Model organisms: Mouse

Hans Bluyssen, 07.03.2023

What are model organisms?

Model organisms are non-human species that are used in the laboratory to help scientists understand biological processes.

A model organism is a species that has been widely studied, usually because it is easy to maintain and breed in a laboratory setting and has particular experimental advantages.

Why are model organisms useful in genetics research?

Many model organisms can breed in large numbers. Some have a very short generation time, so several generations can be followed at once.

Mutants allow scientists to study certain characteristics or diseases.

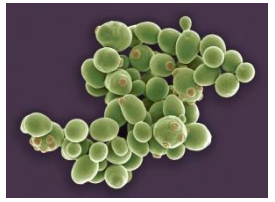
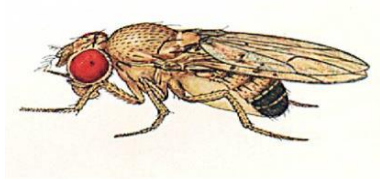
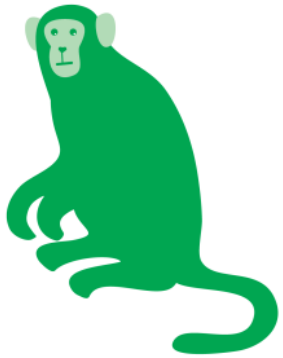
Why are model organisms useful in genetics research?

Some model organisms have similar genes or similar-sized genomes to humans.

Model organisms can be used to create highly detailed genetic maps.

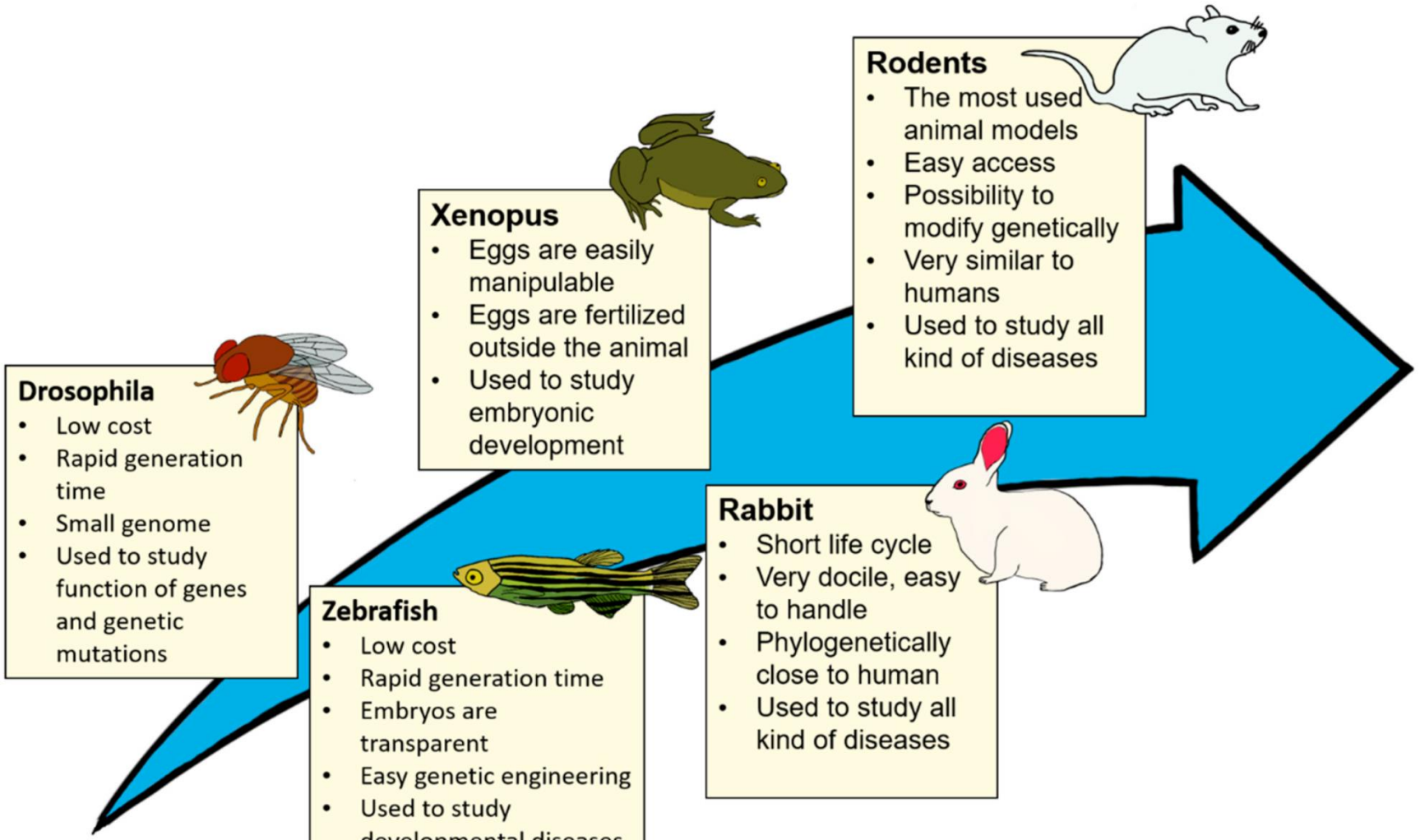
Examples of model organisms used to study genetics

