Genetically modified animals in biomedical research

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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

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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

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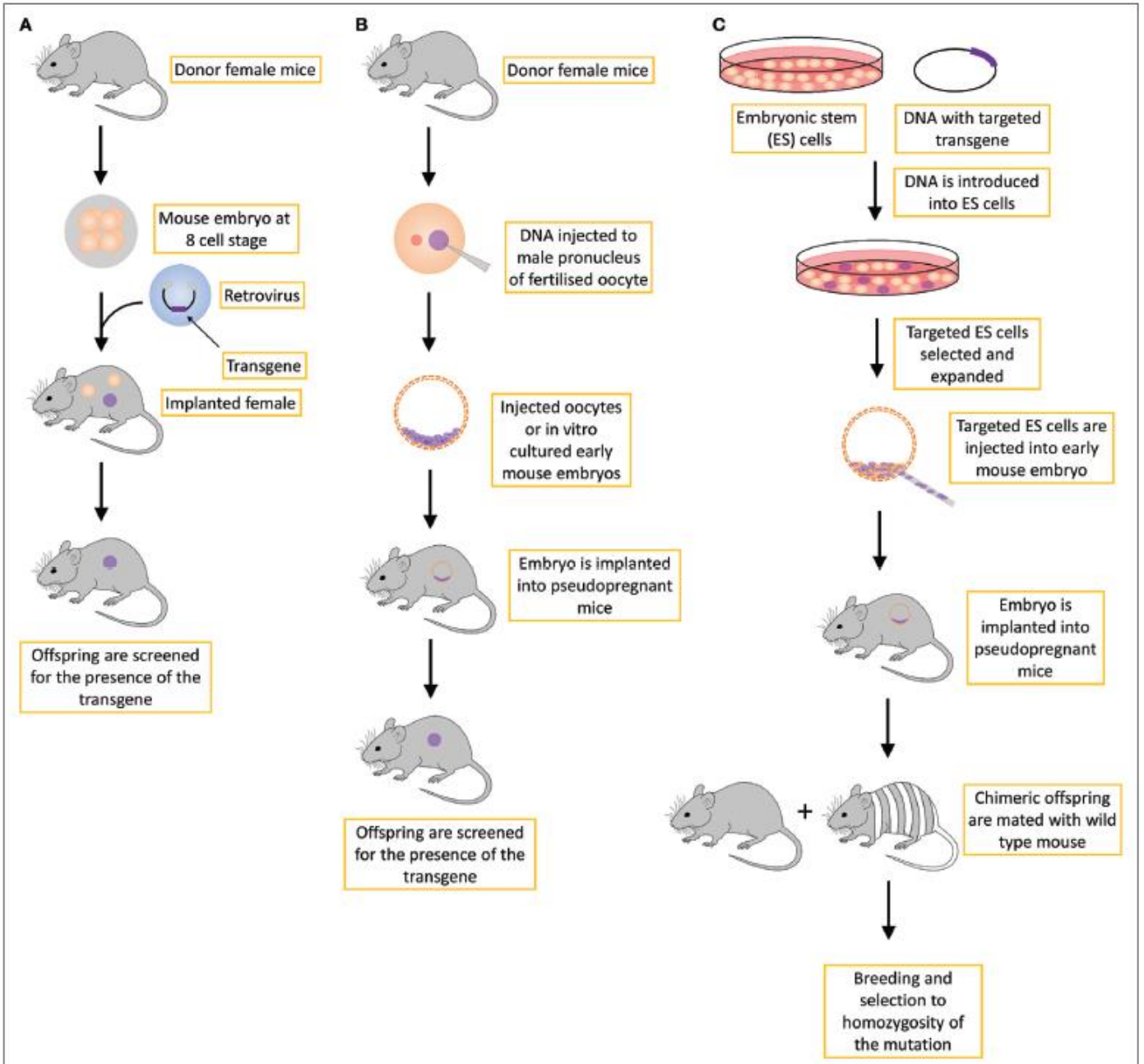
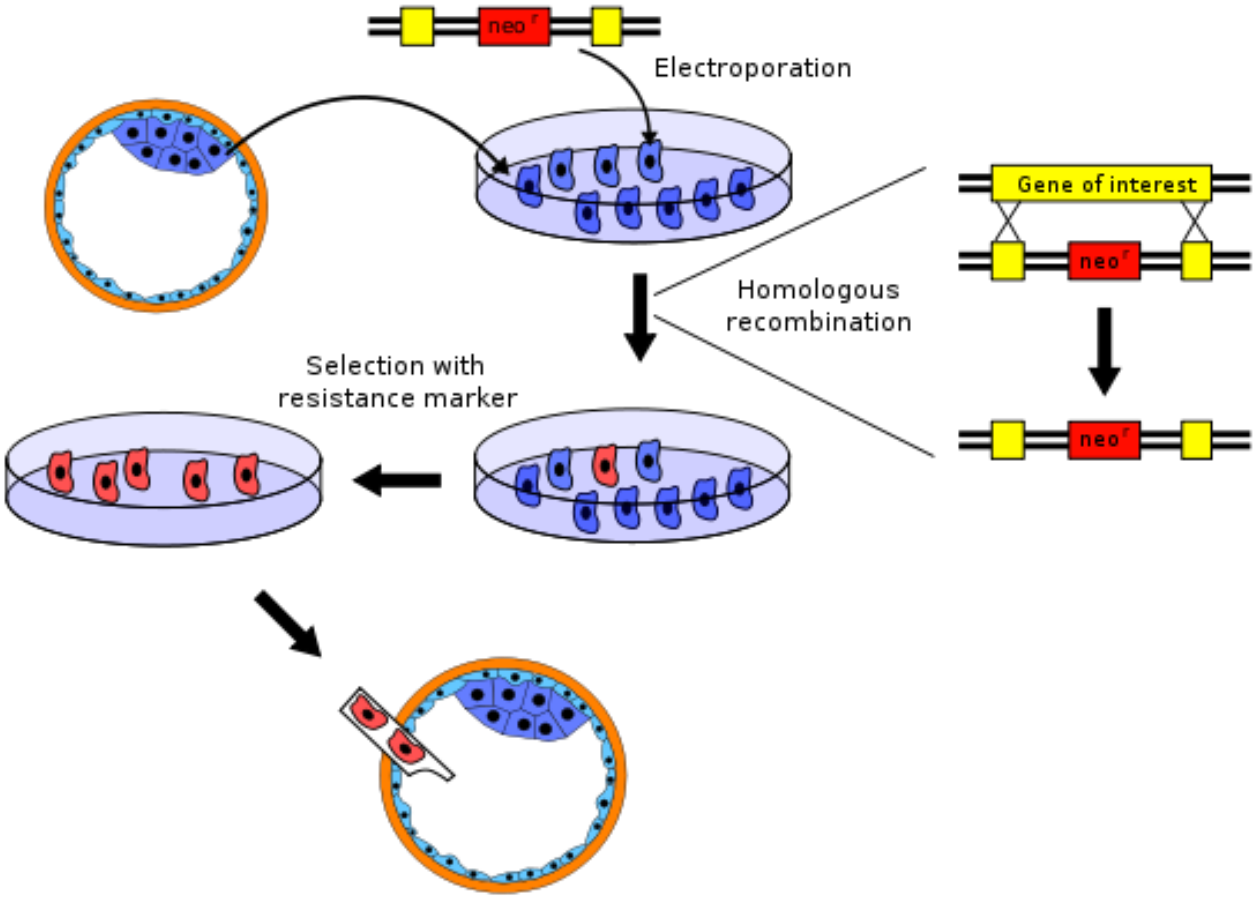
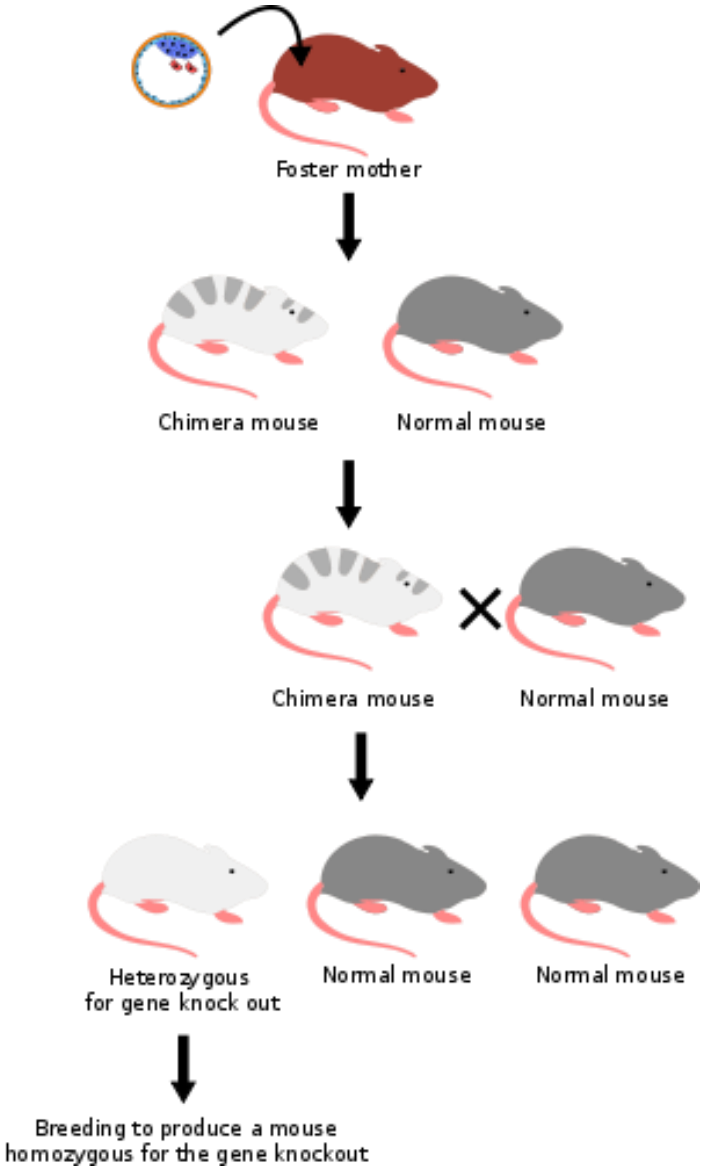


FIGURE 1 | Different strategies for creating transgenic mice include the **(A)** retroviral approach, which is not routinely used; **(B)** standard transgene approach, in which the DNA is inserted into the genome in an unspecific manner; and **(C)** gene-targeted transgene approach, which is an approach 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.

