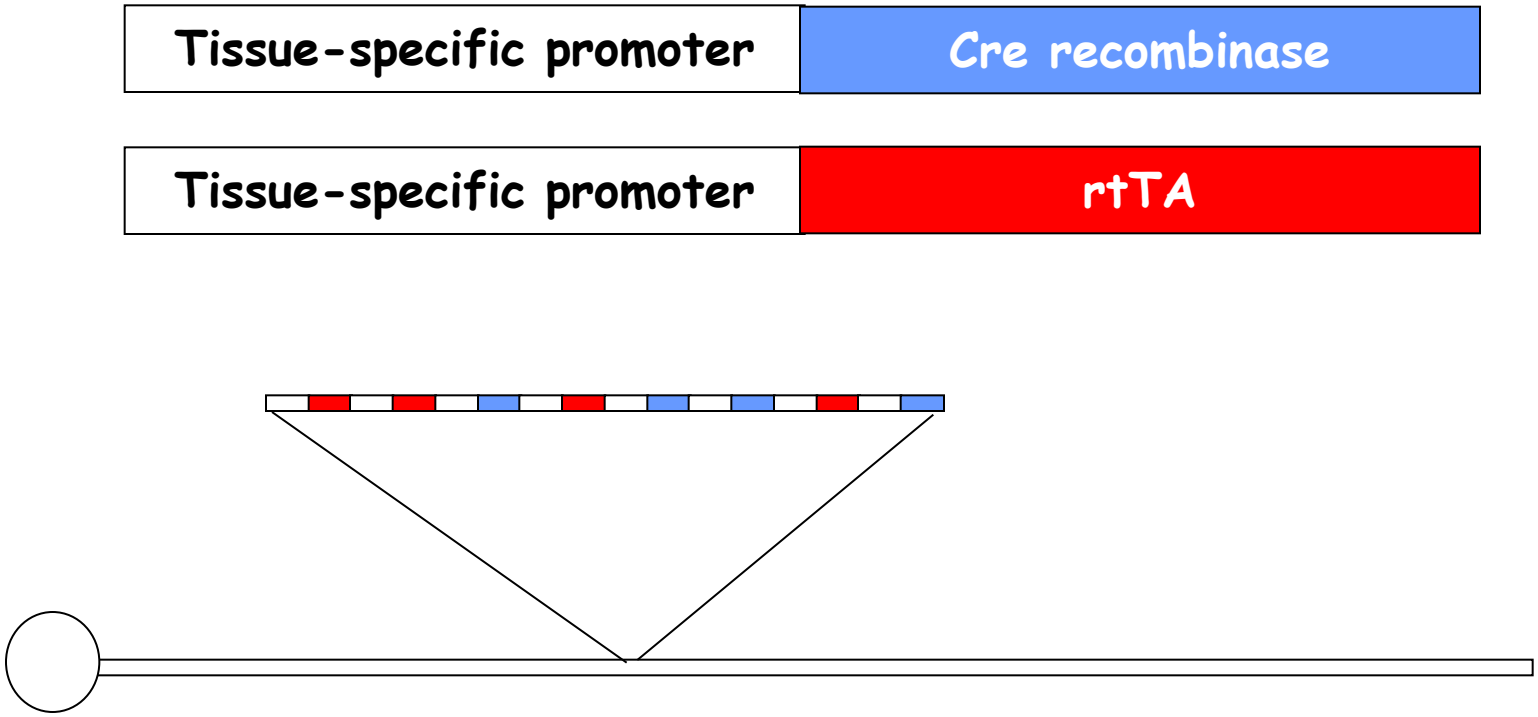


Test your Cre line through *paternal* not *maternal* inhertance of the Cre transgene or allele: because sometime Cre can act as an maternal effect gene as follows:



YFG$^{rec/+}$ (Cre-);
Recombination looks complete & non-specific

Co-injection of two transgenes to generate a dual function trangenic effector line



“Cheating Mendel”

germline-cre ***Flox allele***

Null allele

“Cheating Mendel”

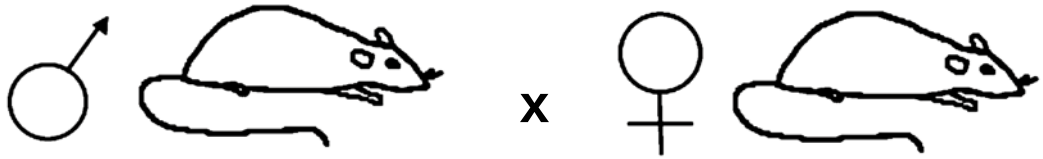
x

→ **50 % homozygous mutants**

germline-cre *Flox allele* / *Null allele*

WT allele / *Null allele*

“Cheating Mendel”

