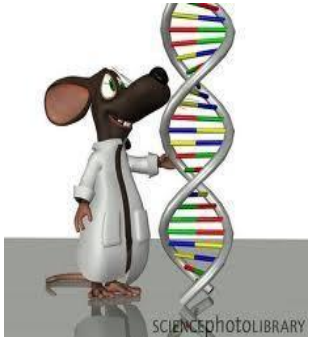

Conditional and induced GM models

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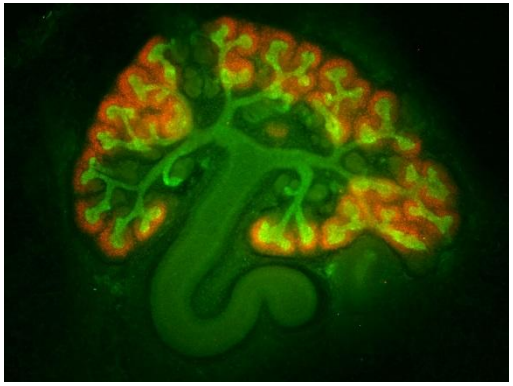
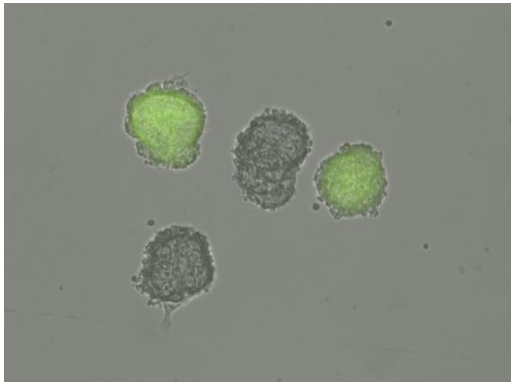




Conditional / Tissue-Specific mutagenesis

- Problem with conventional knock-out mice – the gene of interest is inactivated in ALL cells and tissues

- Might lead to embryonic lethality

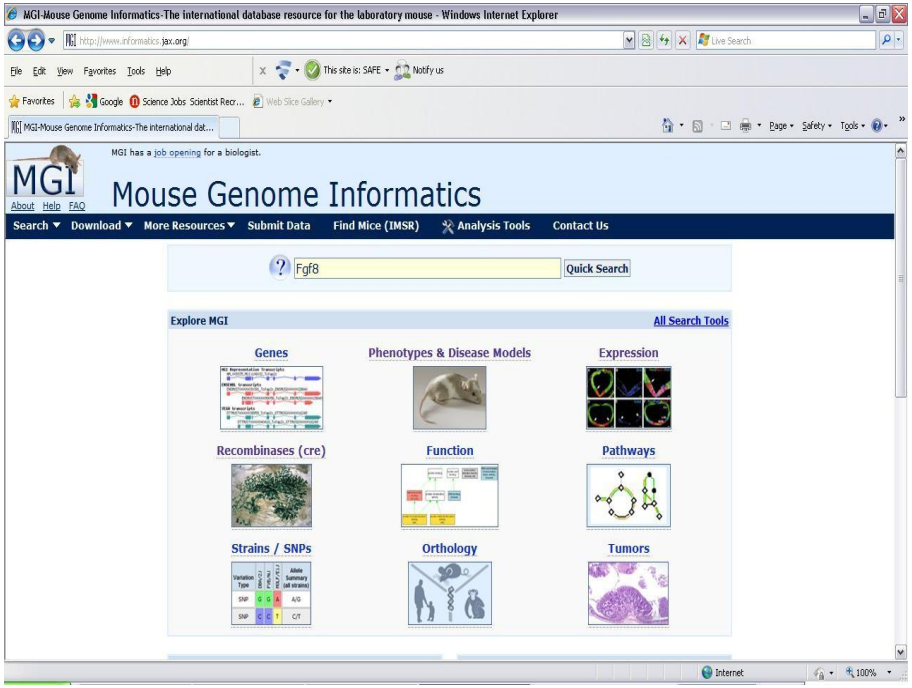
- Many secondary effects may mask the primary effects- difficult to make any conclusions about the gene function and mechanisms of action

Spatial and Temporal Control of Gene Expression;
Conditional and Inducible Systems

Cre-*lox* technology
Flp-*frt* technology

– a site-specific recombinase enzymes

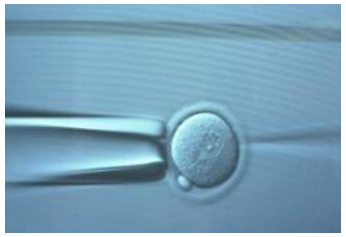
Mouse Genome Informatics;
Existing *floxed/frt* and Cre/Flp mouse models