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".

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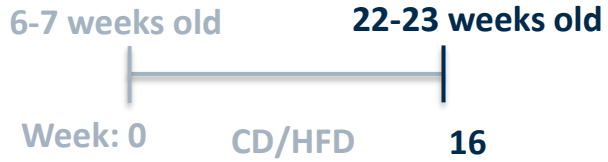
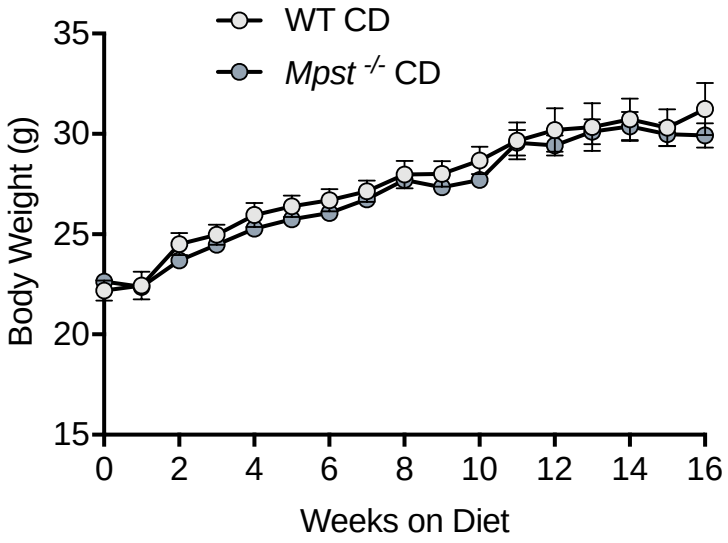
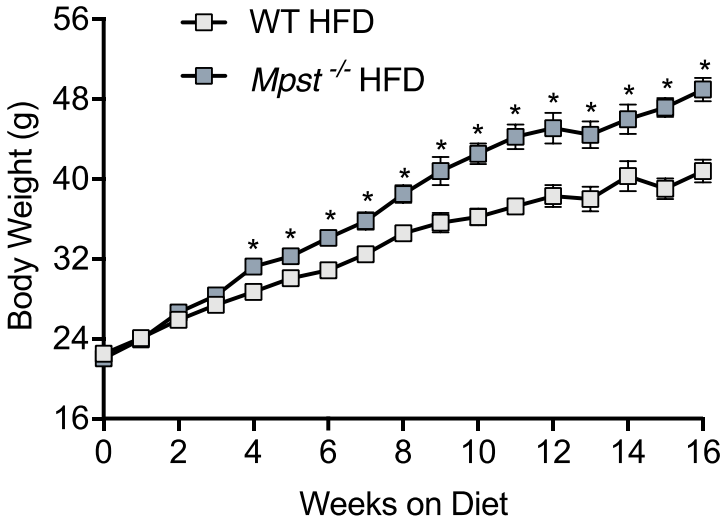
Baseline vs disease conditions

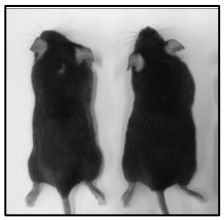
Mpst$^{-/-}$ mice gain more weight on HFD in comparison with WT mice



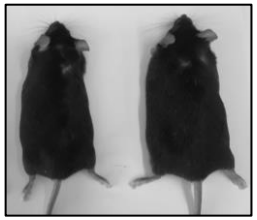




CD

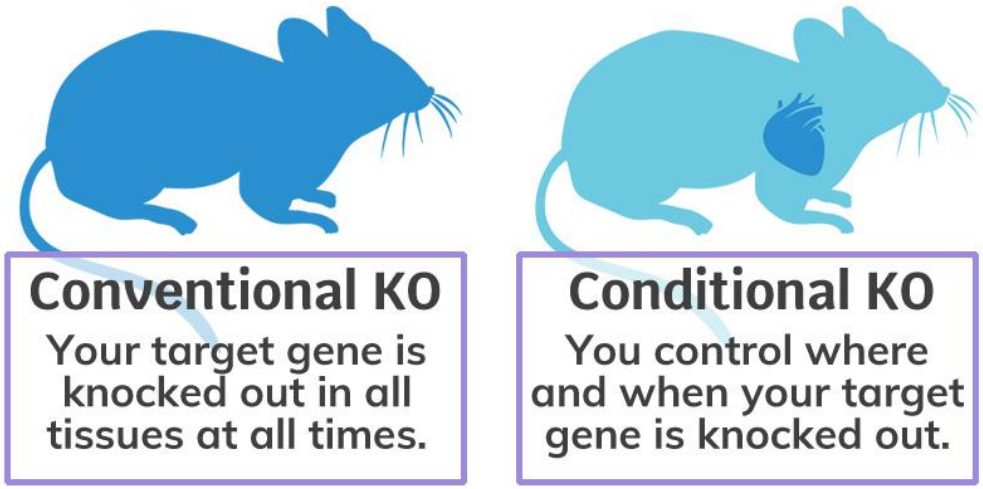


HFD



Problems with global KOs

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



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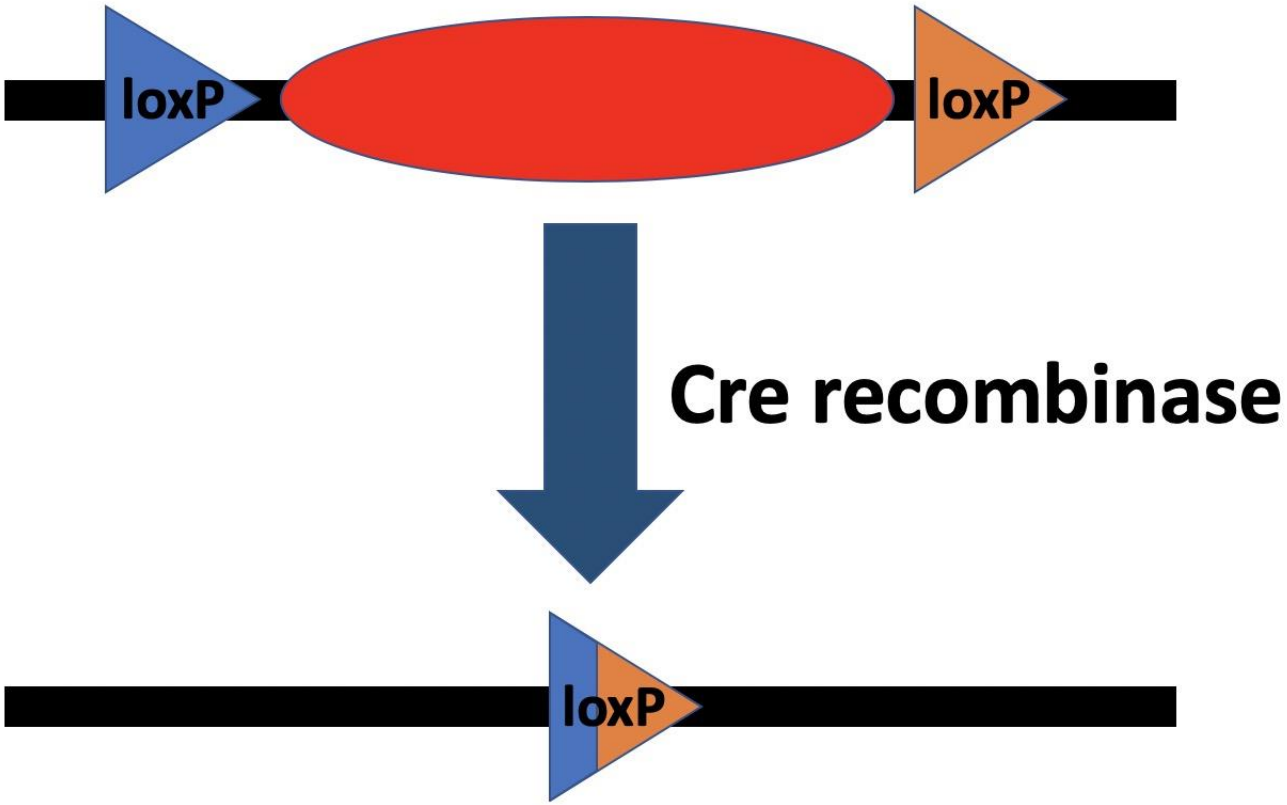
Cre recombinase



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
β-GHRKO	Pancreatic β-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…

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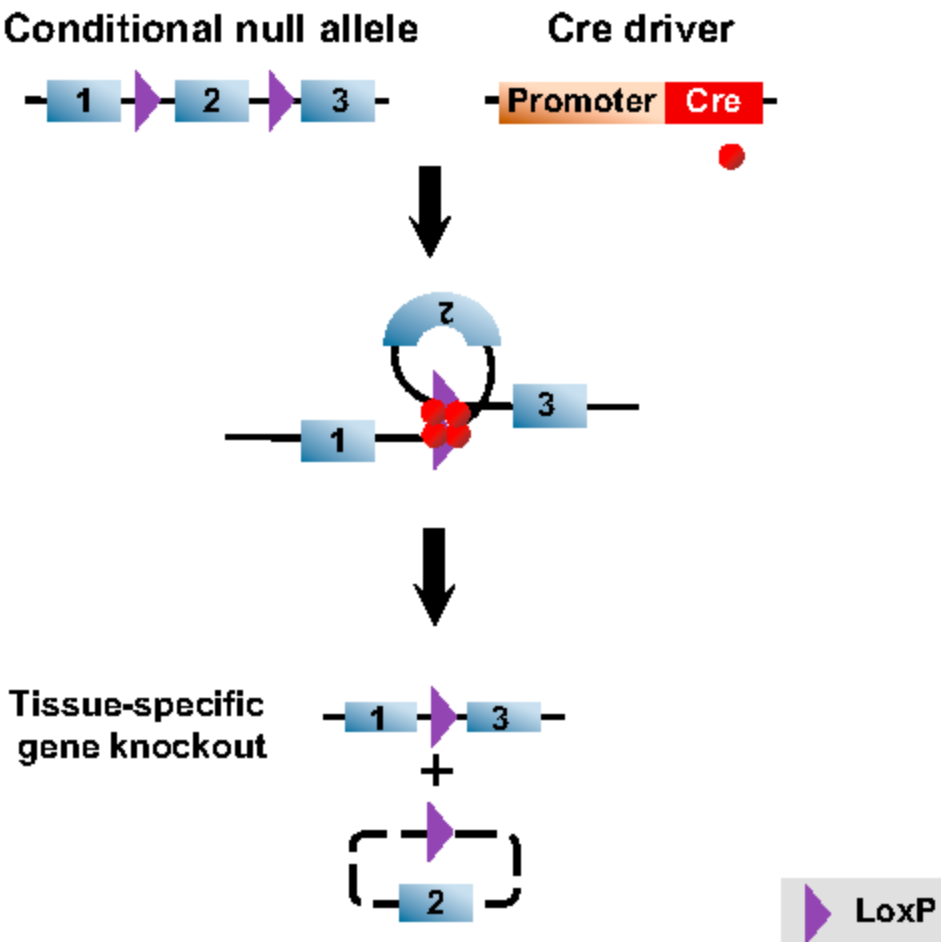
Cre-lox mice (conditional)