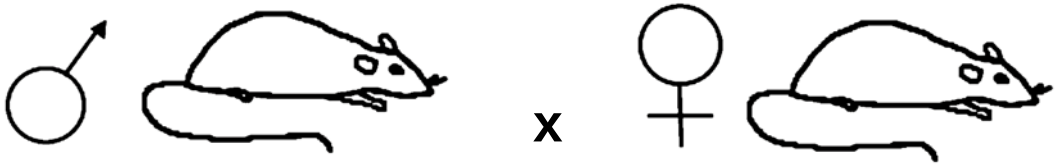


x

germline-cre *Flox allele* / *Null allele*

WT allele / *Null allele*

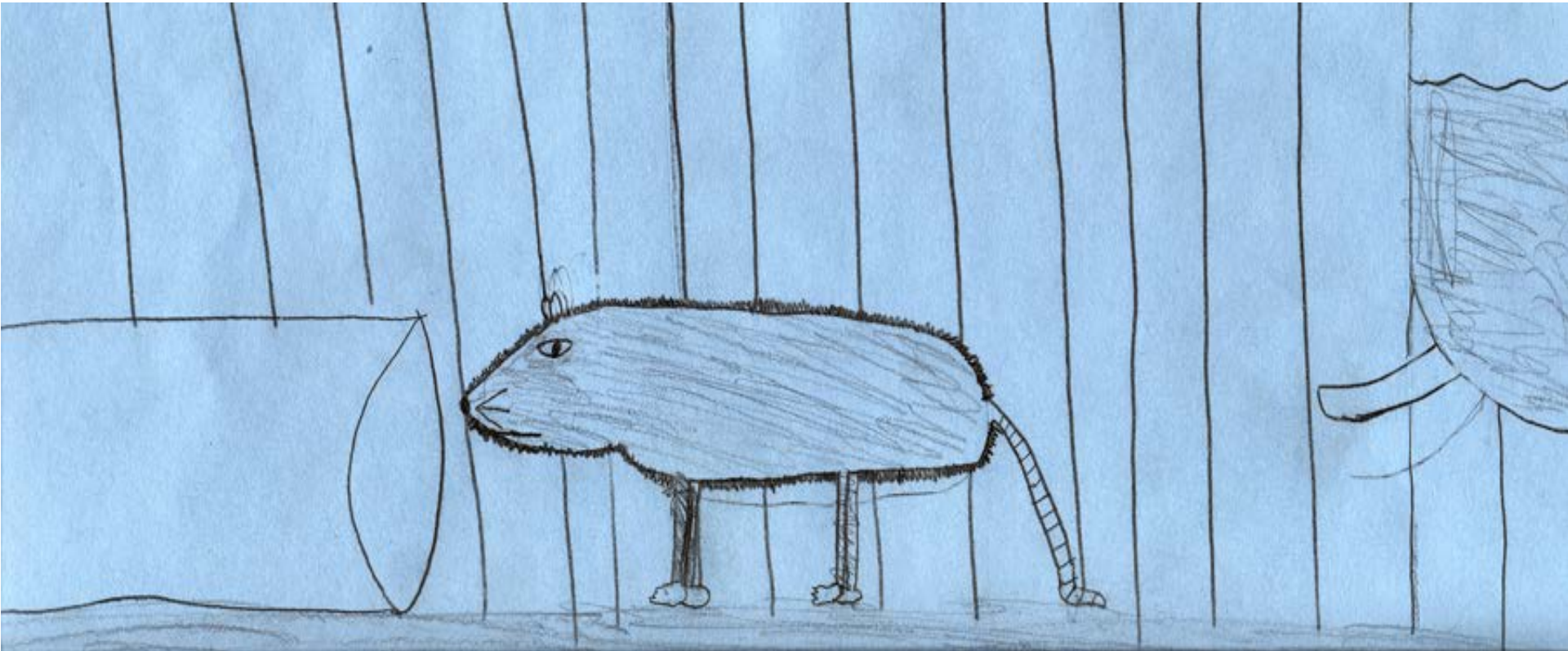
→ **50 % homozygous mutants**



x

germline-cre *Flox allele* / *Null allele*

germline-cre *Flox allele* / *Null allele*

→ **100 % homozygous mutants**

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Lewandom@mail.nih.gov