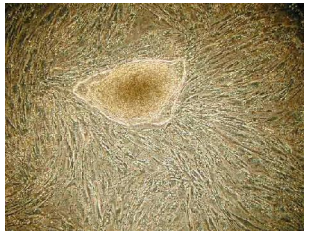


Generation

<u>Cre/Flp lines:</u>
Transgenic or targeted

<u>*Floxed/frt* lines</u>
Targeted, traditional or modern





Cre-*lox* technology

A simple two component system

Cre recombinase

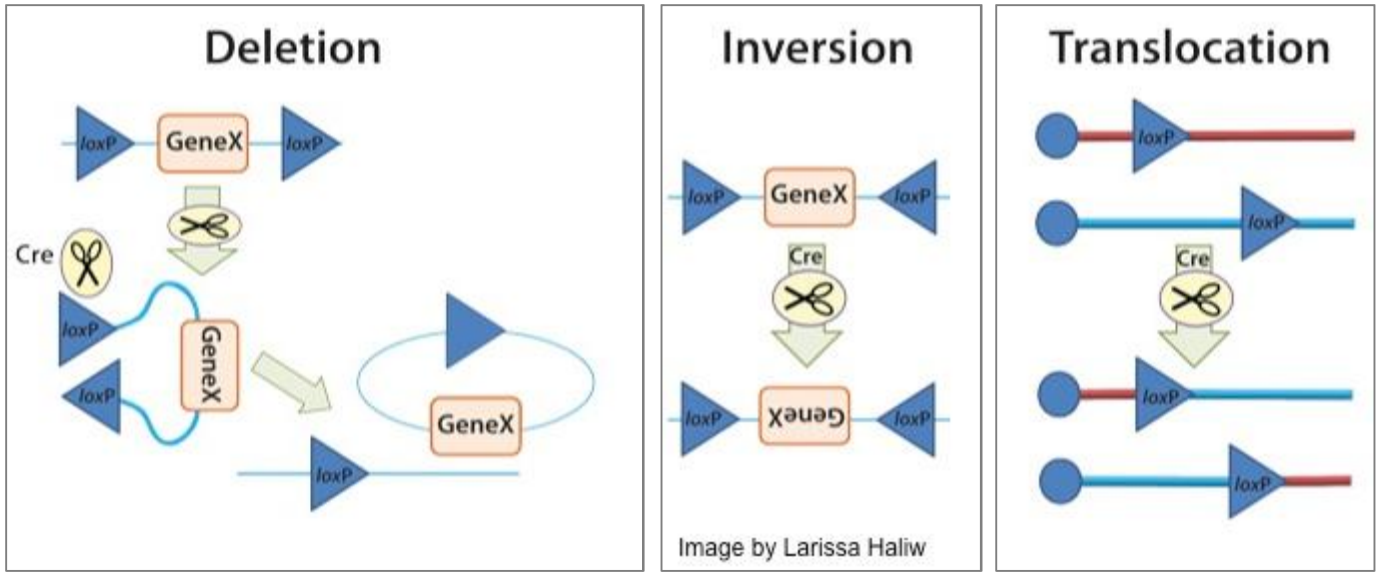
Site specific enzyme, catalyzes recombination between two *loxP* sites

loxP site

1) A 34bp DNA sequence *loxP*

▷

2) Location and orientation determines recombination result:



Cre-*lox* technology (2)

Need to create and maintain <u>two</u> strains (lines) of mice

1) A line of mice in which *loxP* sites have been inserted around the gene of interest. The gene is thus 'floxed'

2) A transgenic or knock-in mouse line expressing CRE under a tissue-specific or inducible promoter