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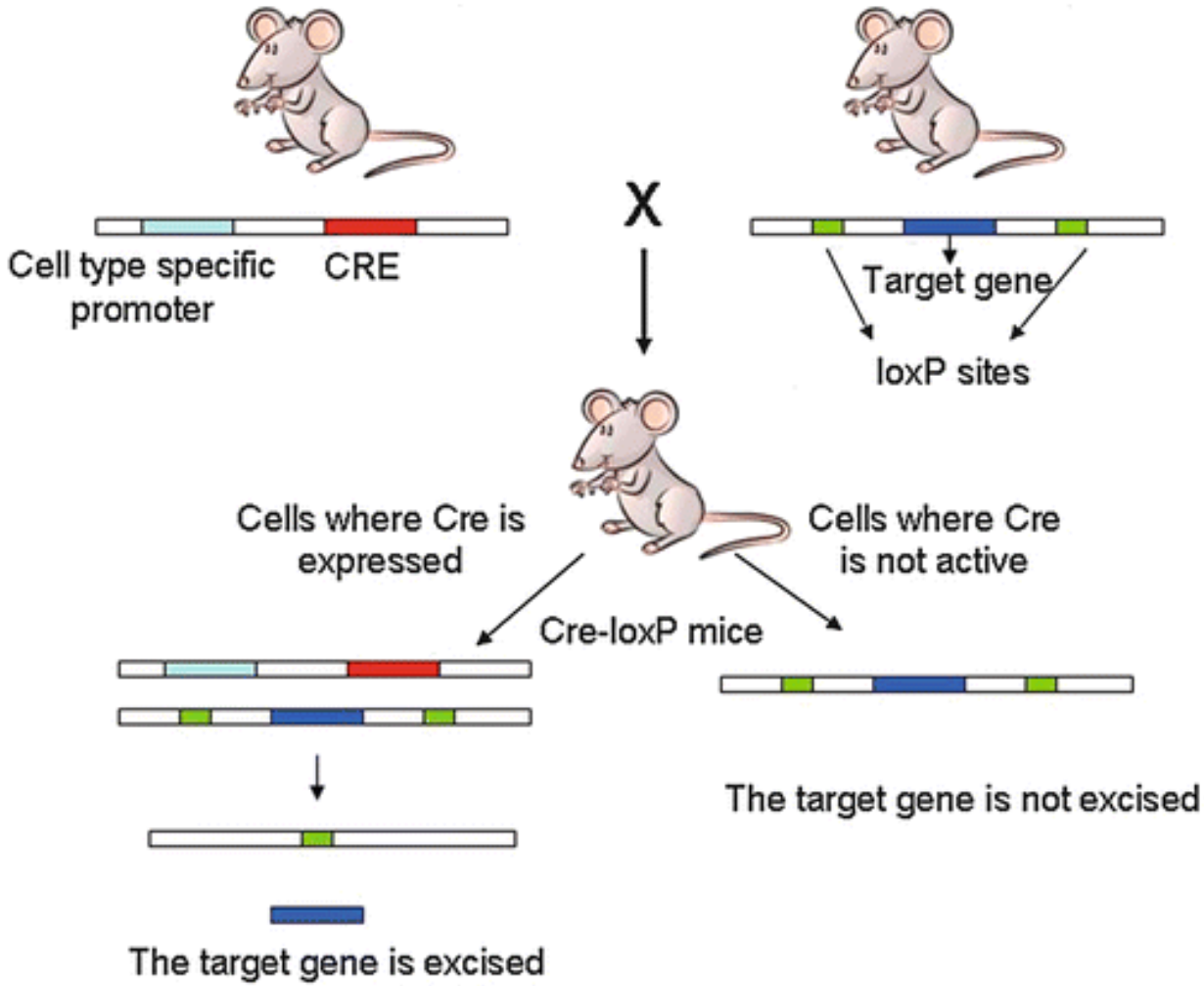
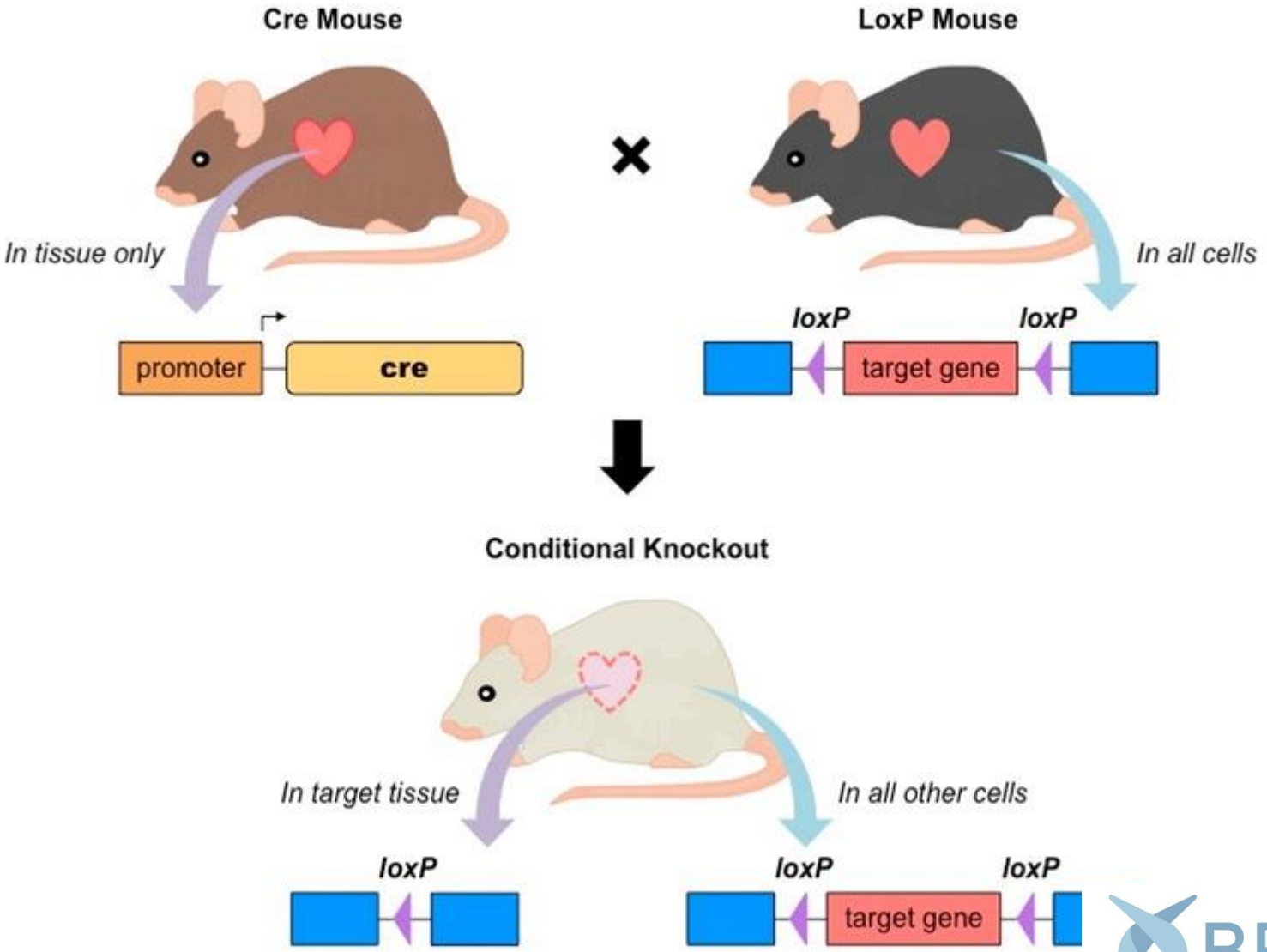


Conditional KO (spatial)



