

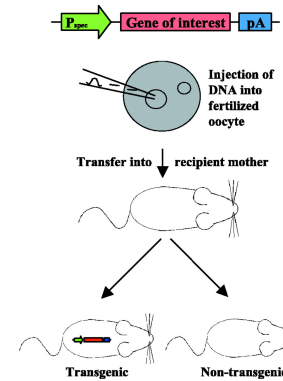
Genetic tools to study gene function *in vivo*

- Mouse as preeminent mammalian model
- Pros and cons
- Why mice?

Gain of function

- Overexpression of a normal gene product
- Overexpression of a mutant gene product
- Partial loss of function: dominant negative, shRNA
- Change spatial pattern
- Change temporal pattern
- Ablate specific cell populations

Gain-of-function approaches in mice

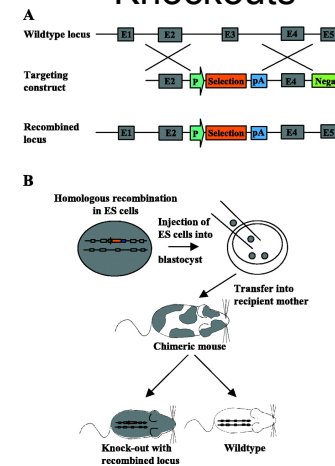


- Pronuclear injection
- Random integration
- Concatamers, multiple sites
- Efficient
- Other methods
 - Retroviral infection
 - Adenoviral transduction
 - Transfect ES cells

Bockamp, E. et al. *Physiol. Genomics* 11: 115-132 2002;
doi:10.1152/physiolgenomics.00067.2002

Loss-of-function approaches in mice:

Knockouts



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Homologous recombination

- Rare event
- Requires positive and negative selection

ES cells

- Derived from inner cell mass of blastocyst
- Immortal
- Totipotent

Conventional knockouts:pros

- Can create null or hypomorphic alleles
- Can create regulatory site mutants
- Can be efficient

Conventional knockouts: cons

- Embryonic lethality
- Compensatory gene expression
- Functional redundancy (big issue!)
- Cytotoxic effects

Conditional Transgenesis

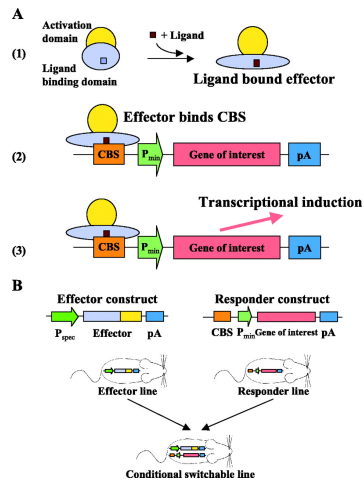
- Induced expression tightly controlled
- Inducing agent nontoxic and specific, good pharmacokinetics
- No leaky expression in absence of inducer
- Fast kinetics of induction

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Conditional transgenesis

- Ligand-mediated transcriptional activation
- DNA binding of transactivator
- activation of a responder gene

Conditional transgenesis: binary control

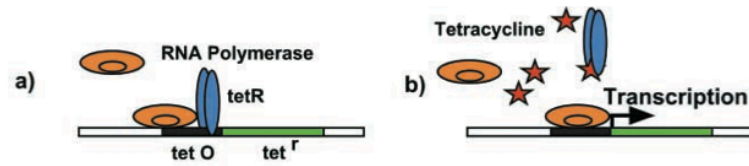


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Tetracycline system

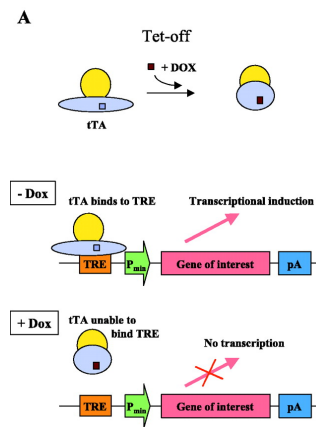
- Derived from *E. coli*
- Tet repressor binds to Tet Operator in absence of tetracycline
- Tet induces conformational change
- Repressor dissociates from operator
- Transcription can proceed

Tetracycline system: Tet-Off



- Tet repressor fused to HSV VP16 transactivation domain
- Tet repressor becomes mammalian transactivator
- TetO-universal promoter (CMV) controls expression of responder gene
- Use Doxycycline (Dox, low cost)
- Responder gene is on until dox is administered

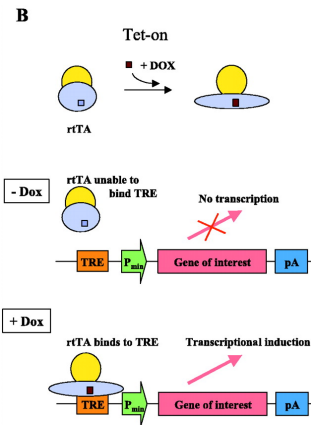
Tetracycline system: Tet-On



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- Based on mutant Tet repressor
- Doesn't bind in absence of Tet
- Make fusion with VP16: Reverse Tet-Transactivator (rtTA)

Conditional gene targeting: tissue-specific knockouts



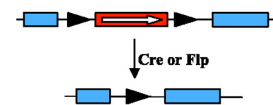
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- Some genes are embryonic lethal when knocked out
- Compensatory gene expression
- Widespread expression: complicated phenotype
- Need to knock out in specific cells

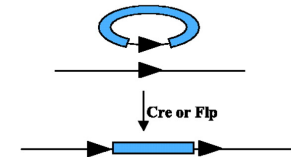
Cre and Flp recombinases

- Site-specific recombination
- Cre recombinase: P1 bacteriophage
- Acts on 34-bp loxP sites
- Effect depends on orientation of loxP sites

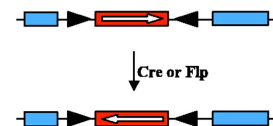
A Excision



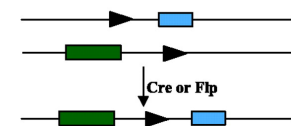
C Insertion



B Inversion



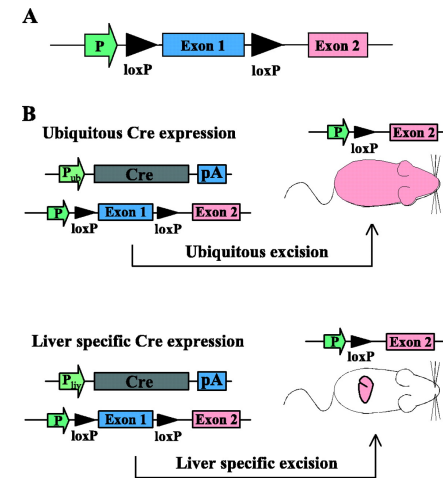
D Translocation



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Conditional Cre-mediated knockout

- Using homologous recombination in ES cells, flox gene of interest
- Generate floxed allele & homozygose
- Generate Cre-expressing transgenic line
- Mate, intercross double-transgenic F_{1s}
- Identify homozygous floxed gene-of-interest, Cre-positive in F_2



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