

# Genetically modified animals in biomedical research

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**National and Kapodistrian University of Athens, Greece**



# Outline

- 1. Historical perspectives**
- 2. Global knockouts**
- 3. Conditional knockouts**
- 4. Inducible knockouts**
- 5. Knock-in mice**
- 6. Lineage tracing**
- 7. Worms, flies and fish**



# Spontaneous mutant mice

1. SHR vs WKY rats
2. Zucker rats (lean vs obese)
3. NOD mice
4. db/db mice
5. nude mice



60s-80s



# Early gene knockout attempts

1. 1978 yeast
2. 1985 mouse cells
3. ES cells

**1980 First transgenic mouse**

**1989 First knockout mouse**

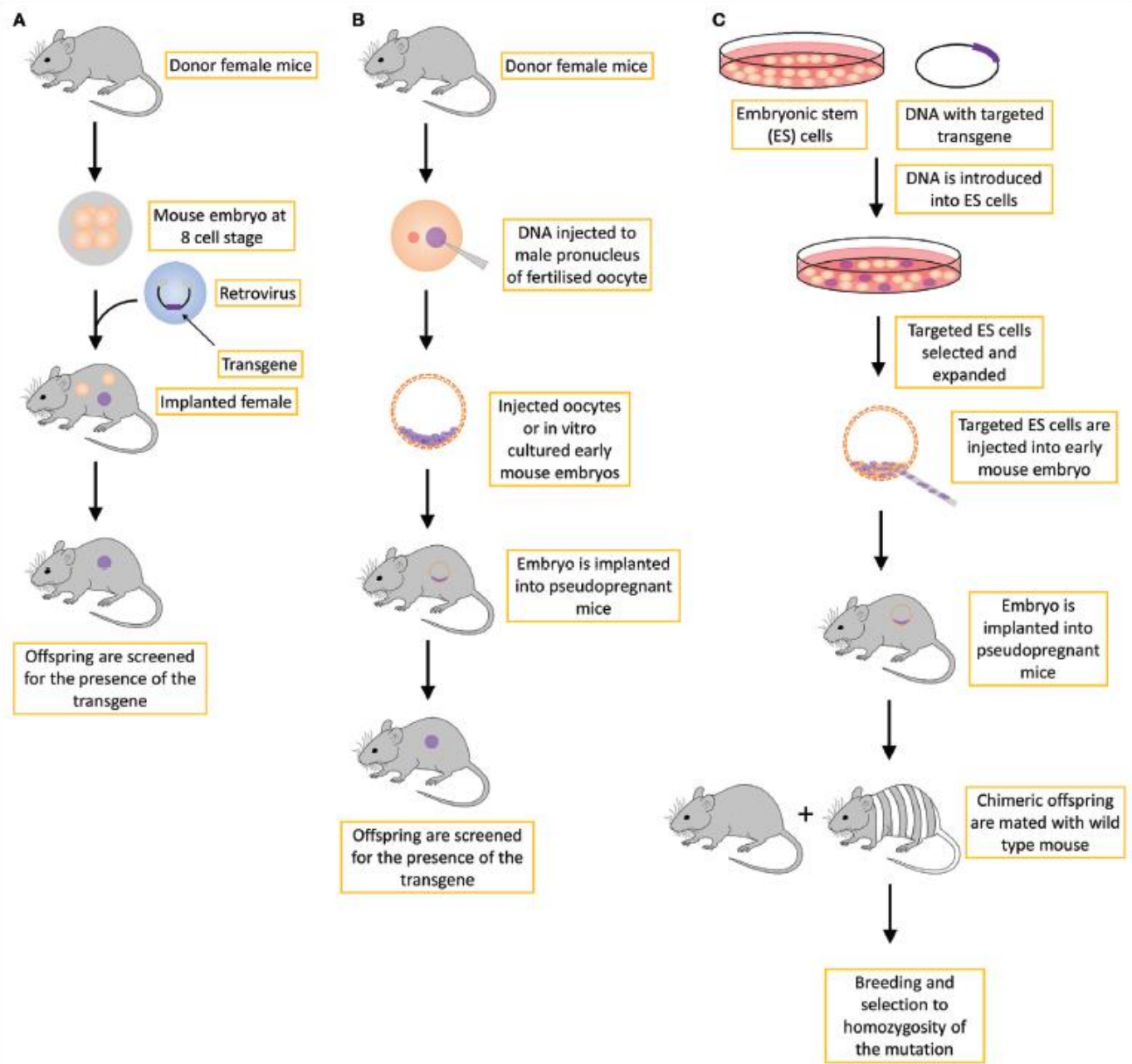
**1990s Conditional/inducible KO mice**



# Outline

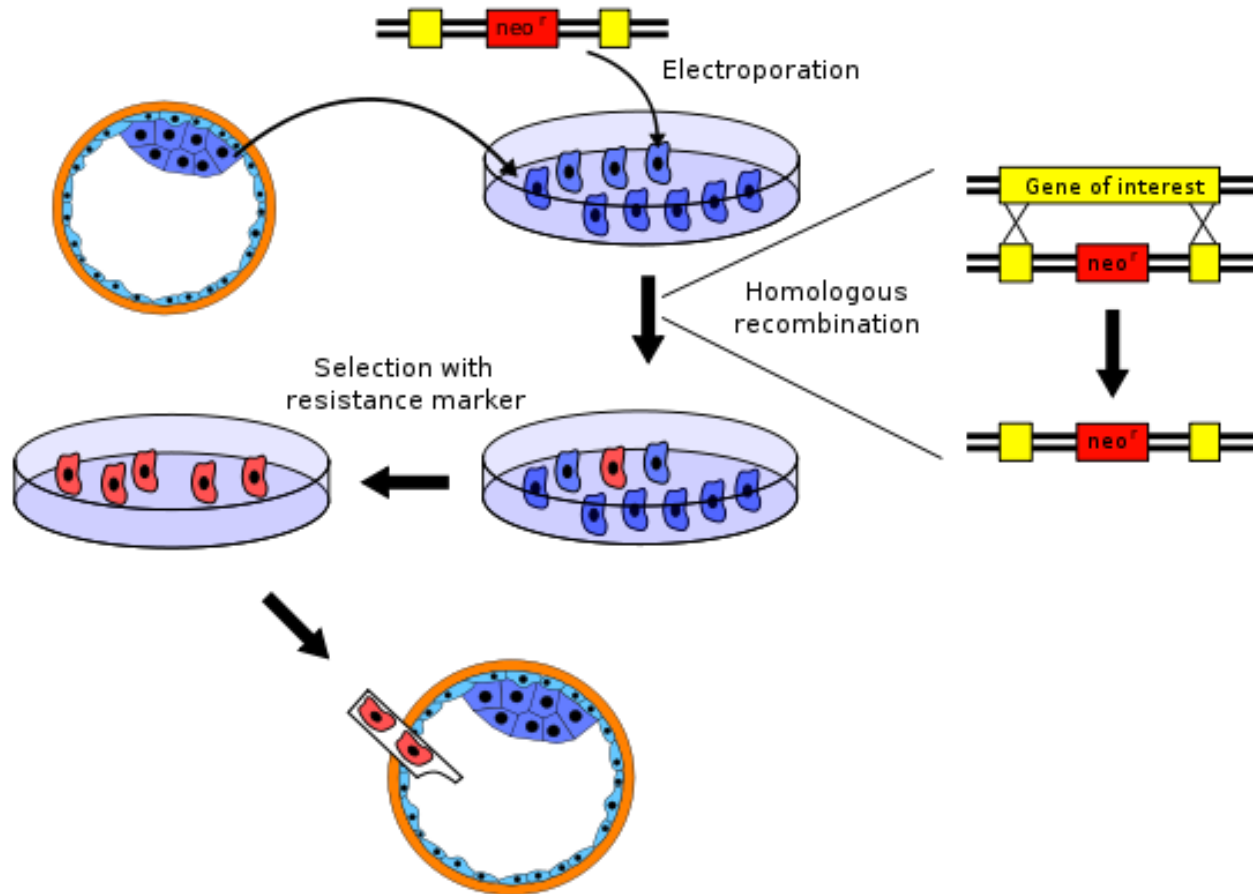
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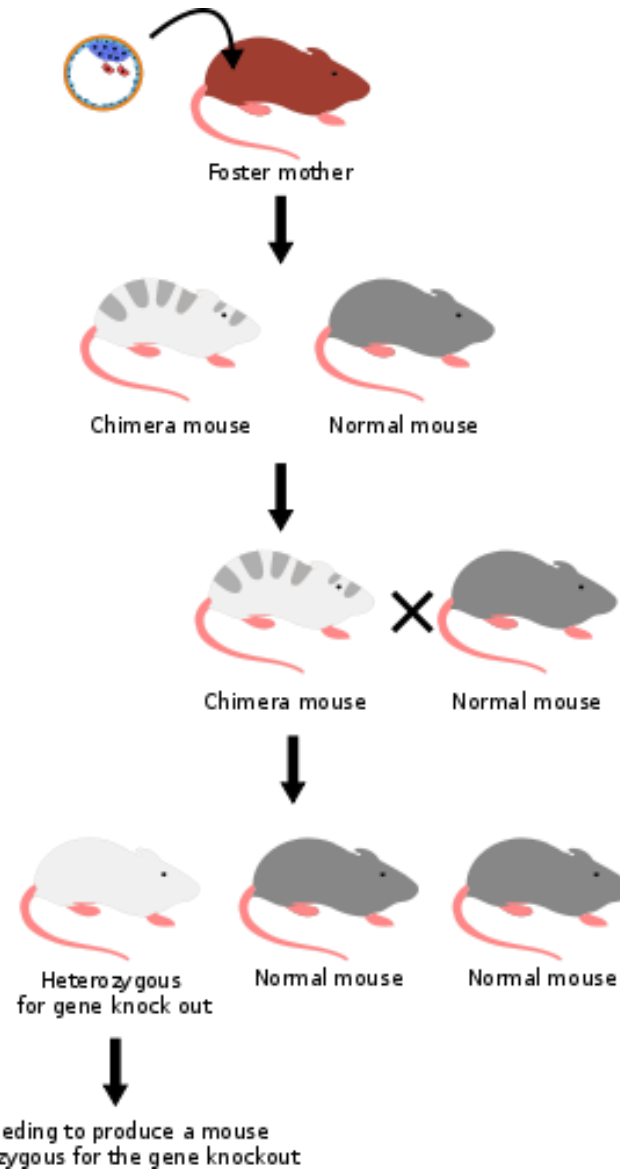


**FIGURE 1** | Different strategies for creating transgenic mice include the **(A)** retroviral approach, which is not routinely used; **(B)** standard transgene approach, in which the DNA is inserted into the genome in an unspecific manner; and **(C)** gene-targeted transgene approach, which is an approach that is routinely used to create conventional knockout transgenic mice, usually with a constitutive loss-of-function mutation.