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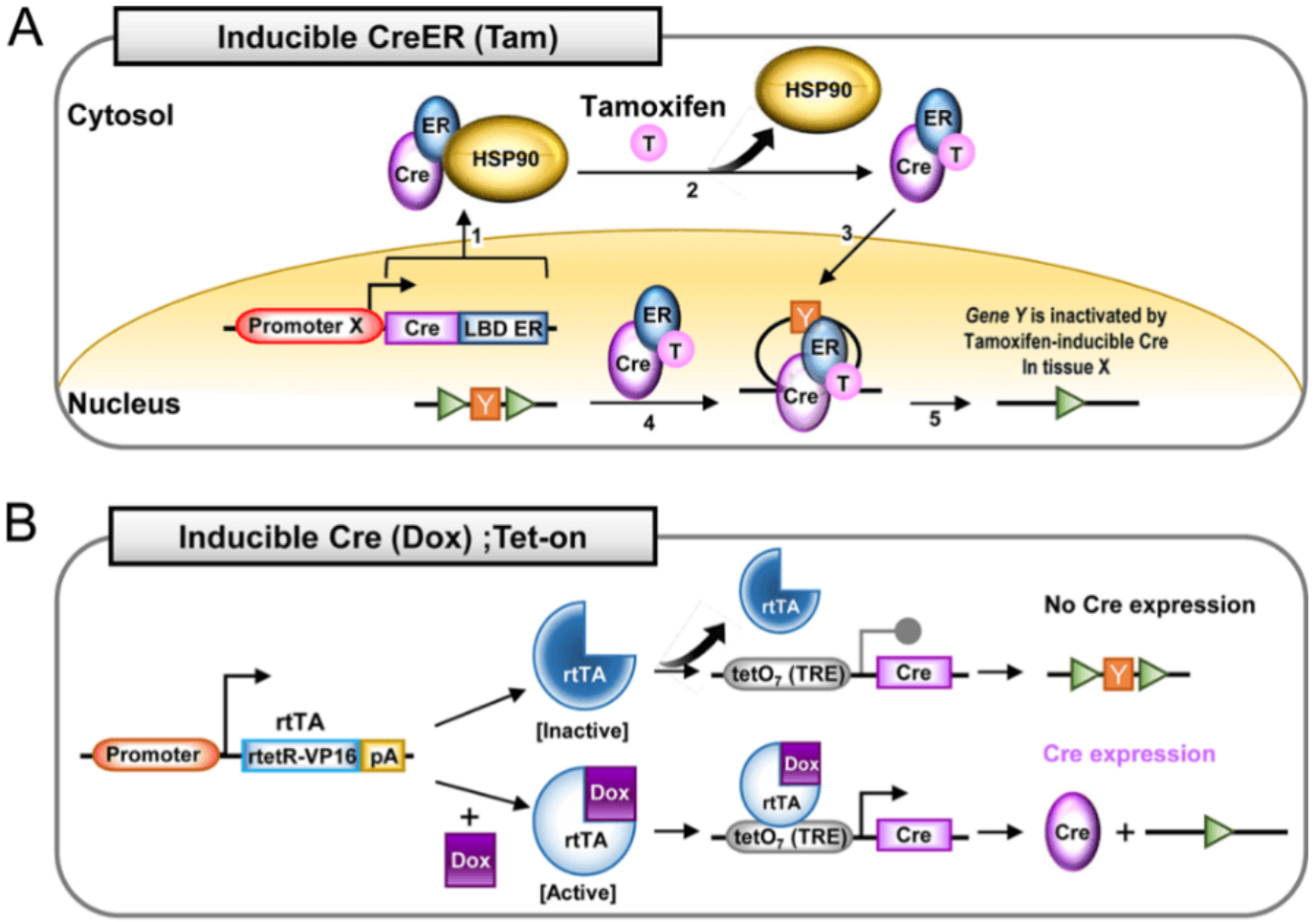
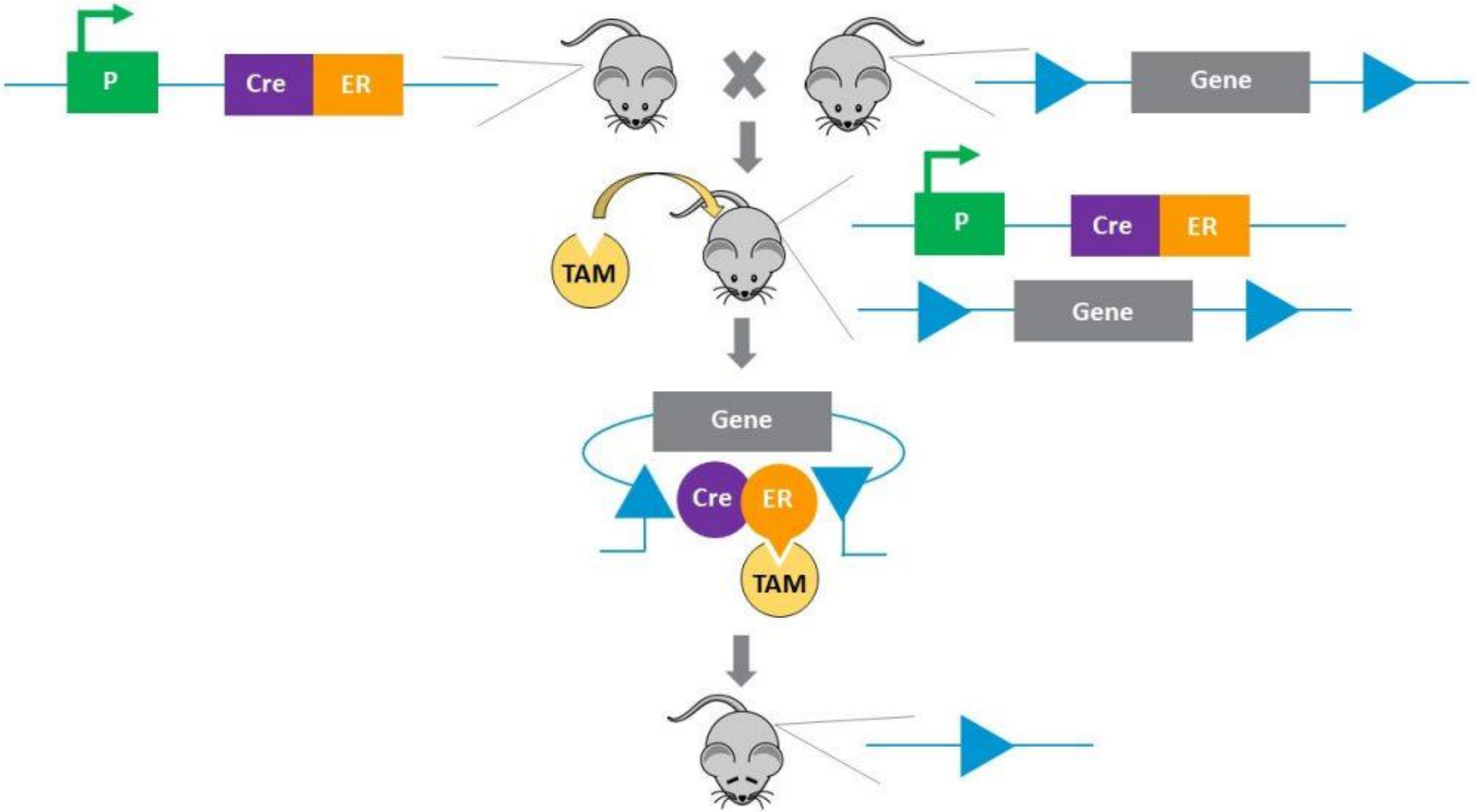
Inducible KO (temporal)

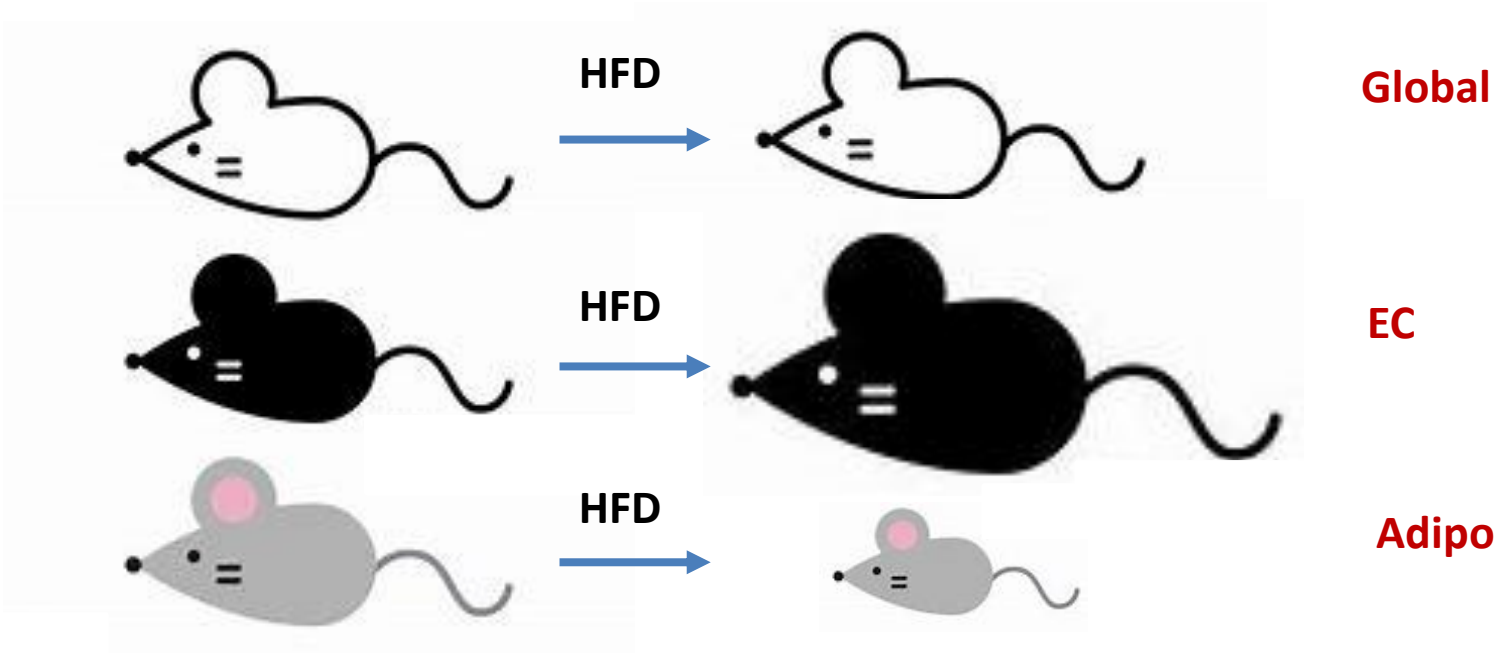


Inducible KO (temporal)