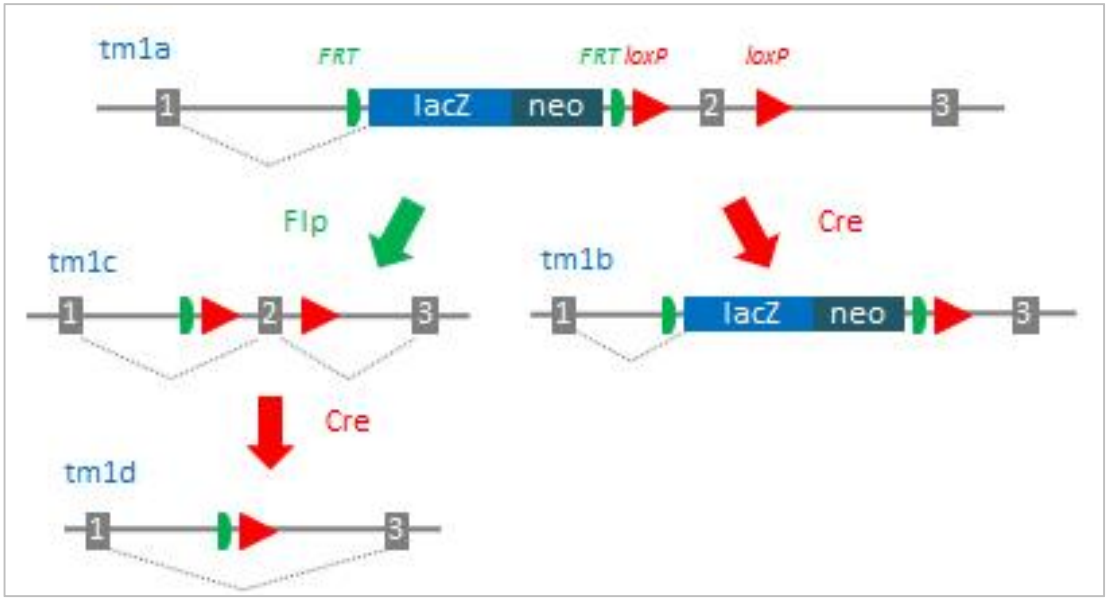
The FLP-*frt* system

Analogous to the Cre-lox system

Becoming more popular in rodent based research

Flipase recombinase (FLP) recognizes a pair of target sequences (*frt*) flanking a genomic region of interest

EUCOMM targeting strategy



Applications of Cre-Mediated Recombination

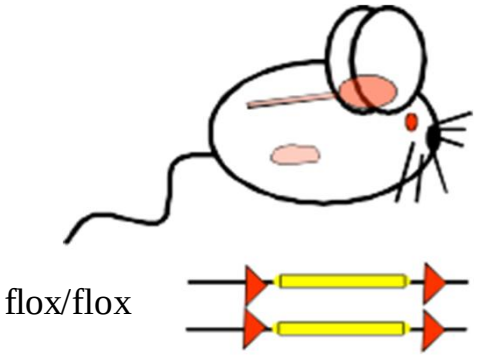
- Tissue-specific deletion of genes
- Activation of genes, by removal of intervening sequences
- Point mutations
- Chromosomal engineering
- Inducible conditional deletion/activation combination with TET etc.

Issues with induced conditional strategy

- Restricted availability of tissue-specific Cre lines
  - generation of novel lines requires data on regulatory sequences
  - testing new line; reporters & insertion site effects (TG)
- Cre toxicity - homozygosity rarely tolerated
- Cre expression in unexpected tissues (including germline)
- Incomplete recombination efficiency
  - mosaicism; not all target cells express Cre OR loxP sites do not recombine
  - => conditional deletion efficiency may be improved in combination with conventional deletion

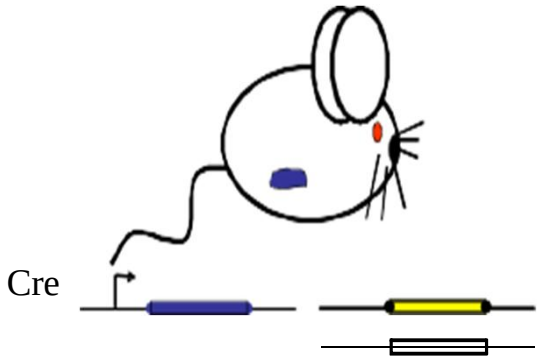
Cre-loxP breeding

1. LoxP sites are around the gene of interest in the genome of every cell in both alleles



X

2. A transgenic or knock-in mouse expressing CRE under a tissue-specific promoter



1st generation: => Cre+; flox/+ (25-50%)

2nd generation: Cre+; flox/+ X flox/flox => 75% Cre+; flox/flox

Improved conditional efficiency:

Cre+ X flox/- => Cre+; flox/+ (25%)