# Targeted disruption-1



# Targeted disruption-2





# Nobel Prize 2007

The Nobel Assembly at Karolinska Institutet has awarded the Nobel Prize in Physiology or Medicine for 2007 jointly to **Mario R. Capecchi, Martin J. Evans** and **Oliver Smithies** for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells.



P. Fetters/HIMI



D. Sears

**Mario R. Capecchi**  
Born 1937  
University of Utah,  
Salt Lake City, USA

**Sir Martin J. Evans**  
Born 1941  
Cardiff University,  
UK

**Oliver Smithies**  
Born 1925  
University of North Carolina at Chapel Hill, USA

This has led to the creation of an immensely powerful technology referred to as gene targeting in mice. It is now widely used to understand the functions of genes in health and disease



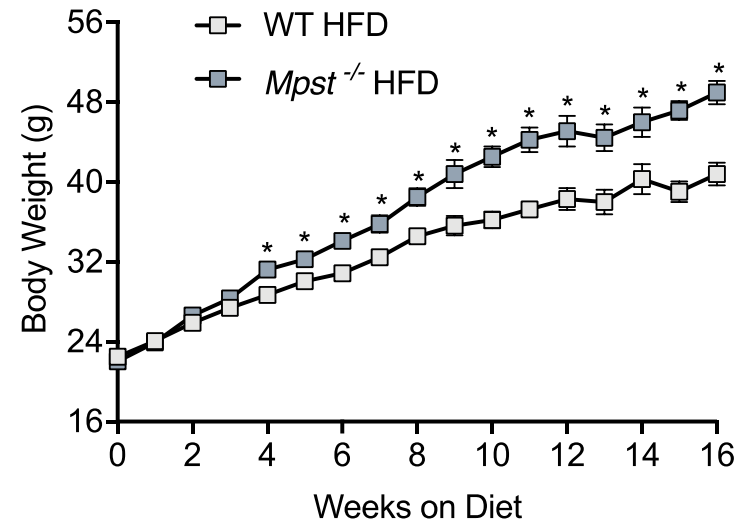
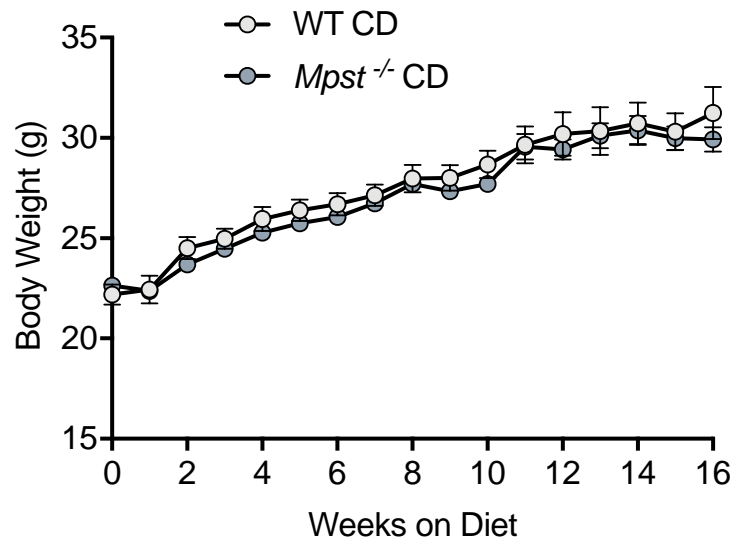
Almost any type of change can be introduced into mouse genes by gene targeting. A common change is to inactivate a gene, thereby creating a knockout "mouse".



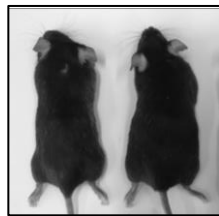
# **Baseline vs disease conditions**

# *Mpst*<sup>-/-</sup> mice gain more weight on HFD in comparison with WT mice

6-7 weeks old  
22-23 weeks old  
Week: 0 CD/HFD 16



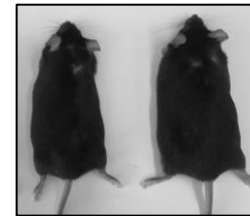
CD



WT

*Mpst*<sup>-/-</sup>

HFD

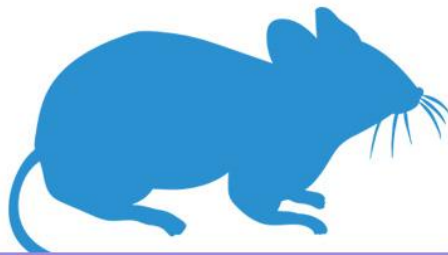


WT

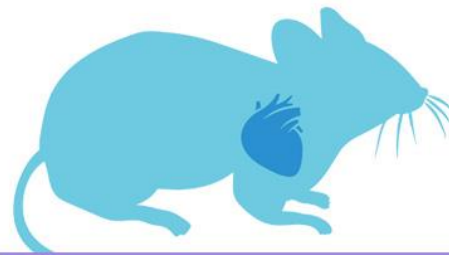
*Mpst*<sup>-/-</sup>

# Problems with global KOs

1. Embryonic lethality (15%)
2. Compensatory changes



**Conventional KO**  
Your target gene is knocked out in all tissues at all times.



**Conditional KO**  
You control where and when your target gene is knocked out.

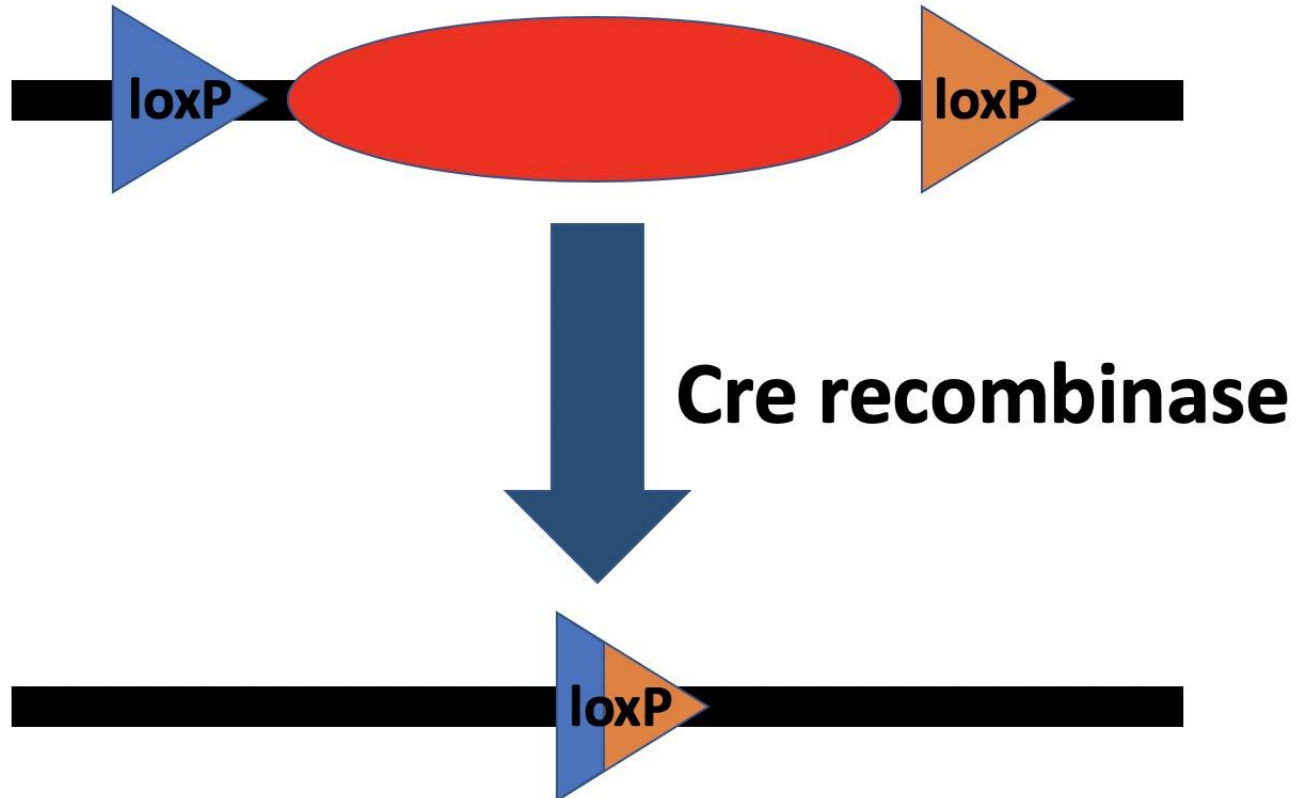


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# Cre recombinase



# Cre-driver lines

Name	Tissue	Cre
GHRKO	Whole-body	
Fat-GHRKO	Adipocytes	aP2
Mac-GHRKO	Macrophages	LysM
Liv-GHRKO	Hepatocytes	Albumin
Liv-GHRKO	Hepatocytes	Albumin
$\beta$ -GHRKO	Pancreatic $\beta$ -cells	Rip
Mus-GHRKO	Muscle cells	Mef-2c
Mus-GHRKO	Muscle cells	Mck

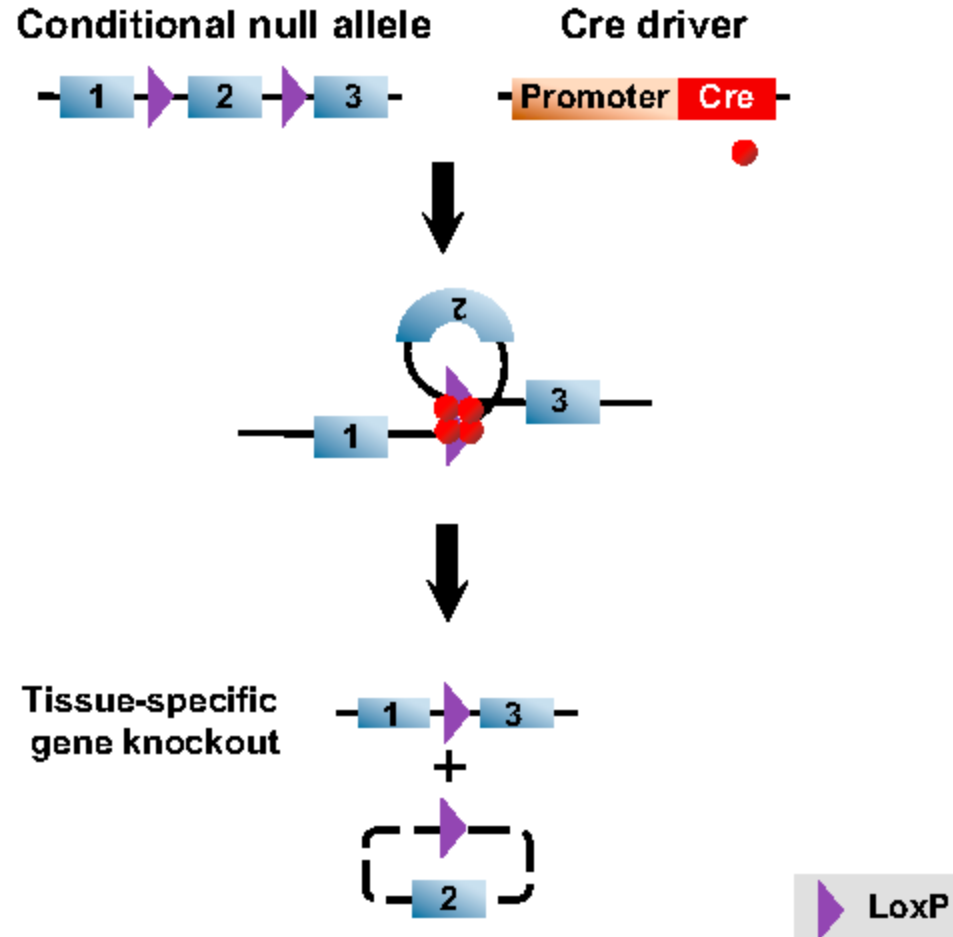
<https://www.jax.org/research-and-faculty/resources/cre-repository/characterized-cre-lines-jax-cre-resource>



and many more...