Differences in global vs conditional KO

The example of cystationine gamma lyase



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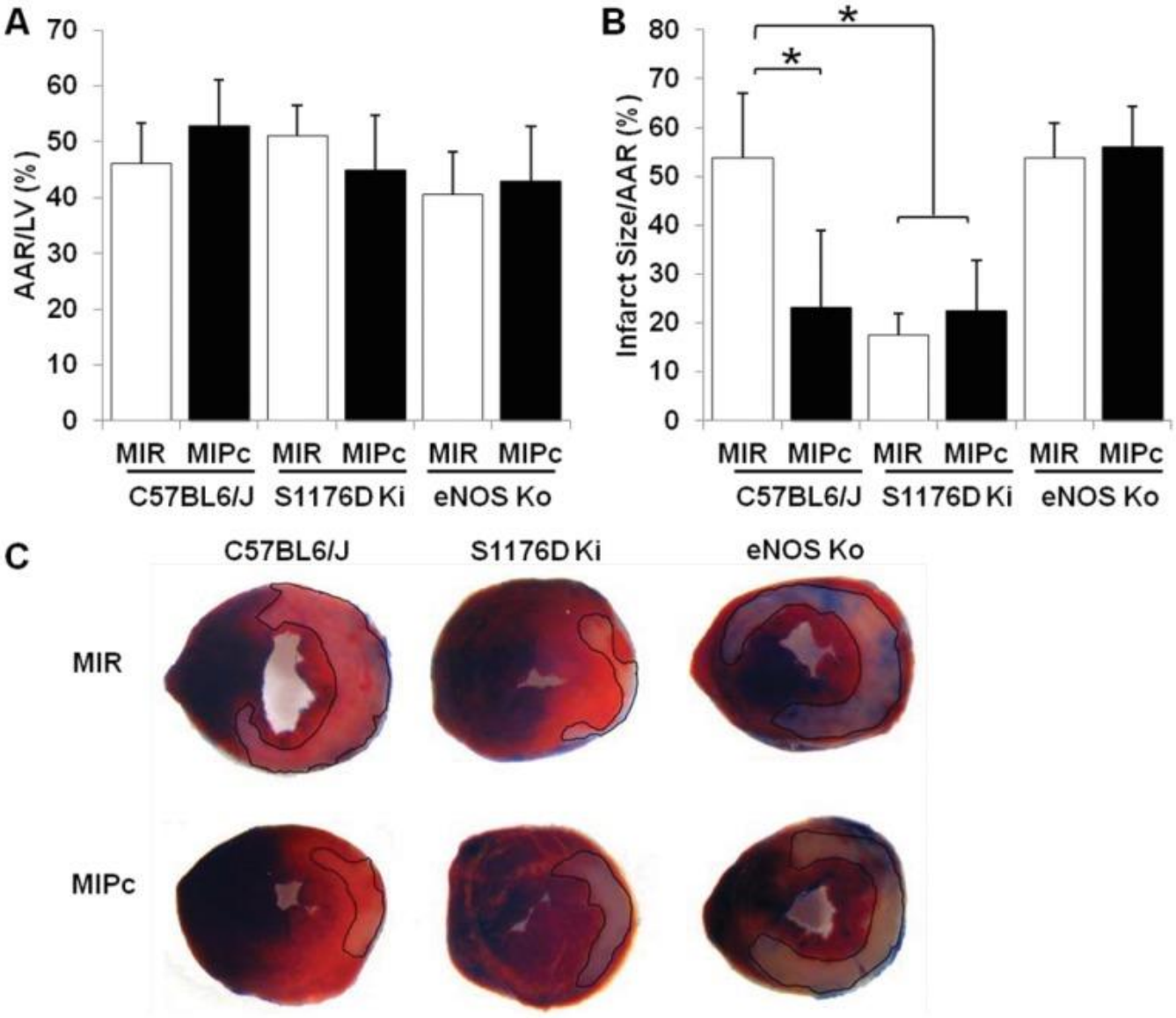
KiKi mice

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 promotors to the production of therapeutically useful humanized antibodies.

4. Might express non-functional or mutated protein


A
AAR/LV (%)
70
60
50
40
30
20
10
0
MIR MIPc MIR MIPc MIR MIPc
C57BL6/J S1176D Ki eNOS Ko
B
Infarct Size/AAR (%)
80
70
60
50
40
30
20
10
0
*
*
MIR MIPc MIR MIPc MIR MIPc
C57BL6/J S1176D Ki eNOS Ko
C
C57BL6/J
S1176D Ki
eNOS Ko
MIR
MIPc

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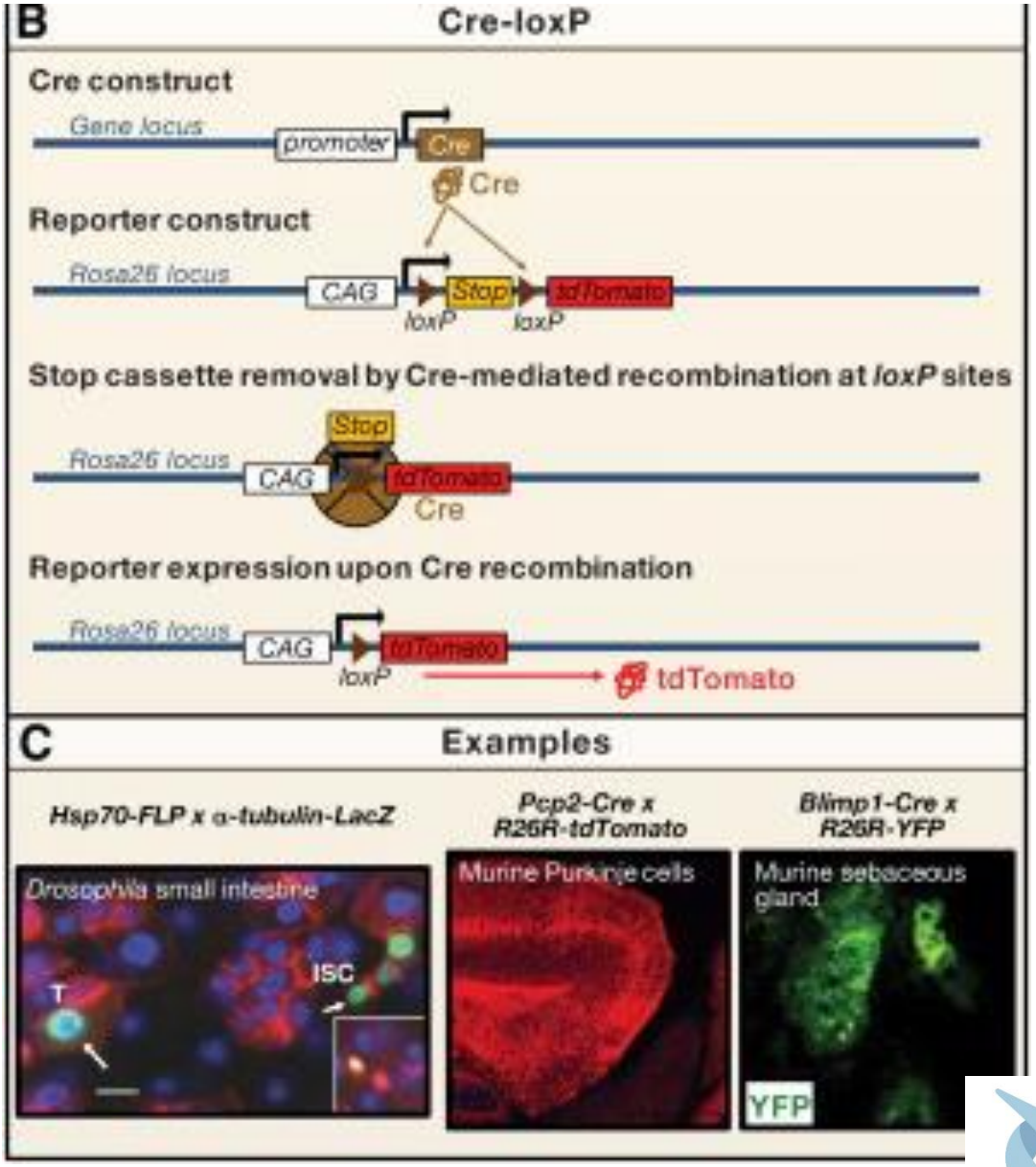
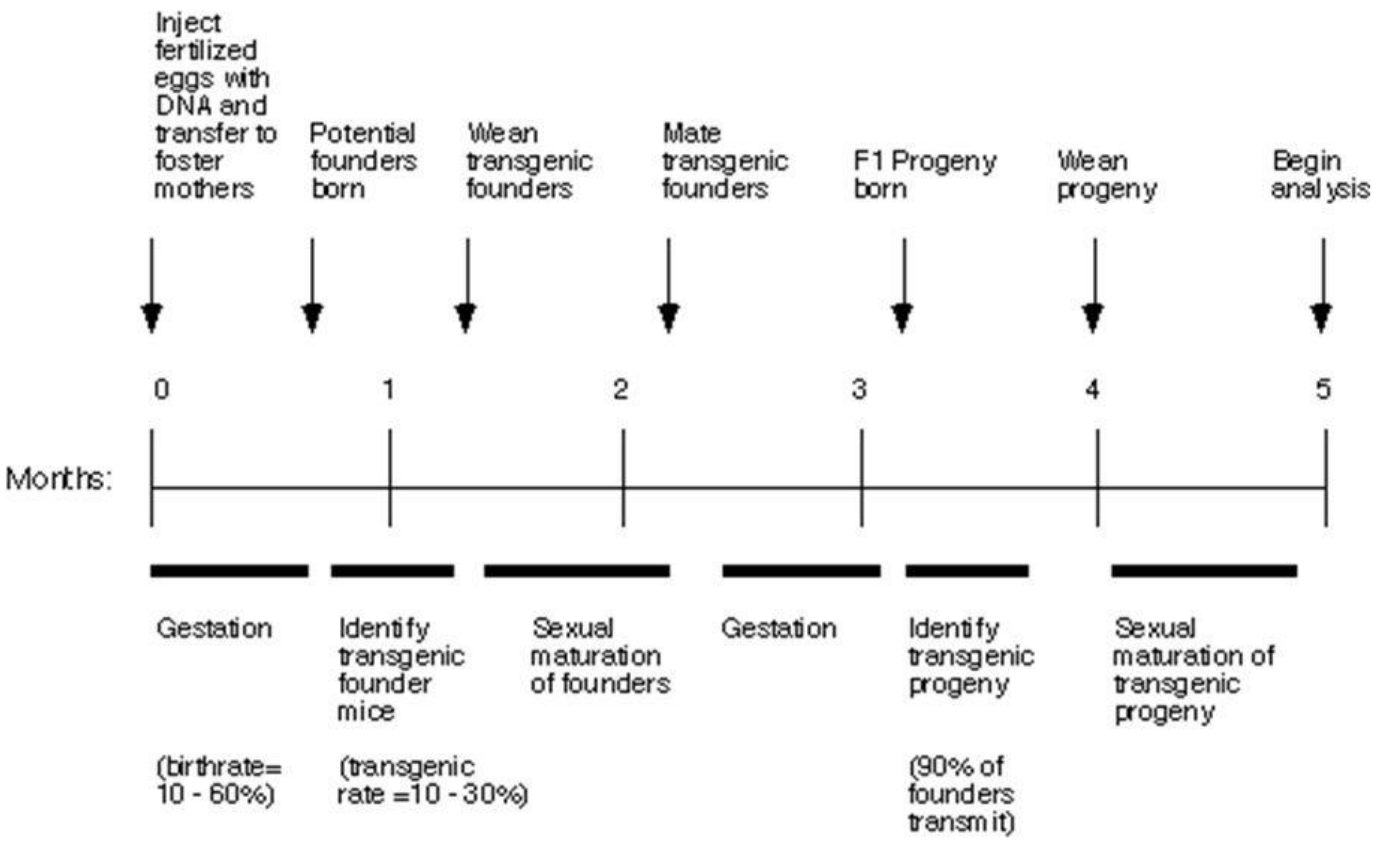