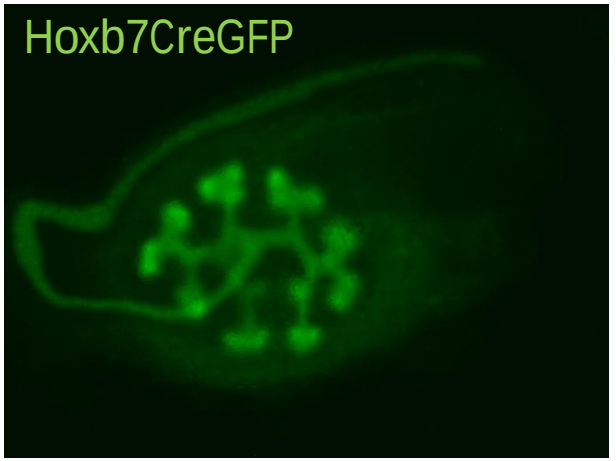
Cre+; flox/+ X flox/- => Cre+; flox/- (25%), Cre+; flox/flox (50%)

Cre-loxP breeding – complex genetics

AIM: deletion of ERK/MAPK activity specifically in UB epithelium

Mek1 exon3 floxed mice
Mek2 conventional ko mice

X



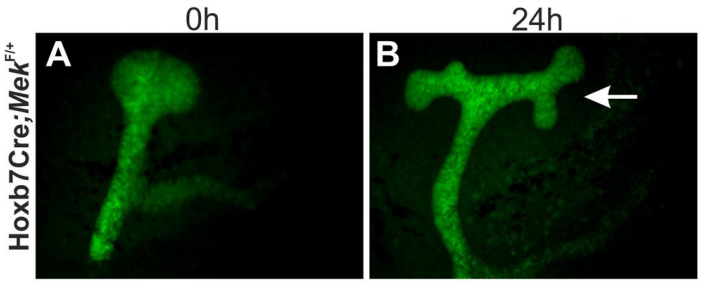
Hoxb7CreGFP;Mek1 F/+;Mek2 +/- male X Mek1 F/F;Mek2 +/- female

or

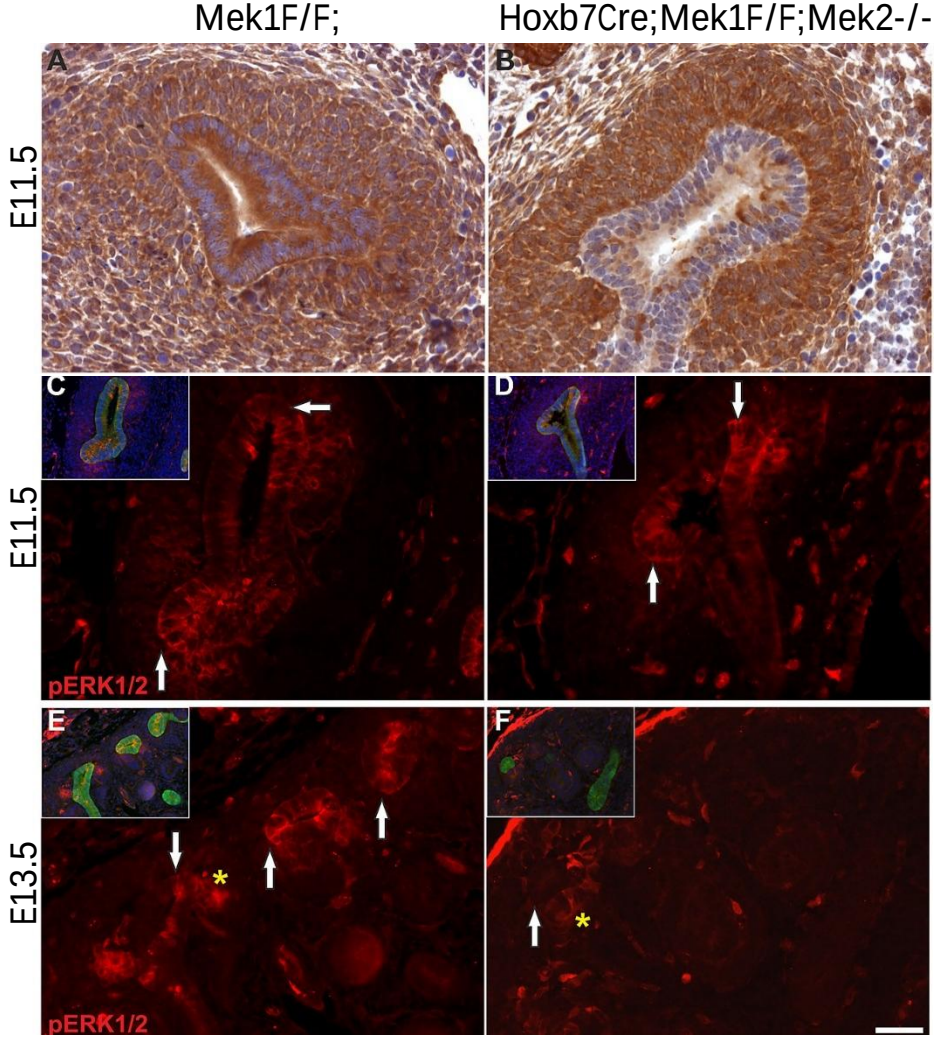
Mek1 F/F;Mek2 -/- male X Hoxb7CreGFP; Mek1 F/+;Mek2 +/- female