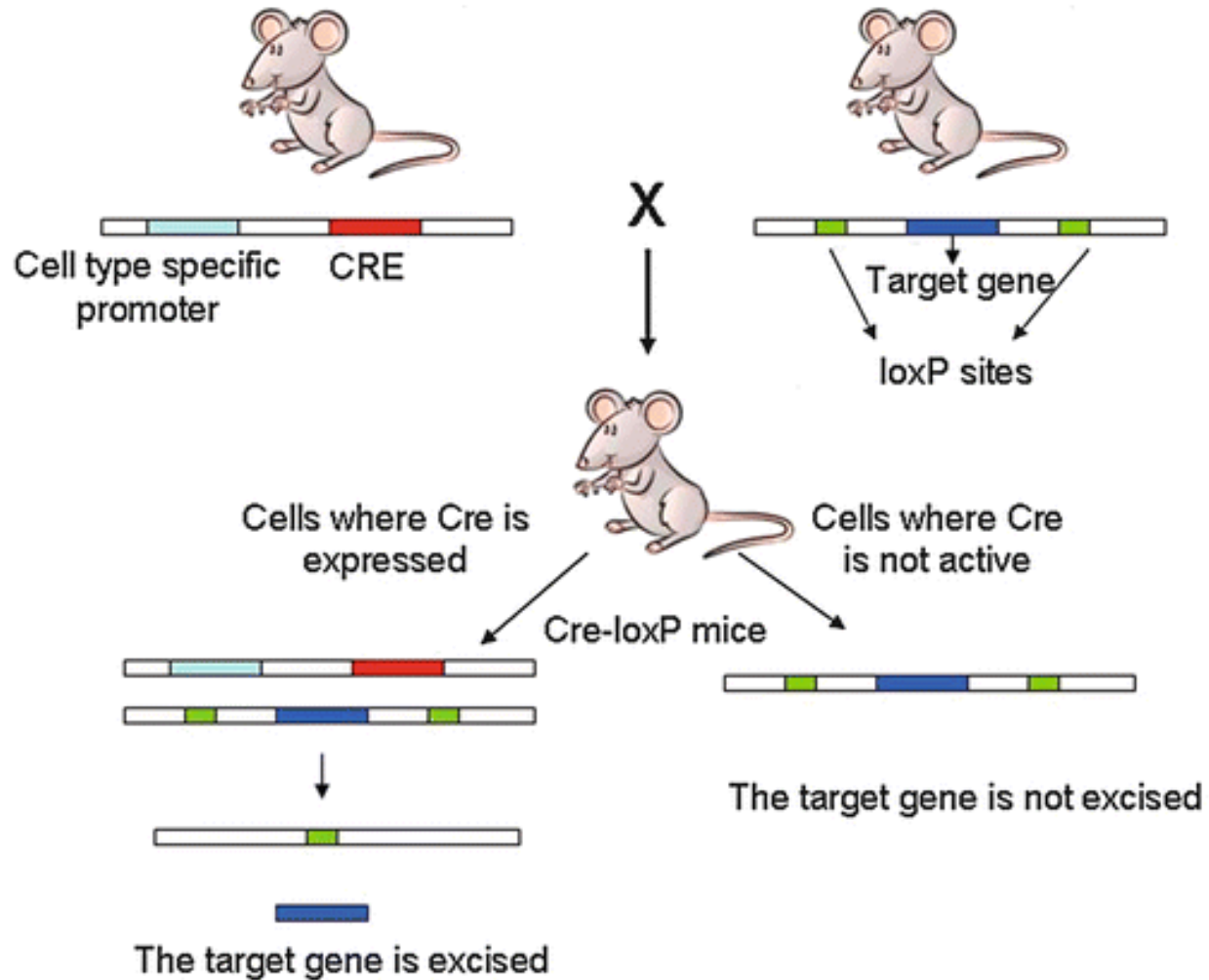


# Cre-lox mice (conditional)

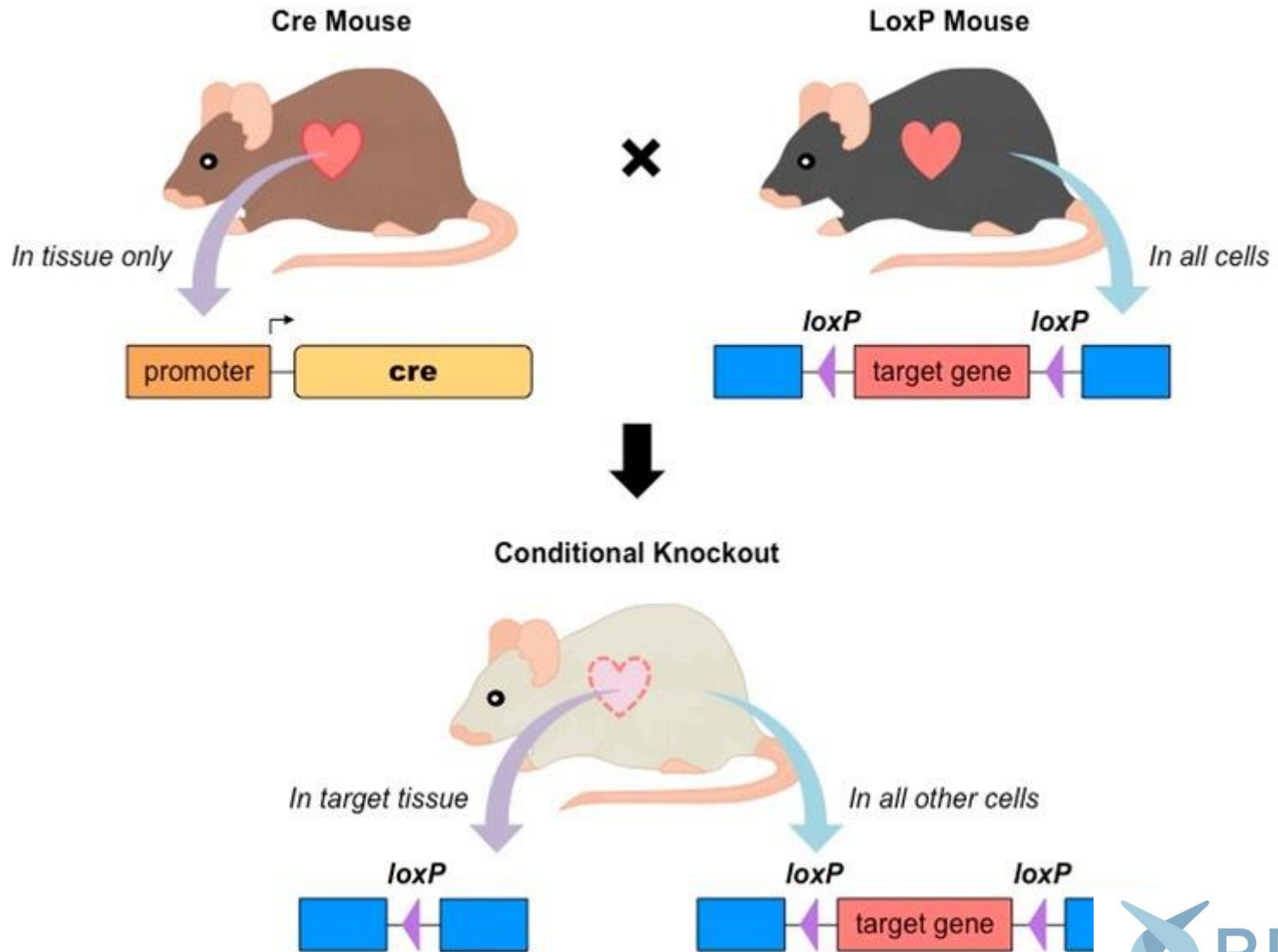




# Cre-lox mice (conditional)



# Conditional KO (spatial)

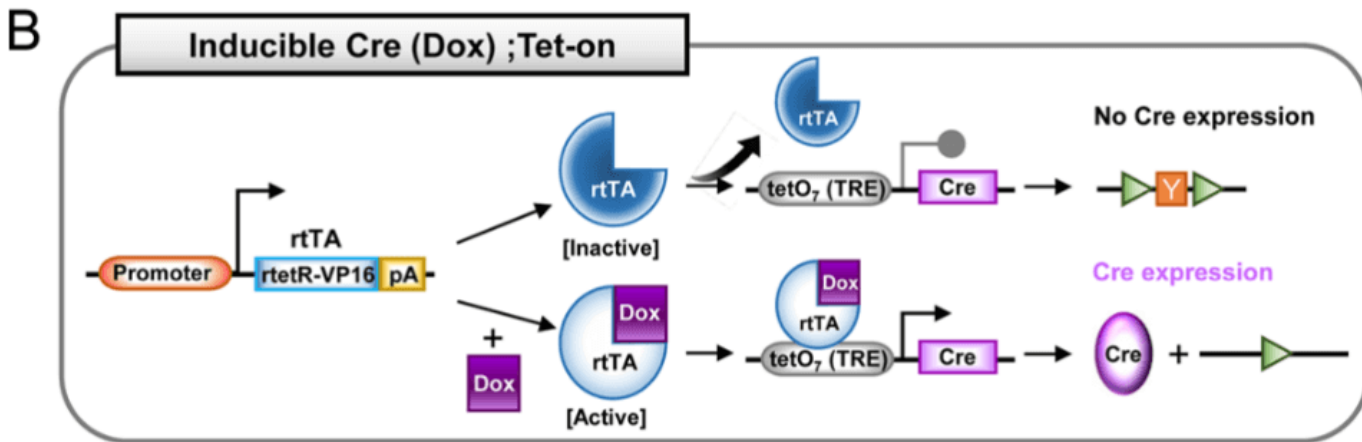
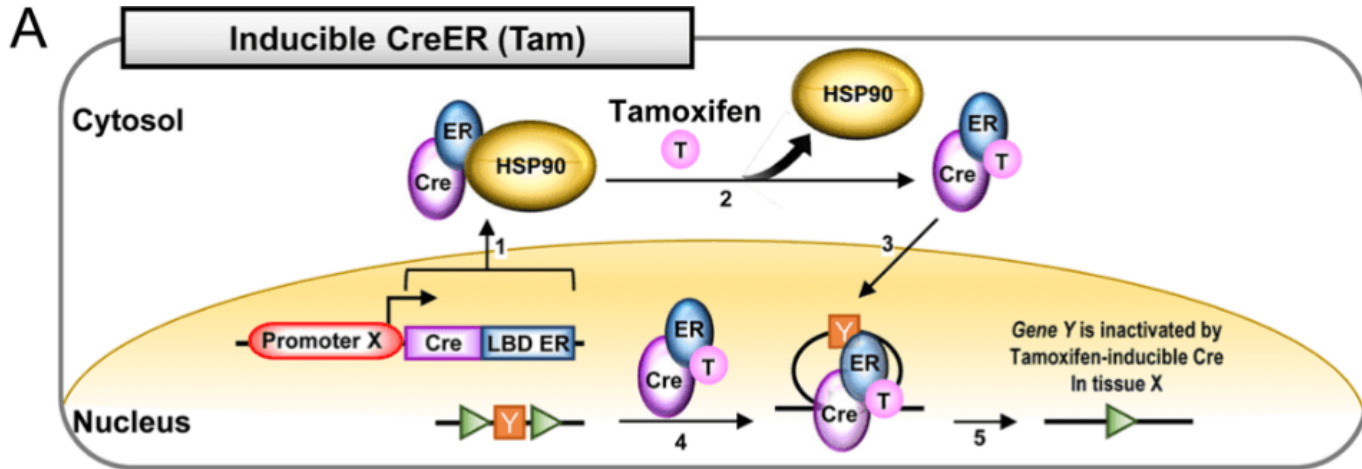


# Outline

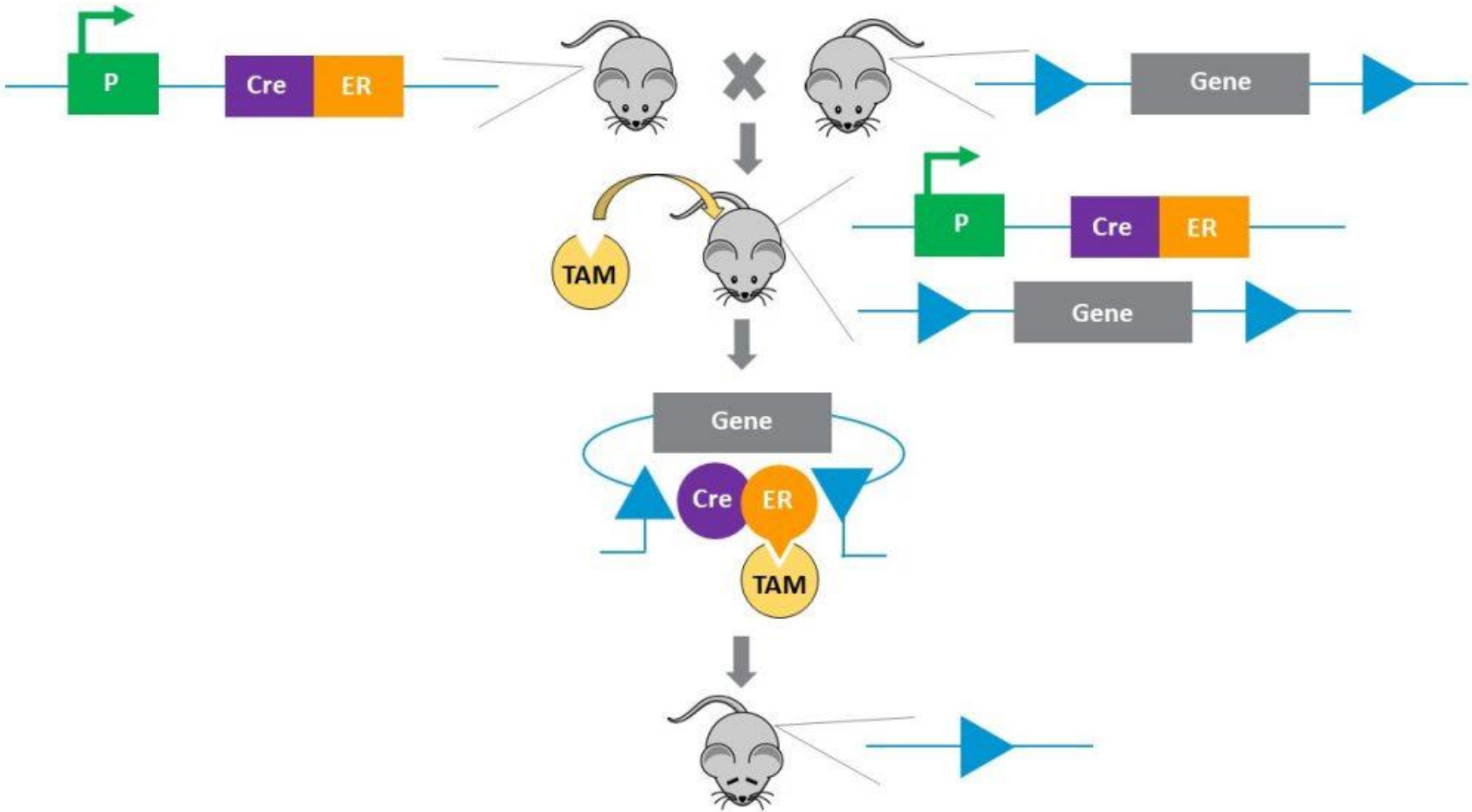
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# Inducible KO (temporal)