Timeline for Transgenic Mouse Analysis



Resources

https://www.mousephenotype.org/martsearch_ikmc_project/

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Drosophila

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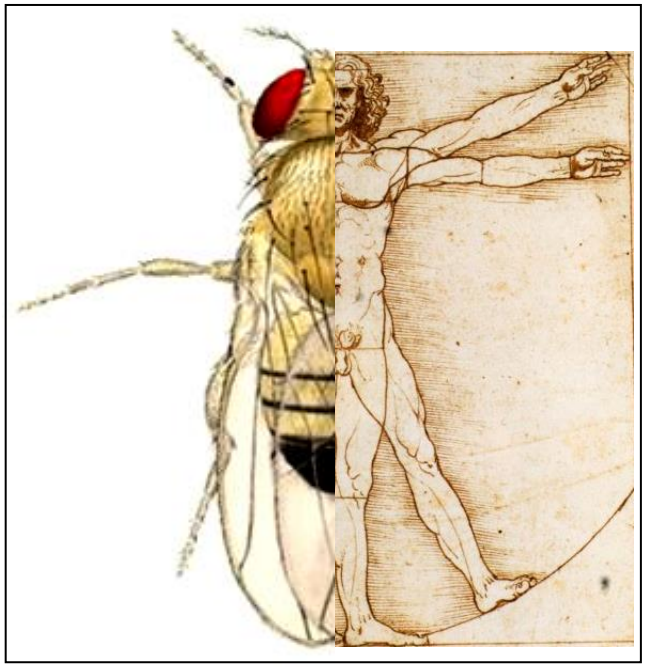
Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery

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.

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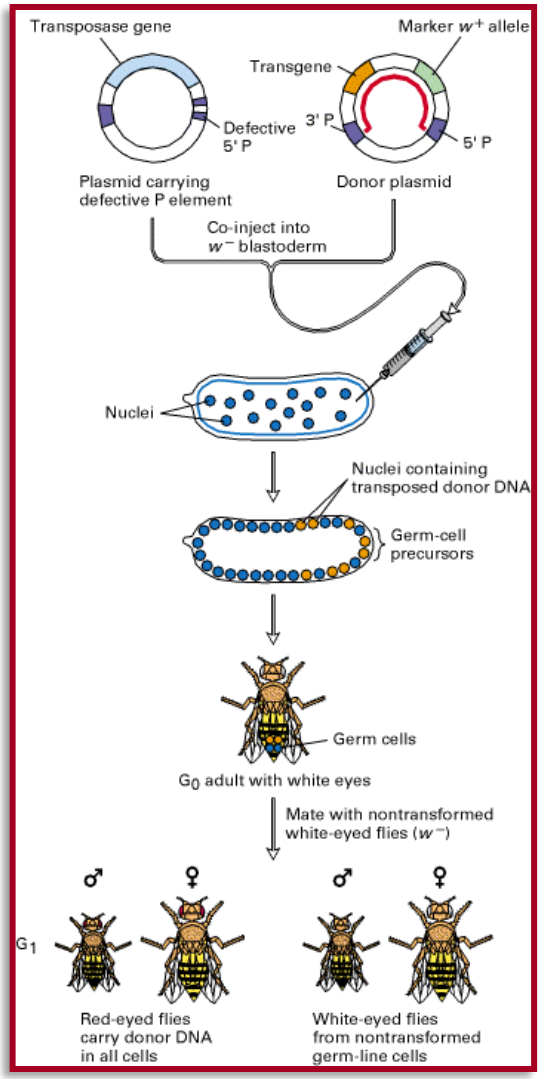
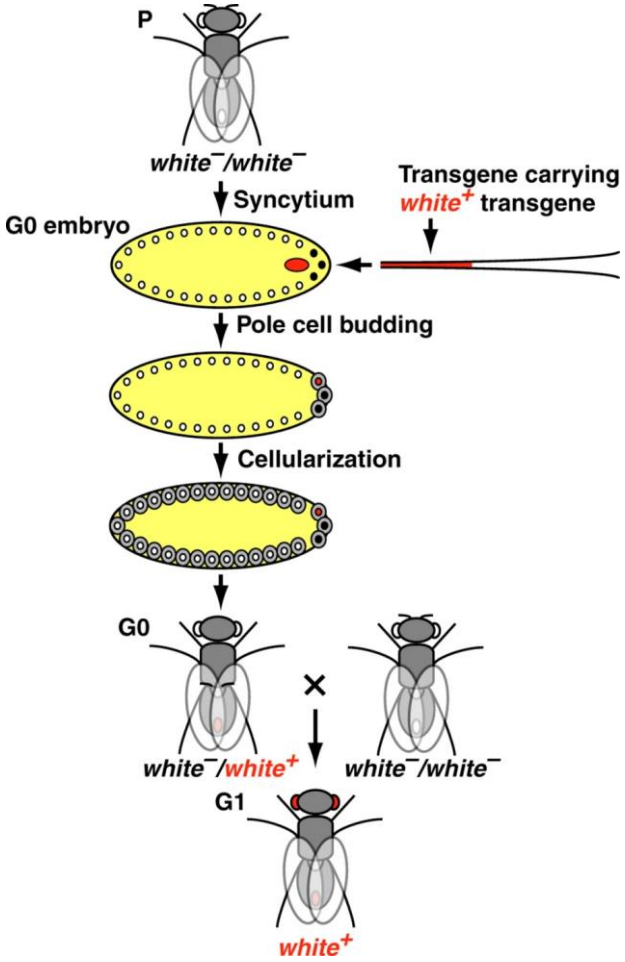
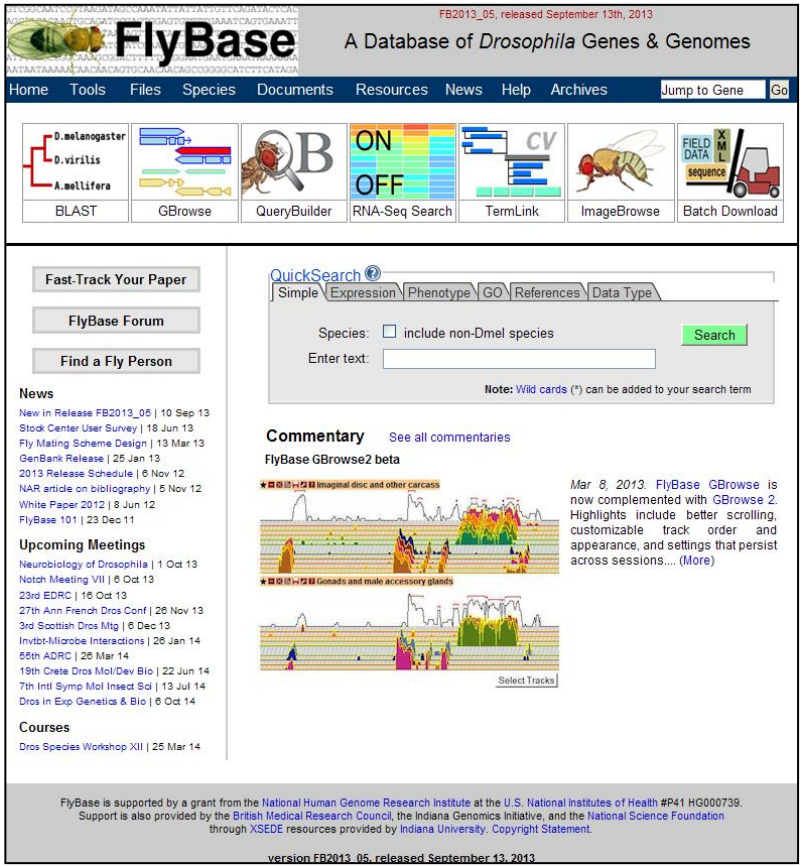
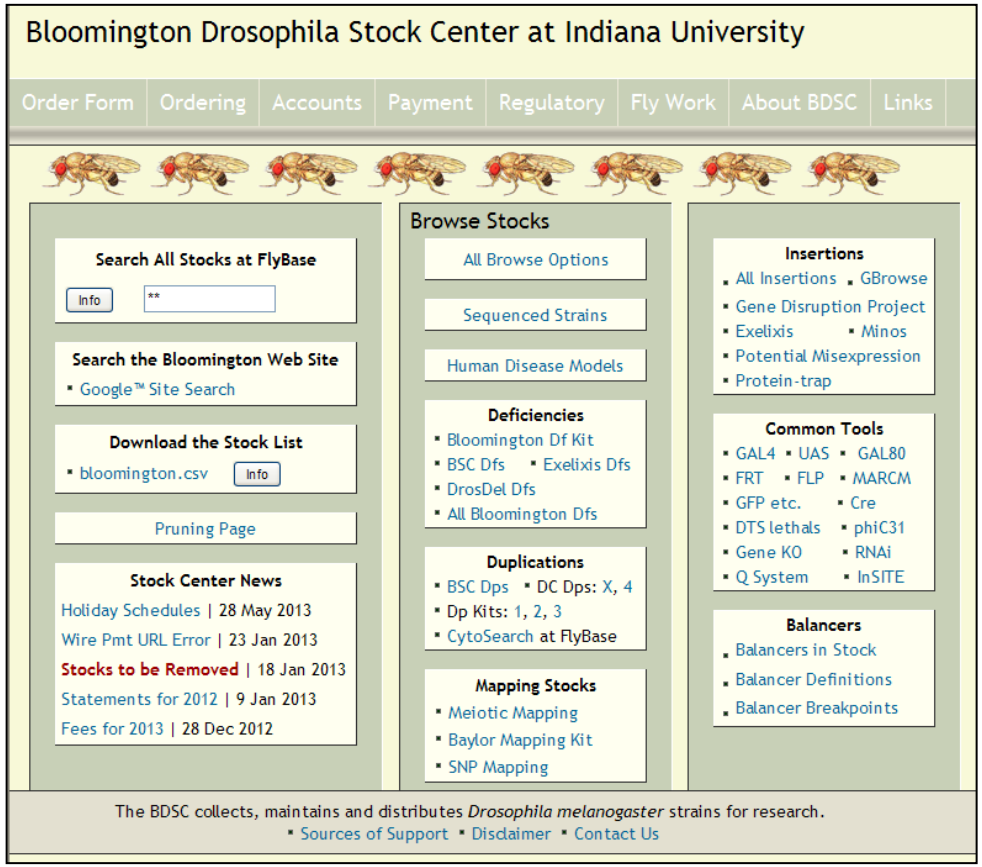


Generation of transgenic fruit flies by P-element transformation. The P element, a mobile genetic element, can move from one place in the genome to another. This movement (transposition) is catalyzed by transposase, which is encoded by the P element; the 3' and 5' ends of the P element are recognized 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 G0 adults that develop from injected embryos have white eyes. Mating of these flies with white-eyed flies will yield some G1 red-eyed progeny carrying the transgene and the marker allele *(w+)* in all cells.