=> 12.5% of offspring should be F/F;-/- & half of them null in ureteric epithelium

Cre-loxP: recombination efficiency and dynamics



- Uniform Cre expression from E10
- Mosaic deletion of target gene (*Mek1*)
- Delayed inactivation of signaling



Ihermann-Hella et al 2014

Inducible systems

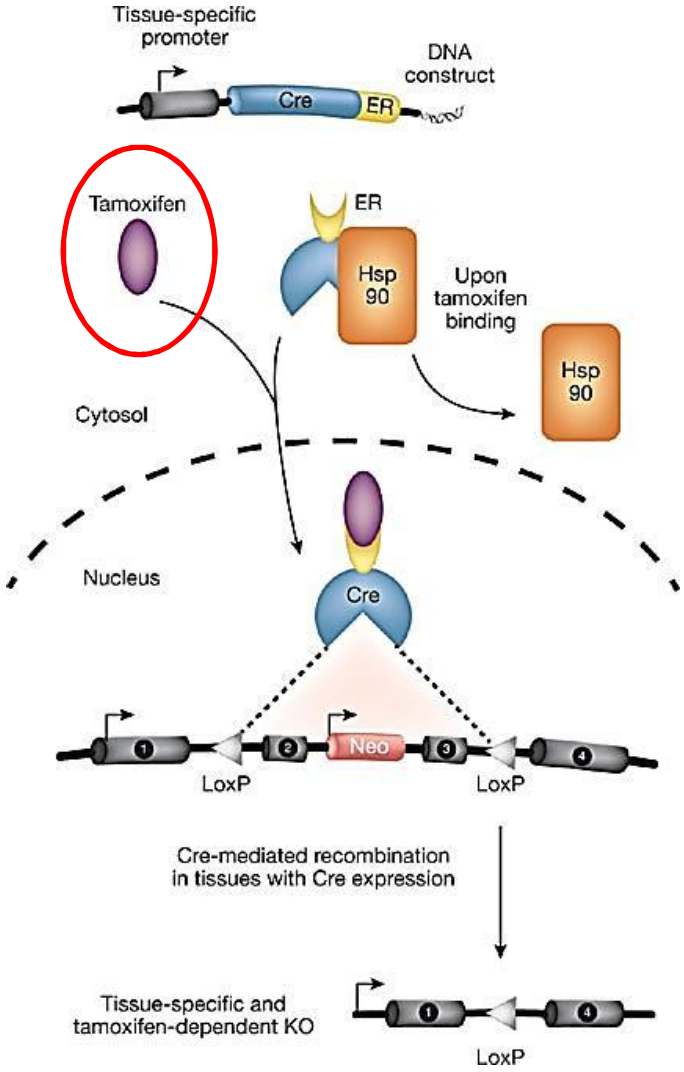
- For induction of endogenous or transgenic promoters, two main systems:
  - -Tetracycline; Tet-On and Tet-Off
  - -Tamoxifen; ER/4-OHT
- Takes advantage of the ligand binding domain of the estrogen receptor to regulate function of attached domain
- Mutant ligand-binding domain (ERT): binds tamoxifen, estrogen antagonist, with greater affinity than endogenous estrogens
- Cre-ERT protein moves to nucleus with tamoxifen treatment allowing Cre action