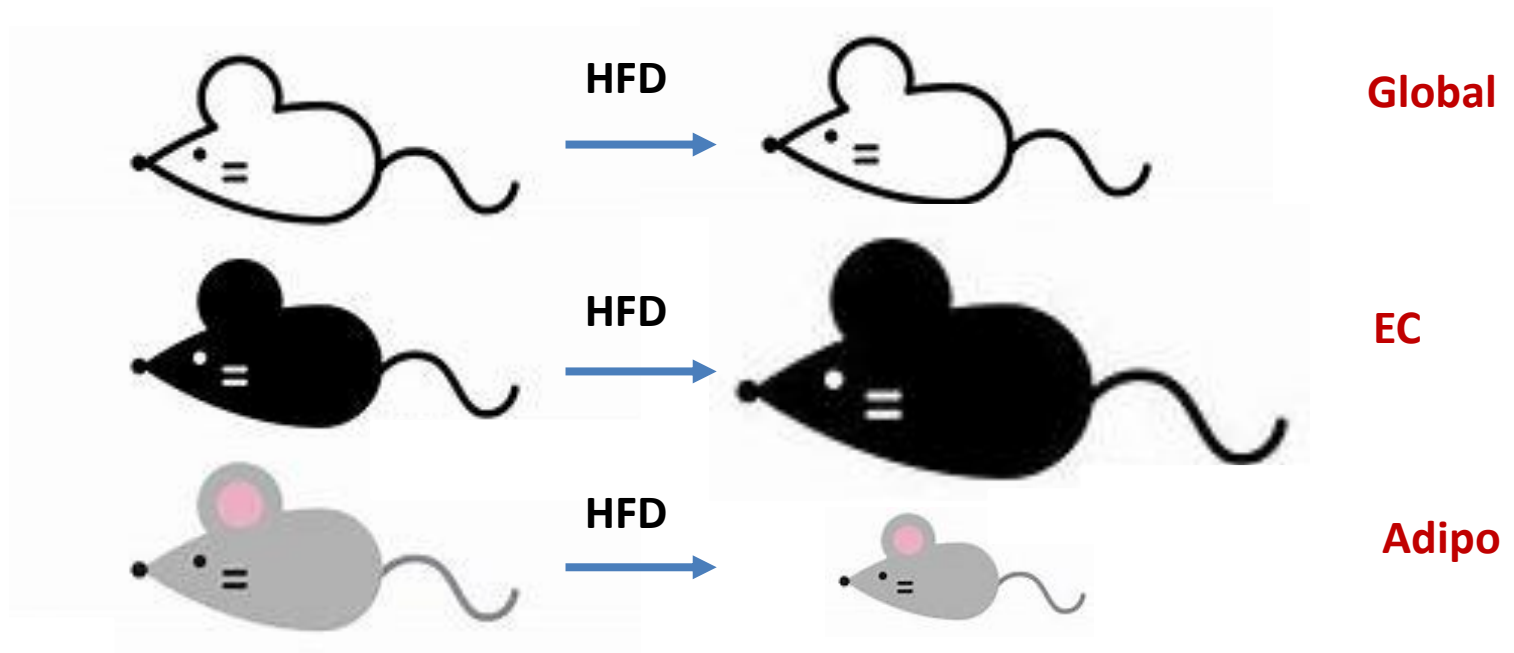


# Inducible KO (temporal)



# Differences in global vs conditional KO

The example of cystationine gamma lyase



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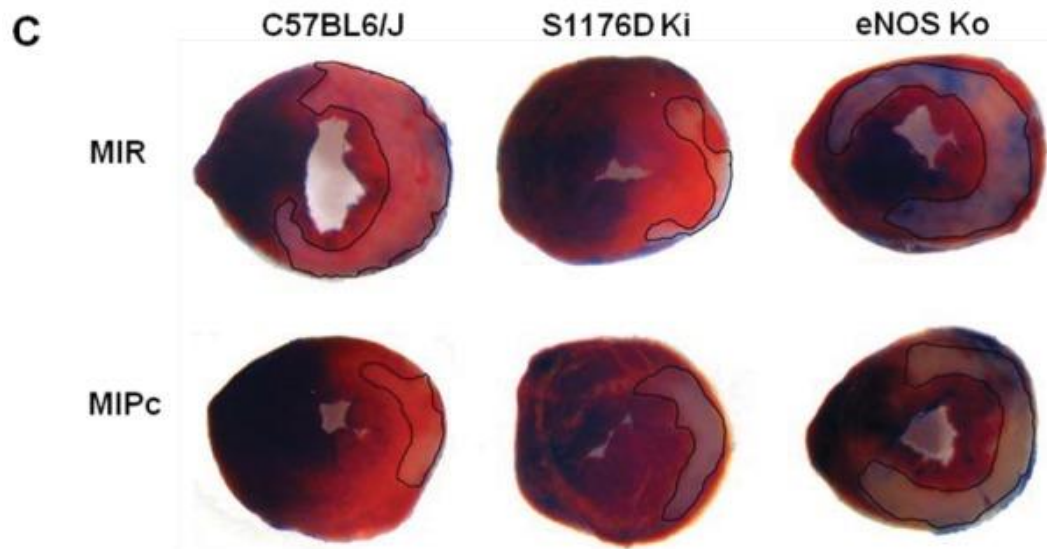
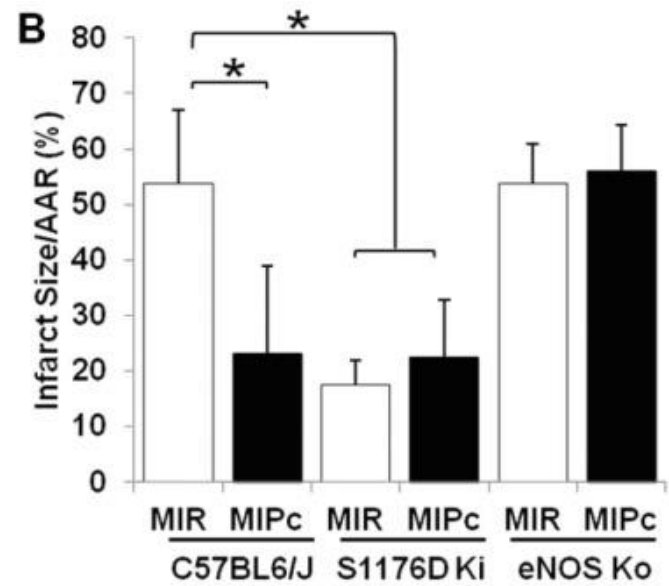
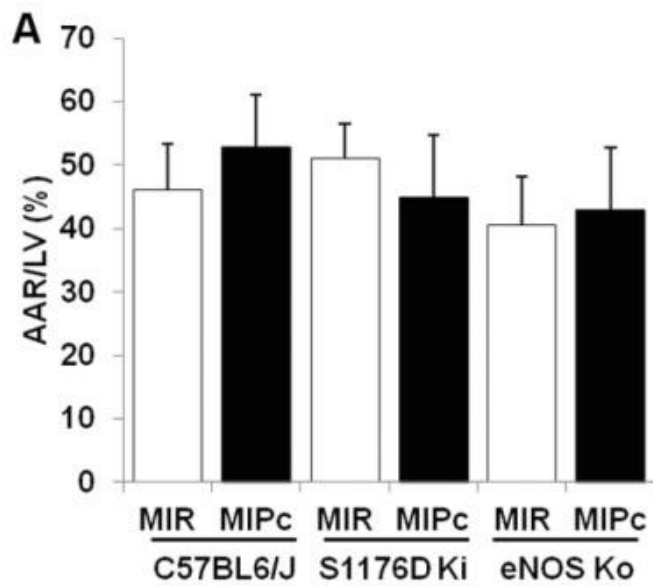


# KiKi mice

1. A Knockin mouse defines an animal model in which a gene sequence of interest is altered by one-for-one substitution with a transgene, or by adding gene sequences that are not found within the locus.