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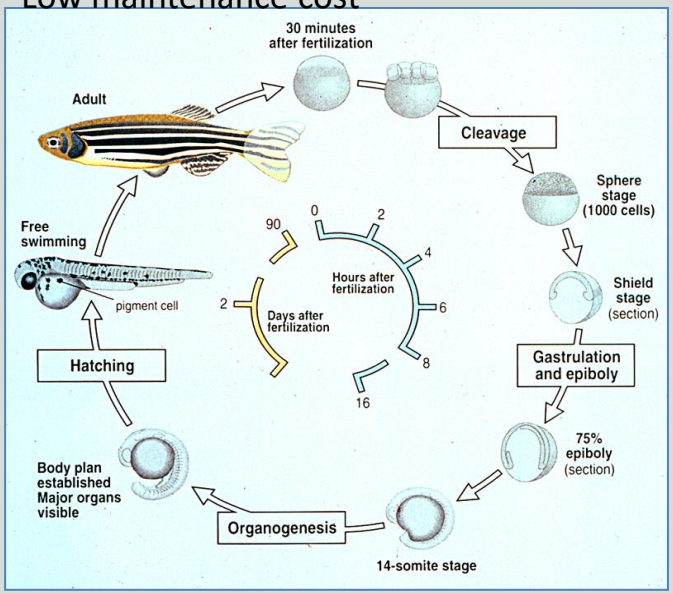
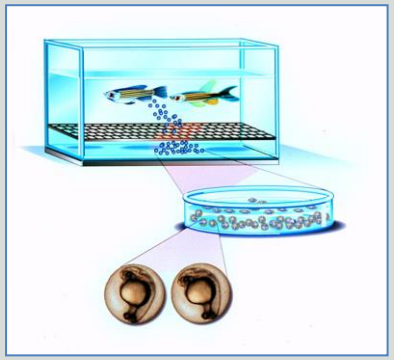
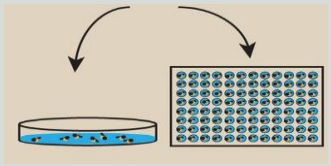
Zebrafish Advantages

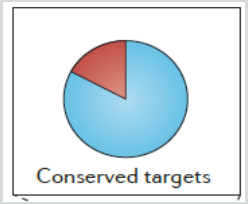
- ✓ Vertebrate
- ✓ Small size
- ✓ Fast reproduction and development
- ✓ External fertilization
- ✓ Transparency
- ✓ Permeability to small molecules
- ✓ Low maintenance cost

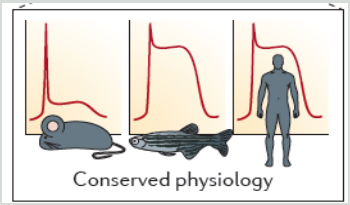






- ✓ High genetic homology with human
- ✓ Conserved targets & physiology
- ✓ Broad range of accessible biology
- ✓ High-throughput screens
- ✓ No need for bioethics approval up to 5dpf





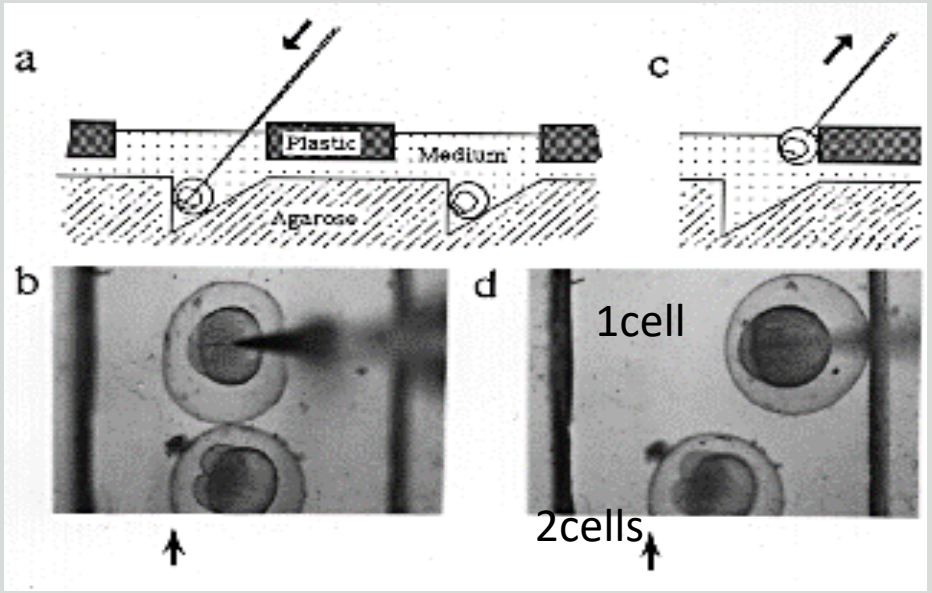
MacRae & Peterson, 2015

RNA injections (for overexpression, gain of function) from 1cell-stage due to external fertilization (30 minutes to 1st 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 <u>targeted</u> integration**

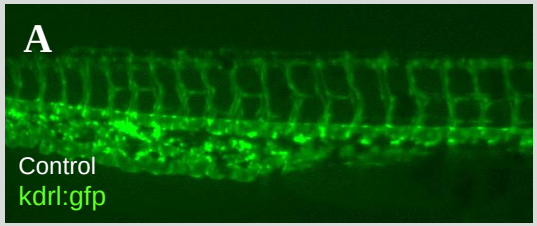


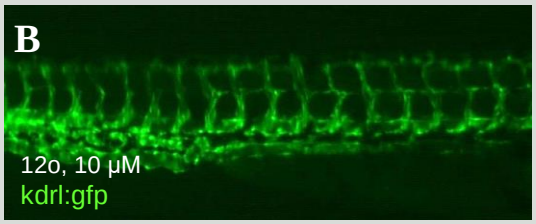
- Zebrafish allows for non invasive in vivo imaging of development and cardiovascular function.
- Endothelial shear-stress and intracardiac flow dynamics affect cardiac valve morphogenesis.
- Zebrafish valves can regenerate and Notch signaling induction is necessary for regeneration.

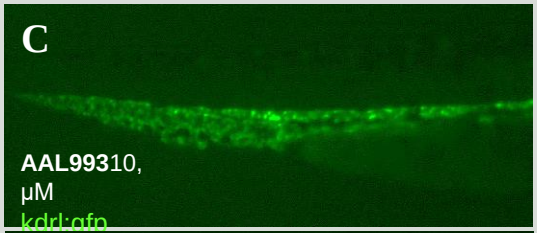
Chemical genetic screens in zebrafish embryos can be used to identify novel bioactive compounds or optimize the activity of an existing compound.

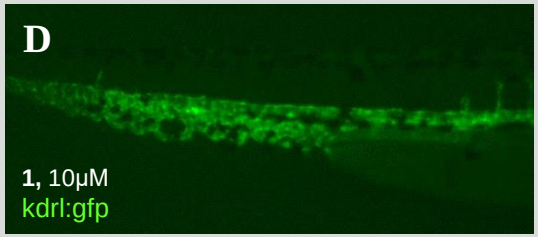
- Anti-Anxiety assays
- Inflammation response and resolution (neutrophils and macrophages kinetics)
- Cardiac Regeneration, Cardiac function, heart rate, arrhythmias, long QT syndrome
- Wound healing.
- Notch, Wnt and other reporter lines for signaling pathway modulators
- *In situ* hybridization assays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

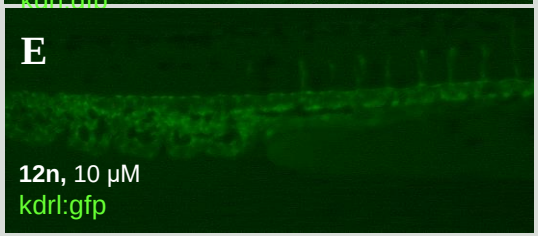
A set of 90 compounds gave 7 new antiangiogenic molecules, using the transgenic line *kdrl:eGFP (made in 2003, naked DNA injections)*