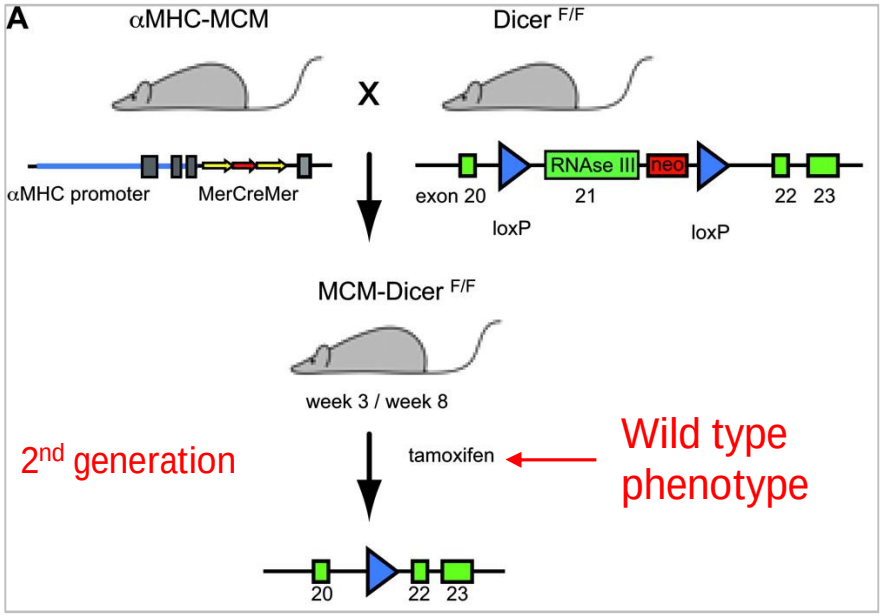
Tamoxifen systems - function

- Site-specific recombinase in combination with inducible system (Spatial and temporal control)

CreERt fusion protein

- Without tamoxifen, CreERT fusion protein remains in the cytosol
- Upon tamoxifen induction translocated to nucleus => Cre-mediated recombination;

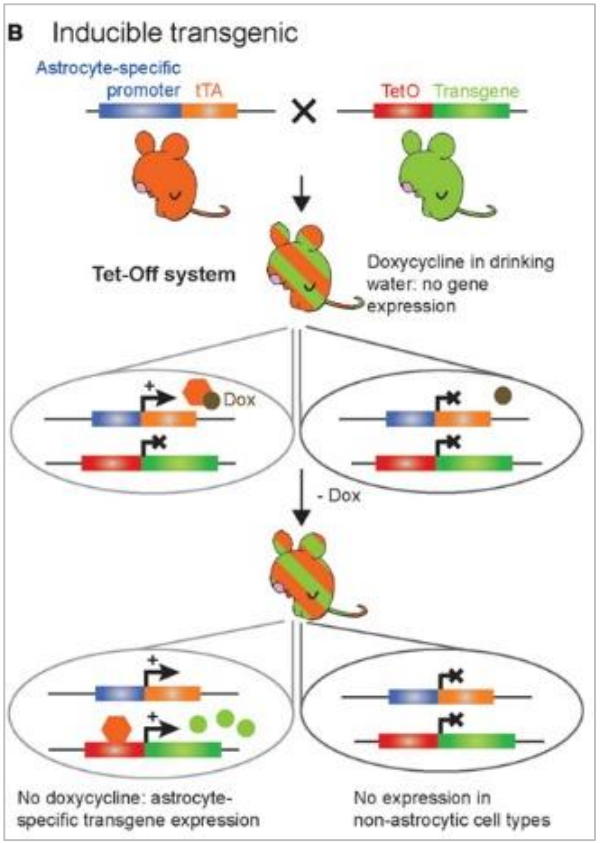




da Costa Martins et al 2008

- MCMCre;floxed/floxed mice can be maintained with this genotype
- Only upon tamoxifen application deletion is induced

Alternative: tetracyclin system (Tet-on) Tet-off



Davila et al 2013

Issues with induced conditional strategy

- Leakiness of inducible system → Cre expression regardless of inducer

- Tamoxifen dosage
  *huge variation, depending on use of tamoxifen / 4-OHT, time and (embryo, pup, adult) and type of administration

- Tamoxifen side effects
  - -premature bone fusion
  - -liver tumors in some mouse strains
  - -breeding problems
  - -failure to deliver

GM animal colony maintenance

Aim: Choose a Breeding Scheme that produces the desired genotypes in most efficient way

- What genotypes do I need?
  - –most efficient way to produce them?
- Will appropriate controls be available?
  - –genetic background
- Reproductive issues (infertility)
- Phenotype issues (lethality)

Transgenic mating scheme

Alleles	Tg	+ (wt)
+	Tg/+	+/+
+	Tg/+	+/+

=> 50% of offspring carriers

Knockout mating scheme

Alleles	+	-
+	+/+	+/-
-	+/-	-/-

=> 25% of offspring carriers

Thanks, QUESTIONS?