2. The insertion of a transgene is typically done in specific loci. This "targeted" approach causes less disturbances of the transcription-active genetic environment.
3. Knockin mice are suited to a wide range of application, from the study of regulatory elements such as promoters to the production of therapeutically useful humanized antibodies.
4. Might express non-functional or mutated protein



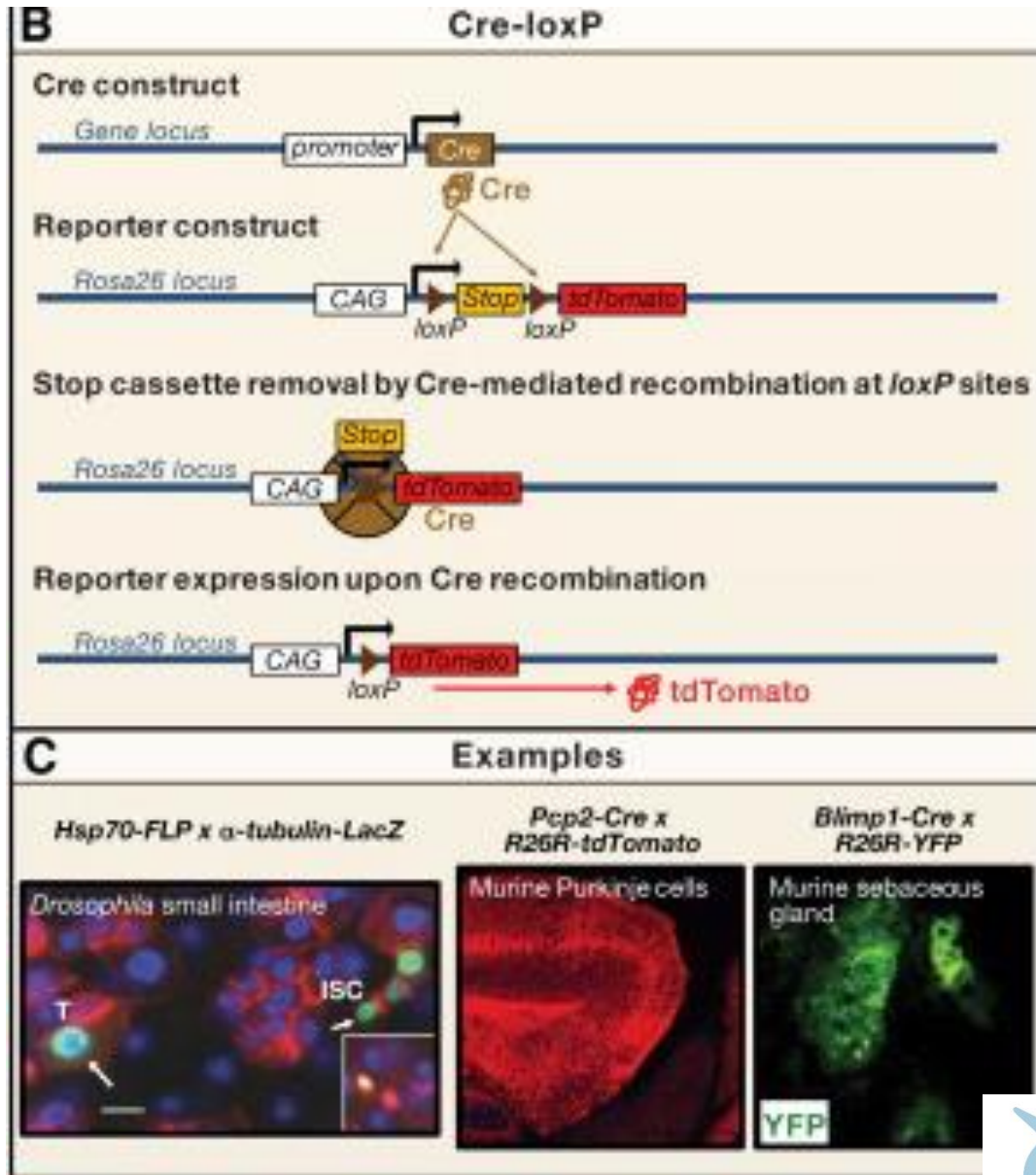




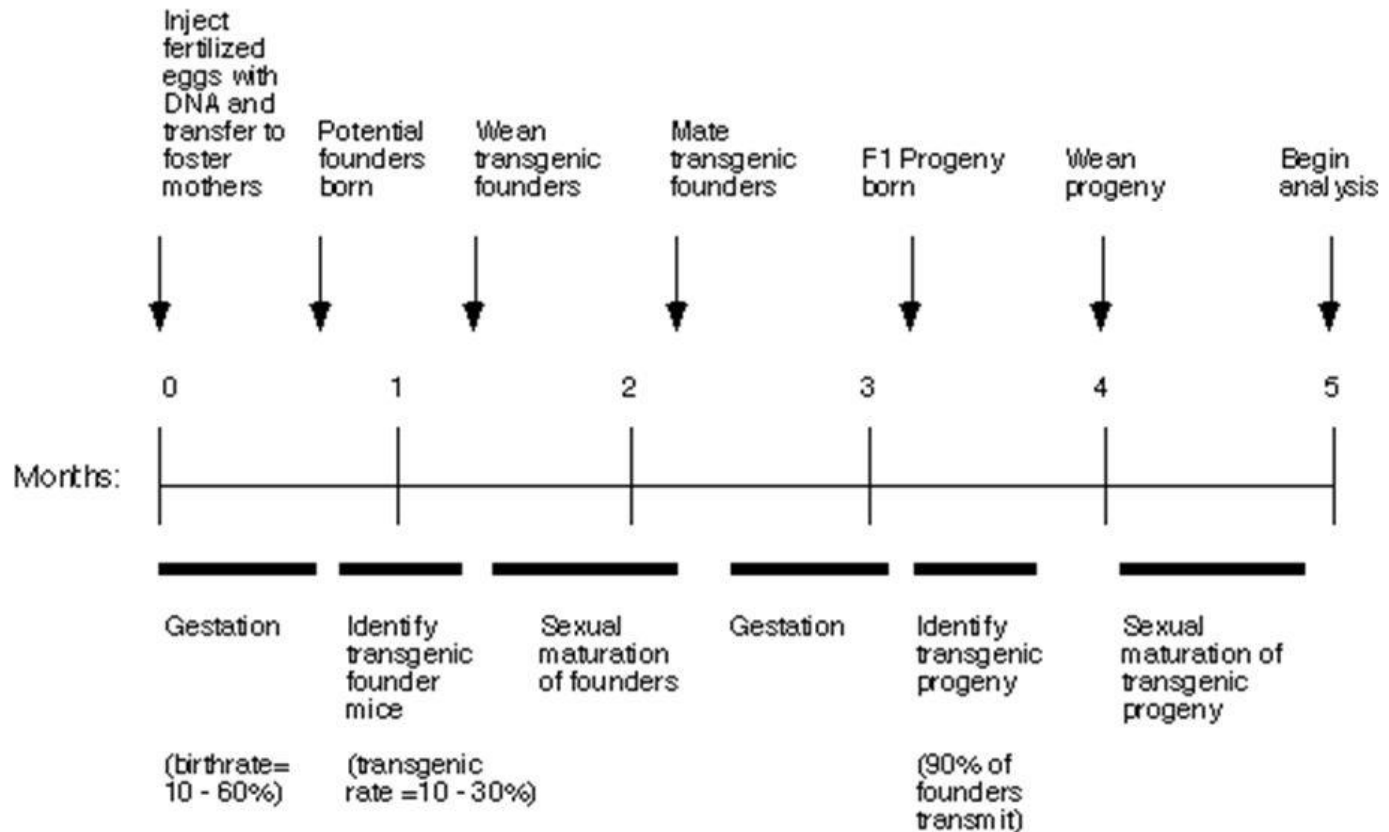
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# Timeline for Transgenic Mouse Analysis