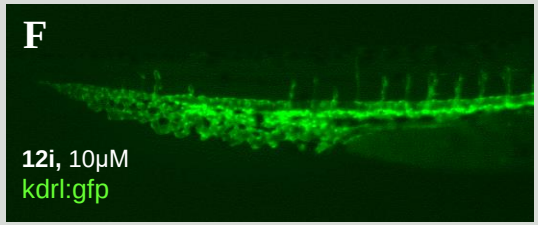


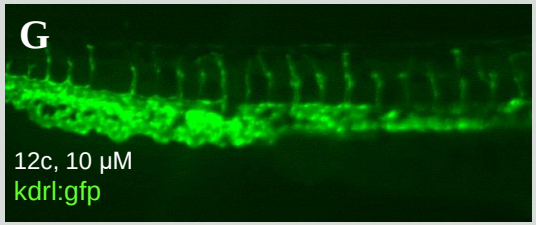


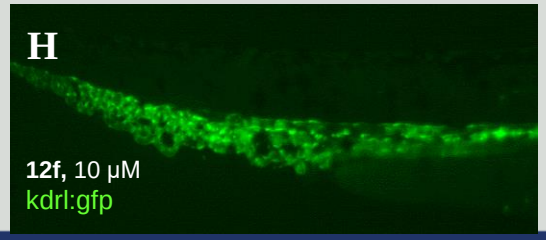


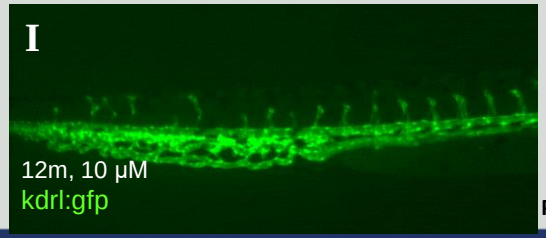




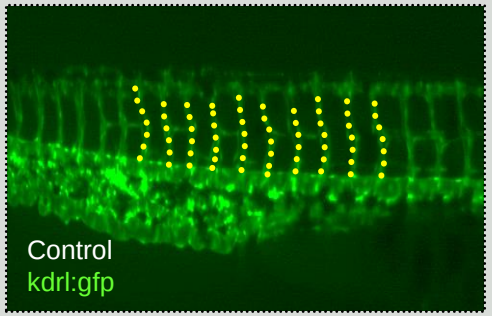




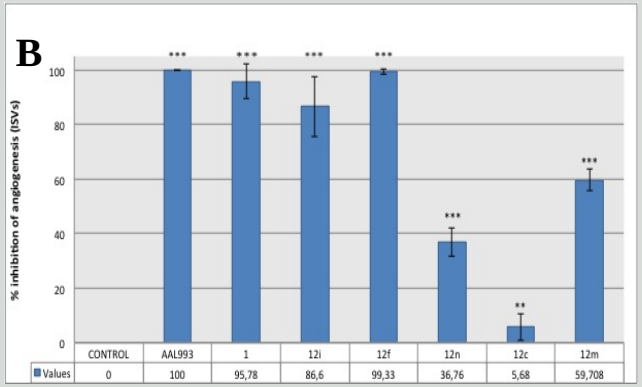




A



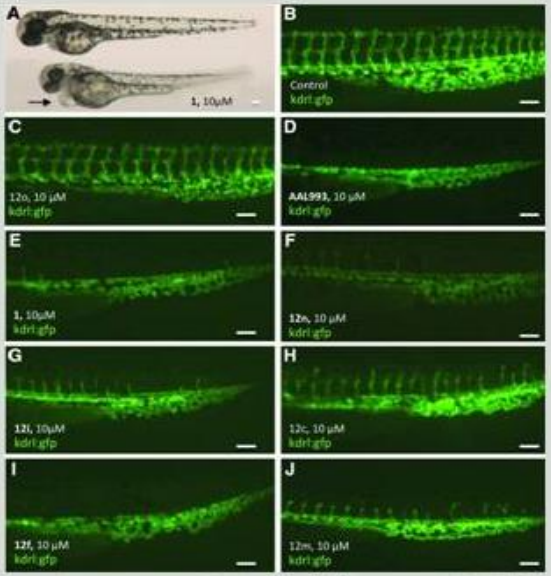
B



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

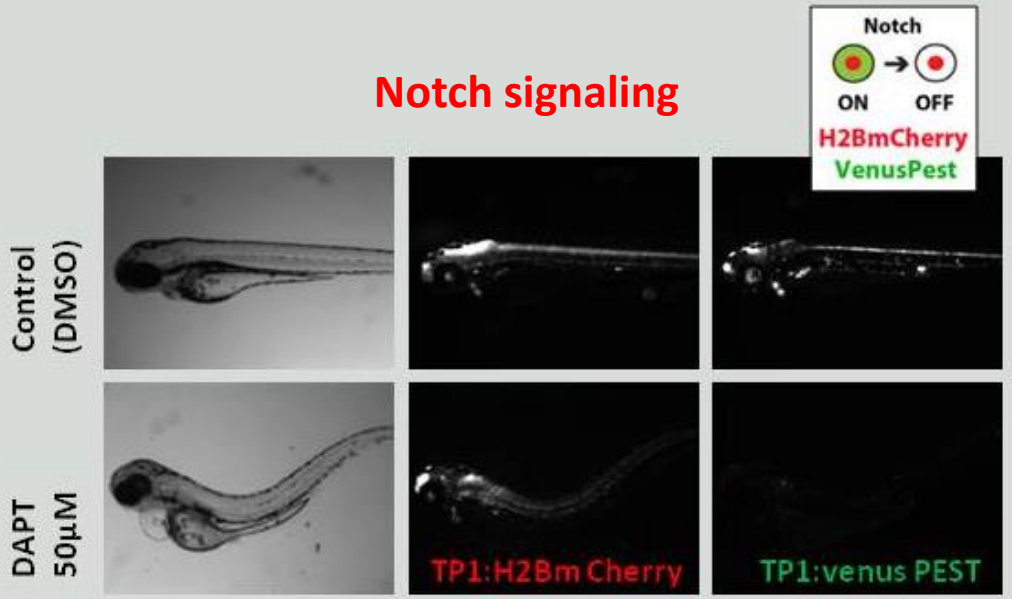
Examples of *in vivo* phenotypic screening assays

Angiogenesis

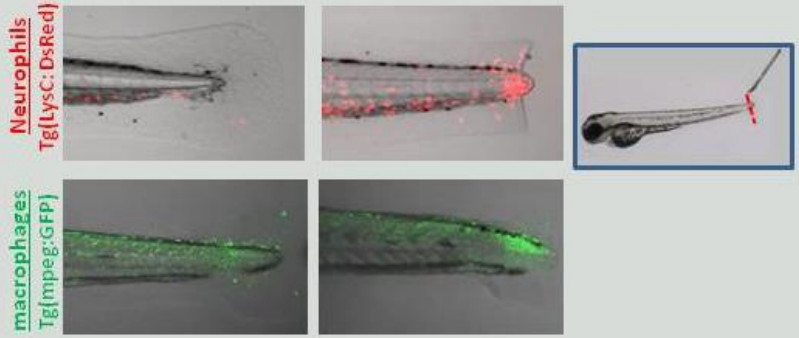


Papakyriakou et al 2014

Notch signaling

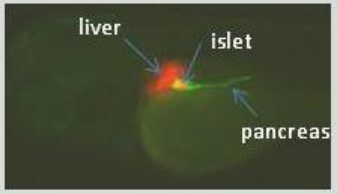


Inflammation / wound healing

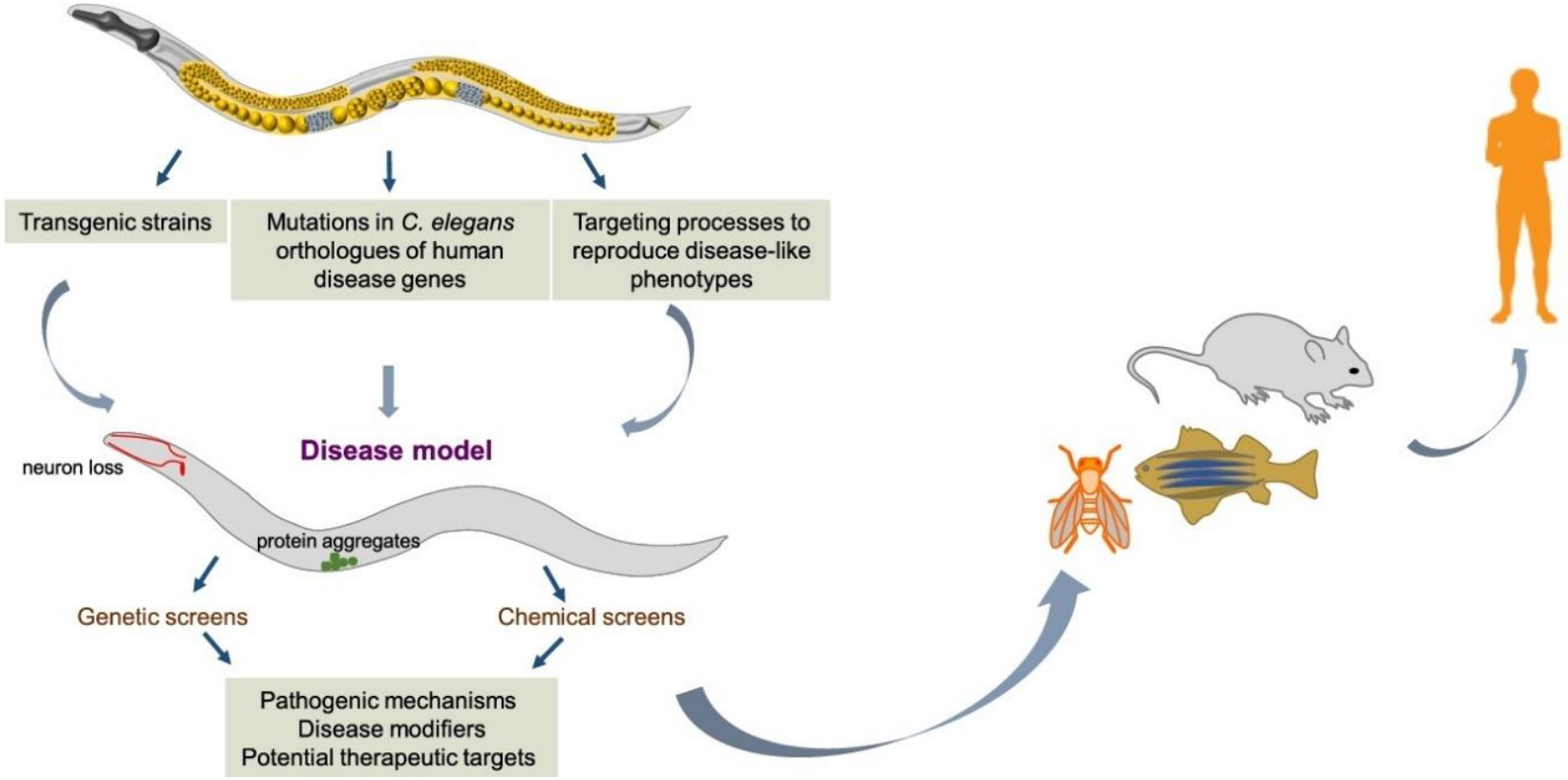


Pancreas & Liver development

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.

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