# Resources

[https://www.mousephenotype.org/martsearch\\_ikmc\\_project/](https://www.mousephenotype.org/martsearch_ikmc_project/)



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# Drosophila

0031-6997/11/6302-411-436\$25.00  
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Pharmacol Rev 63:411-436, 2011

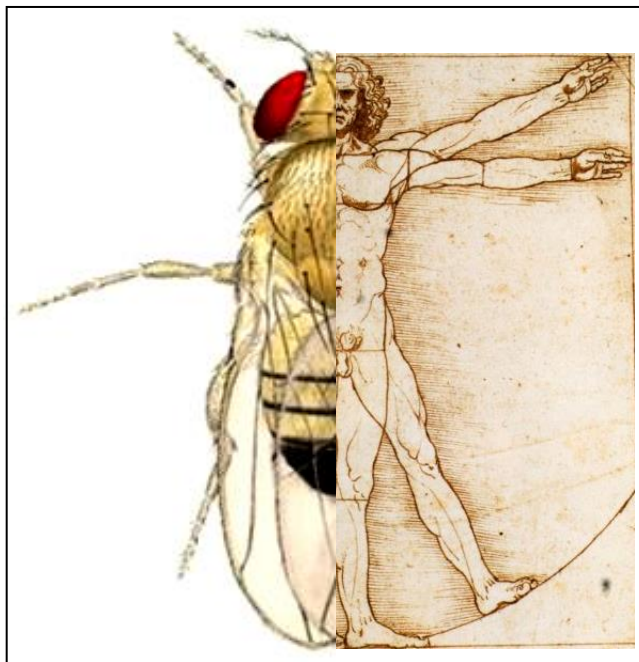
Vol. 63, No. 2  
3293/9678928  
Printed in U.S.A.

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## Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery

Udai Bhan Pandey and Charles D. Nichols

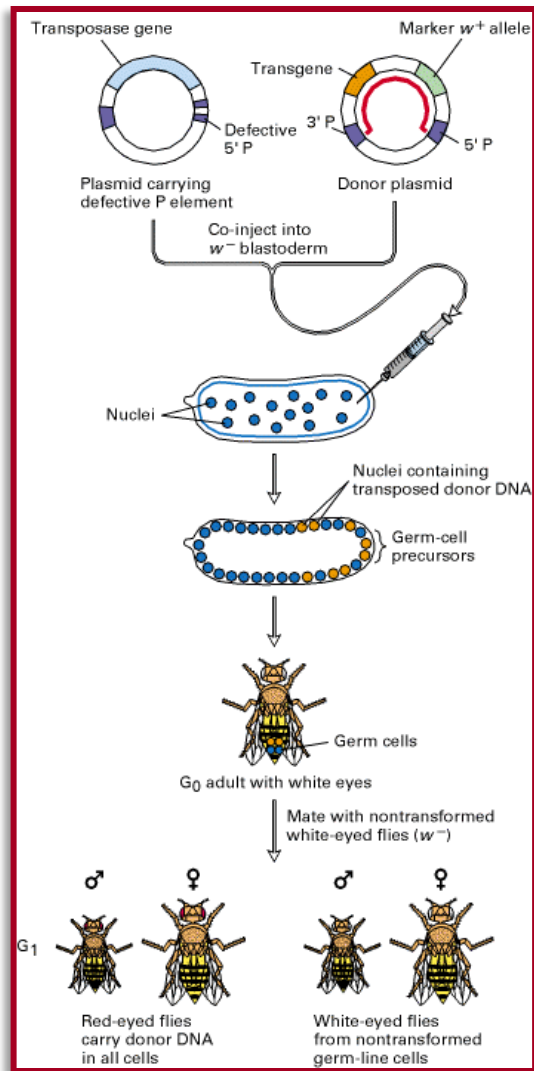
Departments of Genetics (U.B.P.) and Pharmacology and Experimental Therapeutics (C.D.N.), Louisiana State University Health Sciences  
Center, New Orleans, Louisiana



- Completely sequenced and annotated genome
- Encodes for ~14,000 genes
- ~ 75% of disease-related genes in humans have functional orthologs in the fly
- Overall identity at the nucleotide level or protein sequence between fly and mammal is usually approximately 40% between homologs; however, in conserved functional domains, it can be 80 to 90% or higher
- Very rapid life cycle
- Advanced “high tech” genetics – molecular “tools”
- Multiple model organisms (embryo, the larva, the pupa, and the adult)
- The adult fly is a very sophisticated and complex organism not unlike higher organisms. The adult fly has structures that perform the equivalent functions of the mammalian heart, lung, kidney, gut, and reproductive tract.
- The brain of the adult fly is quite remarkable. More than 100,000 neurons form discreet circuits and neuropil that mediate complex behaviors, including circadian rhythms, sleep, learning and memory, courtship, feeding, aggression, grooming, and flight navigation.
- The response of flies to many drugs that act within the CNS is similar to the effects observed in mammalian systems

Although there are many differences between flies and humans, the degree of conserved biology and physiology position *D. melanogaster* as an extremely valuable tool in the drug discovery process.



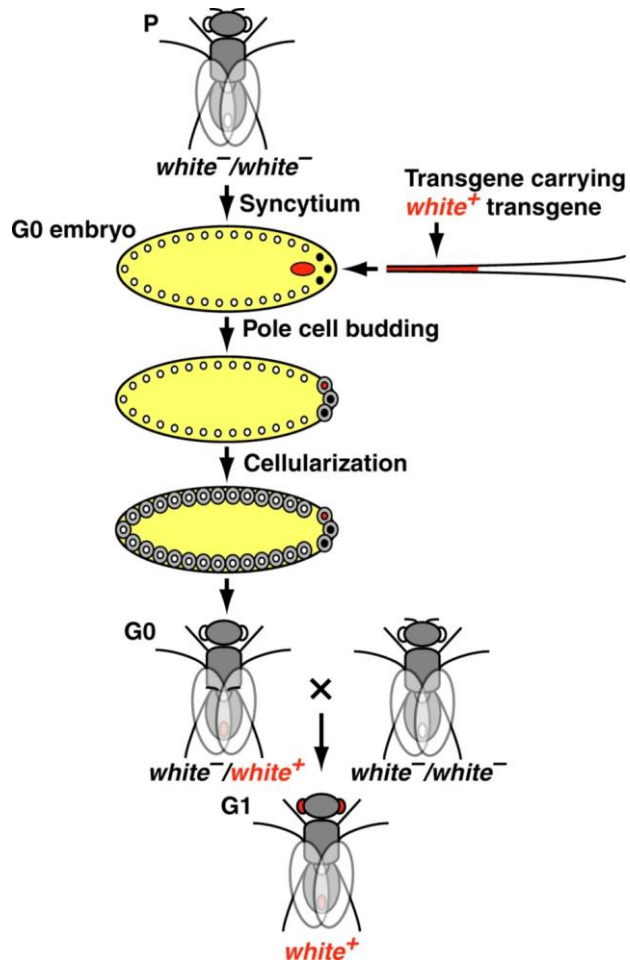


Generation of transgenic fruit flies by P-element transformation. The P element, a mobile genetic element, can move from one place in the genome to another. This movement (transposition) is catalyzed by transposase, which is encoded by the P element; the 3' and 5' ends of the P element are recognized by transposase and are required for transposition to occur. To produce transgenic fruit flies by this method, the functionally different regions of the P element are incorporated into two different bacterial plasmids. The donor plasmid contains three necessary elements: the transgene (orange); a marker gene (green) used to indicate flies in which the plasmid DNA is transposed to a recipient chromosome; and both ends of the P element (dark purple) — 3' P and 5' P — flanking the other two genes. It does not contain transposase.

In this example, the marker is the dominant  $w^+$  allele, which confers red eye color. The red bracket indicates the segment of the donor plasmid that can transpose into the fly genome. The other plasmid carries the P element (encoding transposase) with mutations in one end, which prevent it from transposing. The two plasmids are co-injected into blastoderm embryos homozygous for the recessive  $w^-$  allele, which confers white eye color. Transposase synthesized from the gene on the P-element plasmid catalyzes transposition of the donor plasmid DNA into the fly genome. Because transposition occurs only in germ-line cells (not in somatic cells), all the  $G_0$  adults that develop from injected embryos have white eyes. Mating of these flies with white-eyed flies will yield some  $G_1$  red-eyed progeny carrying the transgene and the marker allele ( $w^+$ ) in all cells.







FB2013\_05, released September 13th, 2013

# FlyBase

A Database of *Drosophila* Genes & Genomes

Home Tools Files Species Documents Resources News Help Archives  Go

**D. melanogaster**

**D. virilis**

**A. mellifera**

BLAST

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**Find a Fly Person**

**QuickSearch**

Simple Expression Phenotype GO References Data Type

Species:  include non-Dmel species

Enter text:

Note: Wild cards (\*) can be added to your search term

**News**

New in Release FB2013\_05 | 10 Sep 13

Stock Center User Survey | 18 Jun 13

Fly Mating Scheme Design | 13 Mar 13

GenBank Release | 25 Jan 13

2013 Release Schedule | 6 Nov 12

NAR article on bibliography | 5 Nov 12

White Paper 2012 | 8 Jun 12

FlyBase 101 | 23 Dec 11

**Upcoming Meetings**

Neurobiology of Drosophila | 1 Oct 13

Notch Meeting VII | 8 Oct 13

23rd EDRC | 16 Oct 13

27th Ann French Dros Conf | 28 Nov 13

3rd Scottish Dros Mtg | 6 Dec 13

Invtb-Microbe Interactions | 26 Jan 14

56th ADRC | 26 Mar 14

19th Crete Dros Mol/Dev Bio | 22 Jun 14

7th Intl Symp Mol Insect Sci | 13 Jul 14

Dros in Exp Genetics & Bio | 6 Oct 14

**Courses**

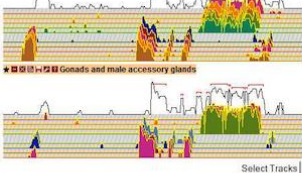
Dros Species Workshop XII | 25 Mar 14

**Commentary** [See all commentaries](#)

**FlyBase GBrowse2 beta**

**Imaginal disc and other carcass**

Mar 8, 2013. FlyBase GBrowse is now complemented with GBrowse 2. Highlights include better scrolling, customizable track order and appearance, and settings that persist across sessions.... (More)



**Gonads and male accessory glands**




Select Tracks

FlyBase is supported by a grant from the National Human Genome Research Institute at the U.S. National Institutes of Health #P41 HG000739. Support is also provided by the British Medical Research Council, the Indiana Genomics Initiative, and the National Science Foundation through XSEDE resources provided by Indiana University. Copyright Statement.

version FB2013\_05, released September 13, 2013

## Bloomington Drosophila Stock Center at Indiana University

Order Form
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Accounts
Payment
Regulatory
Fly Work
About BDSC
Links



**Search All Stocks at FlyBase**

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**Search the Bloomington Web Site**

▪ [Google™ Site Search](#)

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**Download the Stock List**

▪ [bloomington.csv](#)

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[Pruning Page](#)

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**Stock Center News**

[Holiday Schedules](#) | 28 May 2013

[Wire Pmt URL Error](#) | 23 Jan 2013

**Stocks to be Removed** | 18 Jan 2013

[Statements for 2012](#) | 9 Jan 2013

[Fees for 2013](#) | 28 Dec 2012

**Browse Stocks**

[All Browse Options](#)

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[Sequenced Strains](#)

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[Human Disease Models](#)

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**Deficiencies**

- Bloomington Df Kit
- BSC Dfs    ▪ Exelixis Dfs
- DrosDel Dfs
- All Bloomington Dfs

---

**Duplications**

- BSC Dps    ▪ DC Dps: X, 4
- Dp Kits: 1, 2, 3
- CytoSearch at FlyBase

---

**Mapping Stocks**

- Meiotic Mapping
- Baylor Mapping Kit
- SNP Mapping

**Insertions**

- All Insertions    ▪ GBrowse
- Gene Disruption Project
- Exelixis    ▪ Minos
- Potential Misexpression
- Protein-trap

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**Common Tools**

- GAL4    ▪ UAS    ▪ GAL80
- FRT    ▪ FLP    ▪ MARCM
- GFP etc.    ▪ Cre
- DTS lethals    ▪ phiC31
- Gene KO    ▪ RNAi
- Q System    ▪ InSITE

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**Balancers**

- Balancers in Stock
- Balancer Definitions
- Balancer Breakpoints

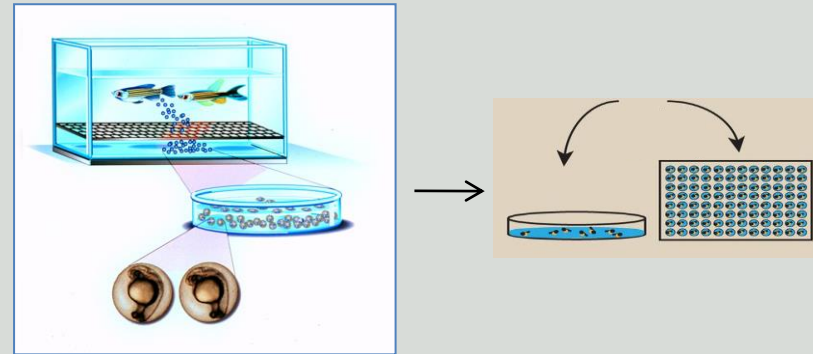
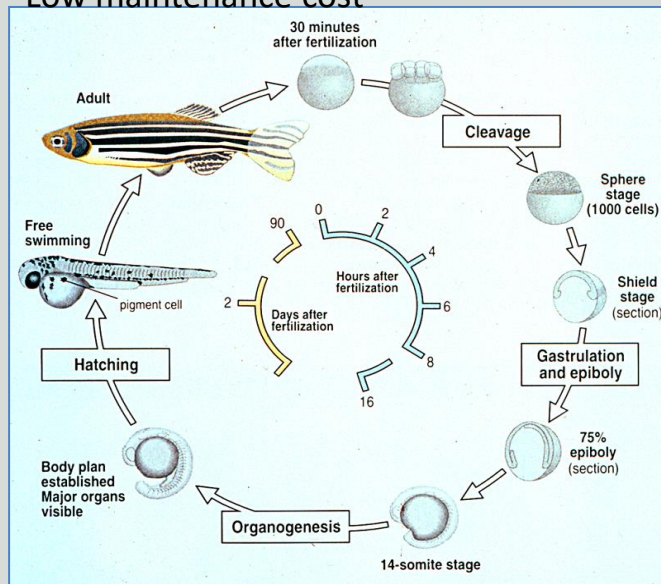
The BDSC collects, maintains and distributes *Drosophila melanogaster* strains for research.

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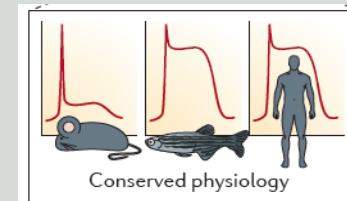
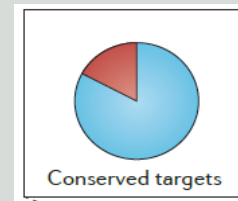


# Zebrafish Advantages

- ✓ Vertebrate
- ✓ Small size
- ✓ Fast reproduction and development
- ✓ External fertilization
- ✓ Transparency
- ✓ Permeability to small molecules
- ✓ Low maintenance cost



- ✓ High genetic homology with human
- ✓ Conserved targets & physiology
- ✓ Broad range of accessible biology
- ✓ High-throughput screens
- ✓ No need for bioethics approval up to 5dpf



MacRae & Peterson, 2015



RNA injections (for overexpression, gain of function) from 1cell-stage due to external fertilization (30 minutes to 1<sup>st</sup> division, enough time to inject 100 embryos)

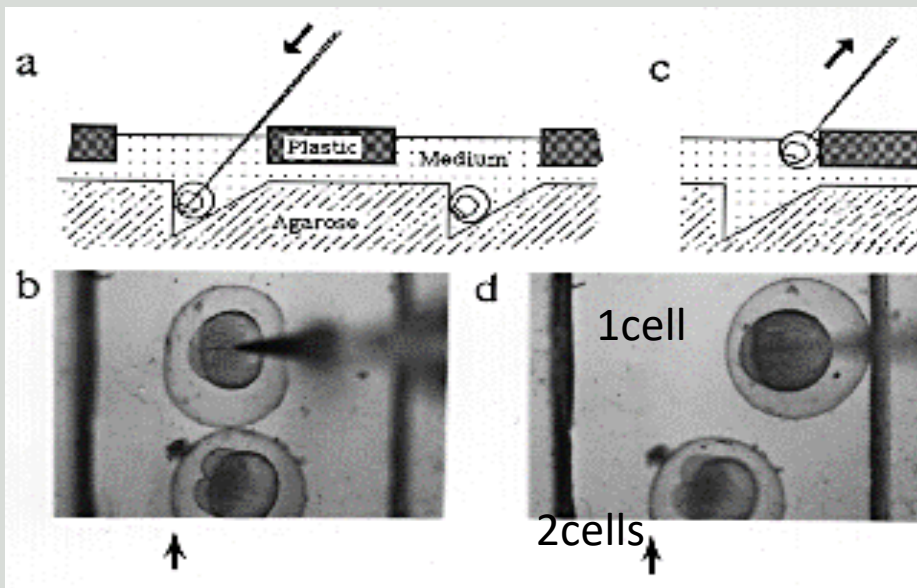
## Reverse genetics

Morpholino (antisense oligonucleotides) injections for knock-down

CRISPR nucleases knock-out / knock-in experiments (loss of function, functional verification of variants of unknown function)

## DNA injections to make transgenics

- naked linear DNA multiple integrations
- Tol2 transposase (from medaka) co-injection favors single integrations
- CRISPR mediated short homology directed targeted integration

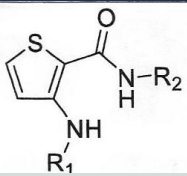


- Zebrafish allows for non invasive in vivo imaging of development and cardiovascular function.
- Endothelial shear-stress and intracardiac flow dynamics affect cardiac valve morphogenesis.
- Zebrafish valves can regenerate and Notch signaling induction is necessary for regeneration.

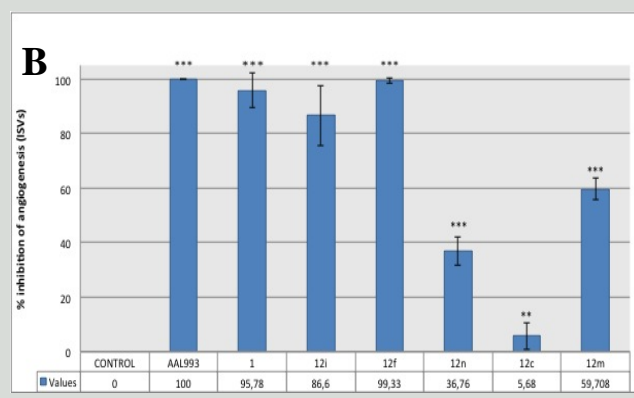
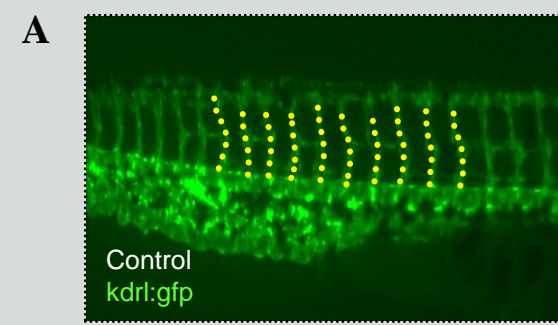
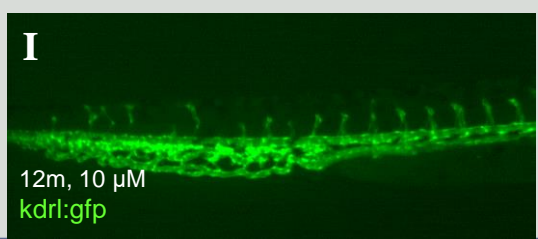
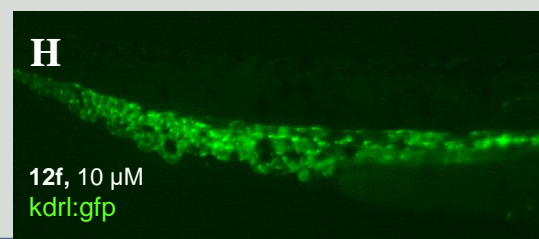
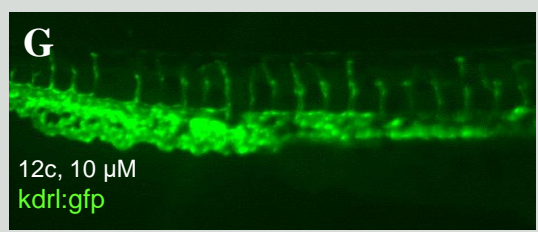
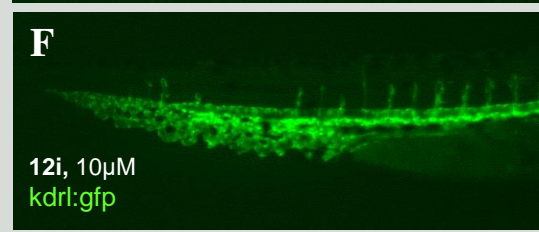
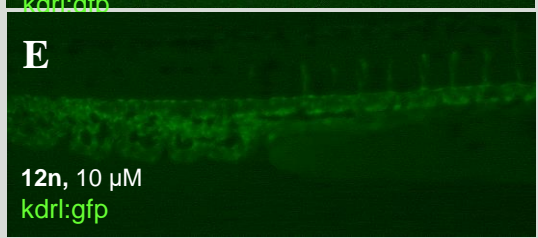
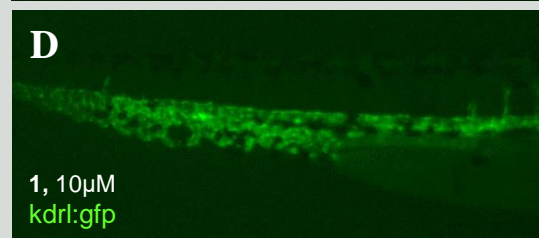
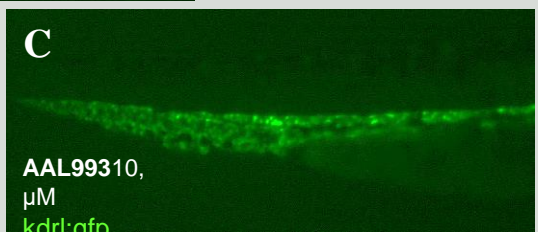
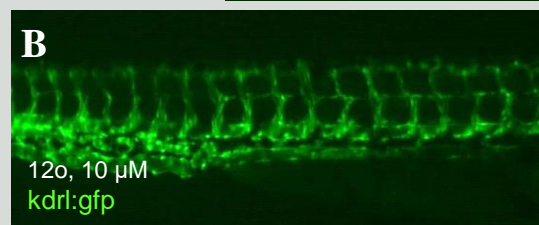
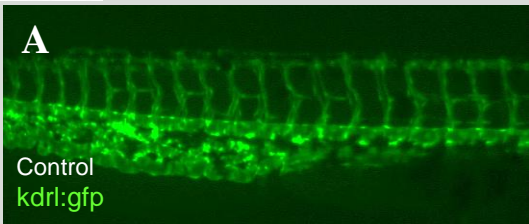
**Chemical genetic screens in zebrafish embryos can be used to identify novel bioactive compounds or optimize the activity of an existing compound.**

- Anti-Anxiety assays
- Inflammation response and resolution (neutrophils and macrophages kinetics)
- Cardiac Regeneration, Cardiac function, heart rate, arrhythmias, long QT syndrome
- Wound healing.
- Notch, Wnt and other reporter lines for signaling pathway modulators
- *In situ* hybridization assays of key transcription factors
- Melanogenesis, melanoma, neural crest development
- Angiogenesis
- Liver toxicity assays
- Alternative model of toxicology





A set of 90 compounds gave 7 new antiangiogenic molecules, using the transgenic line *kdrl:eGFP* (made in 2003, naked DNA injections)

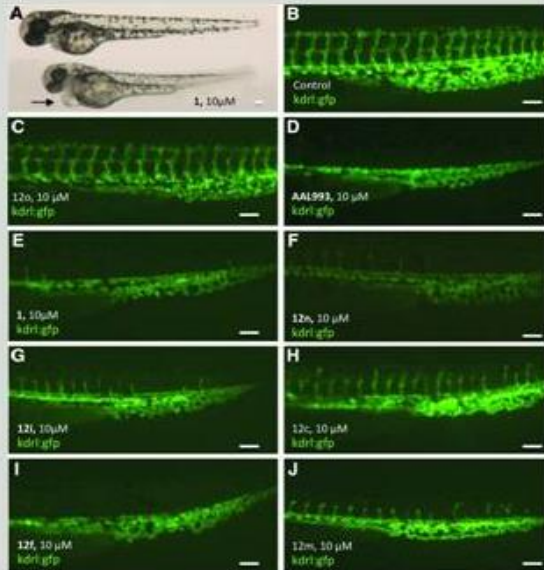


Papakyriakou et al. *Assay and Drug Development Technologies* 2014



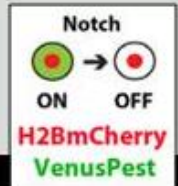
# Examples of *in vivo* phenotypic screening assays

## Angiogenesis

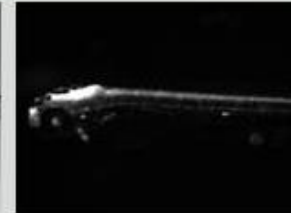


Papakyriakou et al 2014

## Notch signaling



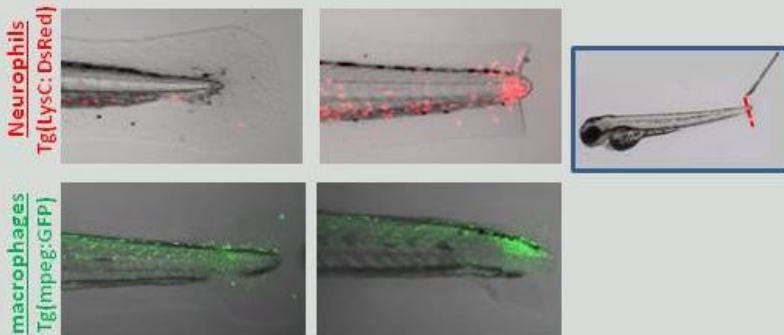
Control  
(DMSO)



DAPT  
50 μM

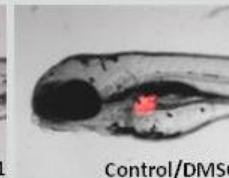
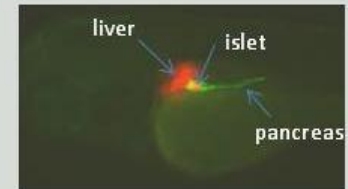


## Inflammation / wound healing

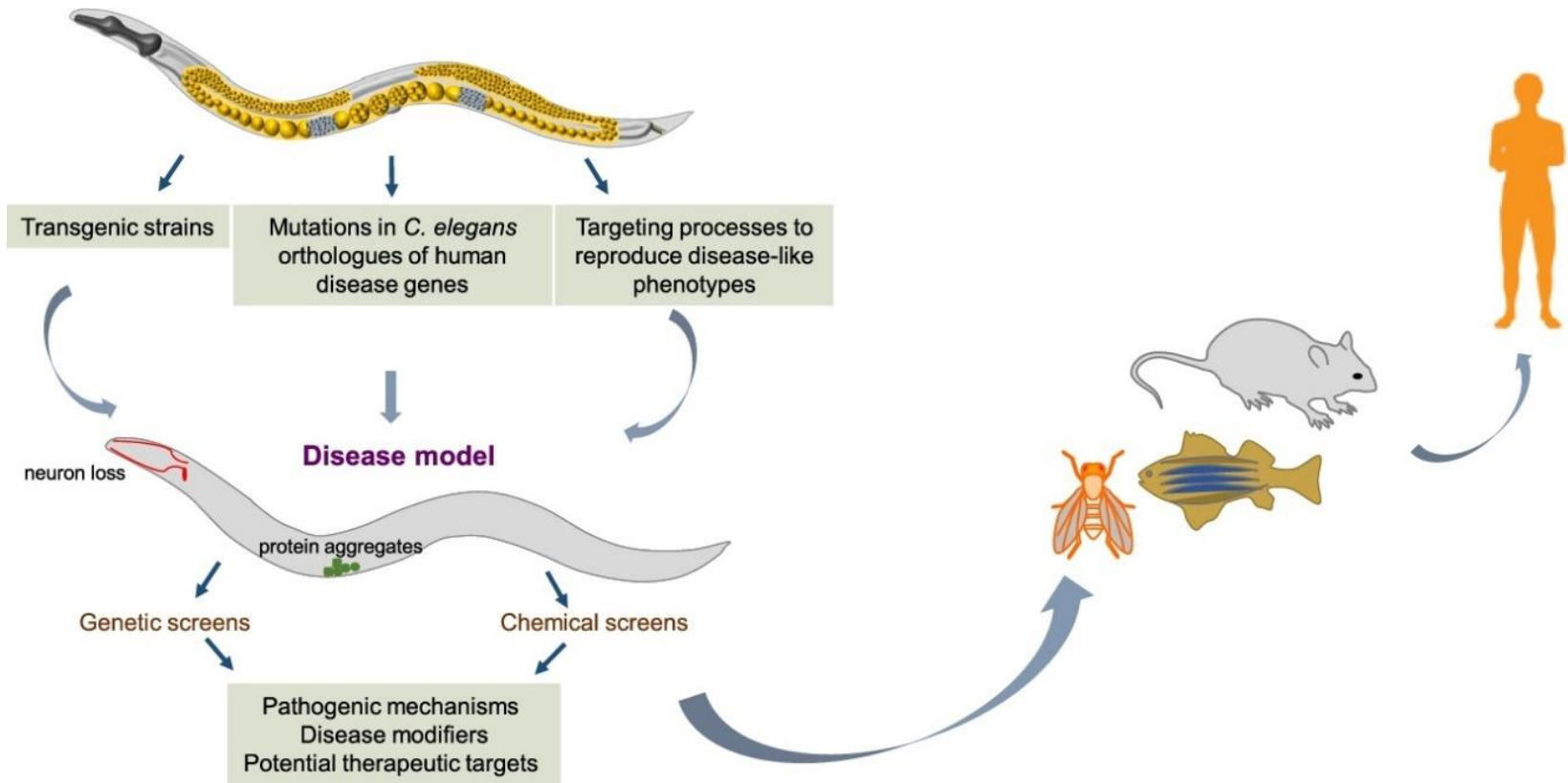


## Pancreas & Liver development

Tg(lfabp10:dsRed) → liver  
 Tg(ela3l:EGFP) → acinar pancreas (exocrine)  
 Tg( ins:dsRed) → pancreas islet (endocrine)



# C. elegans





# C. elegans

*C. elegans* offers a powerful platform to emulate key aspects of human pathology.



Focus on *C. elegans* models of the most common neurodegenerative disorders.



Worm models have provided critical insights into mechanisms underlying disease.



Unravelling disease pathogenesis identifies novel candidate therapeutic targets.



Evolutionary conservation between *C. elegans* and humans offers *in vivo* target validation.