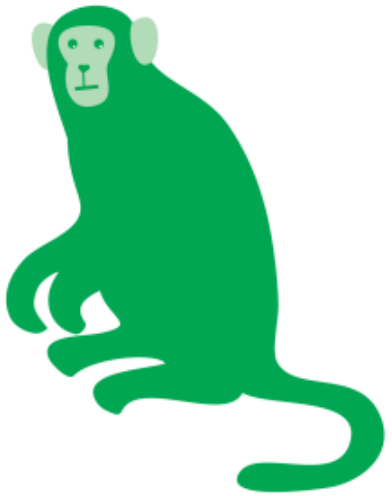
Yeast (*Saccharomyces cerevisiae*)
Fruit fly (*Drosophila melanogaster*)
Nematode worm (*Caenorhabditis elegans*)
Western clawed frog (*Xenopus tropicalis*)
Mouse (*Mus musculus*)
Zebrafish (*Danio rerio*)
Monkey (*Macaca mulatta)*



Examples of model organisms used to study genetics





MACAQUE MONKEY
Macaca mulatta

- Genetically similar to humans
- Similar immune responses to humans
- Useful for longitudinal studies

Examples of model organisms used to study genetics

Disease modeling of human inherited kidney disease

- Pathomechanisms
- Genetics:
  - Forward mutagenic screens
  - Validation of variants pathogenicity
- Precision medicine
- Identification of therapeutic targets and drug testing

(*Mus musculus*)

House mouse: Benefits

-It is relatively easy to manipulate the mouse genome.

-Mice are far better than flies or worms for studying complex biological systems found in humans.

-Immunodeficient mice (mice without a fully functioning immune system) can also be used as hosts to grow both normal and diseased human tissue (Xenografts).

Examples of mouse models?

Transgenic mouse

Mice that have had DNA from another source put into their DNA. The foreign DNA is put into the nucleus of a fertilized mouse egg.

The new DNA becomes part of every cell and tissue of the mouse.

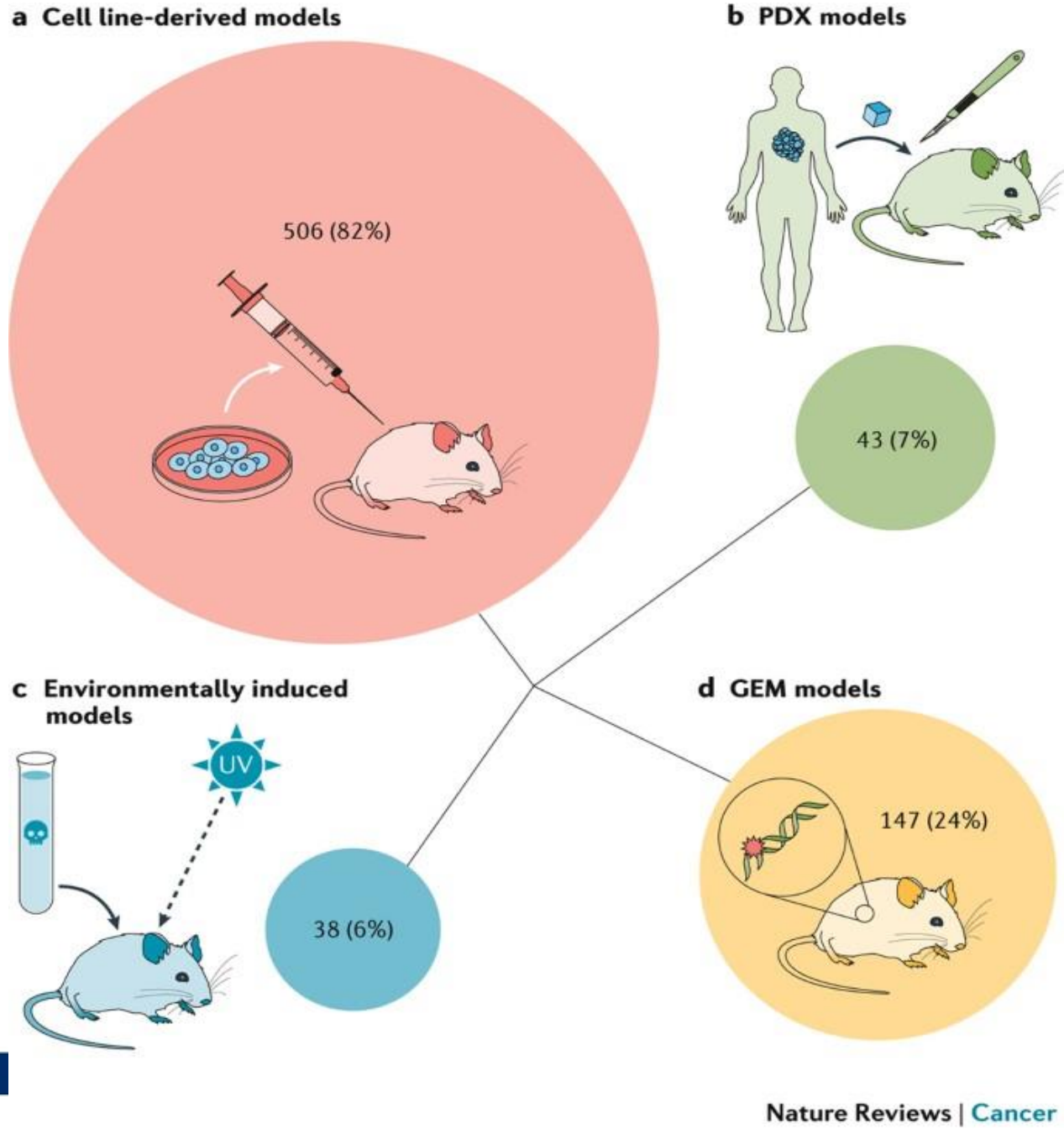
The house mouse (*Mus musculus*)

Genetically modified mouse

A genetically modified mouse or genetically engineered mouse model is a mouse that has had its genome altered through the use of genetic engineering techniques.

Commonly used for research or as animal models of human diseases.

Examples of mouse models

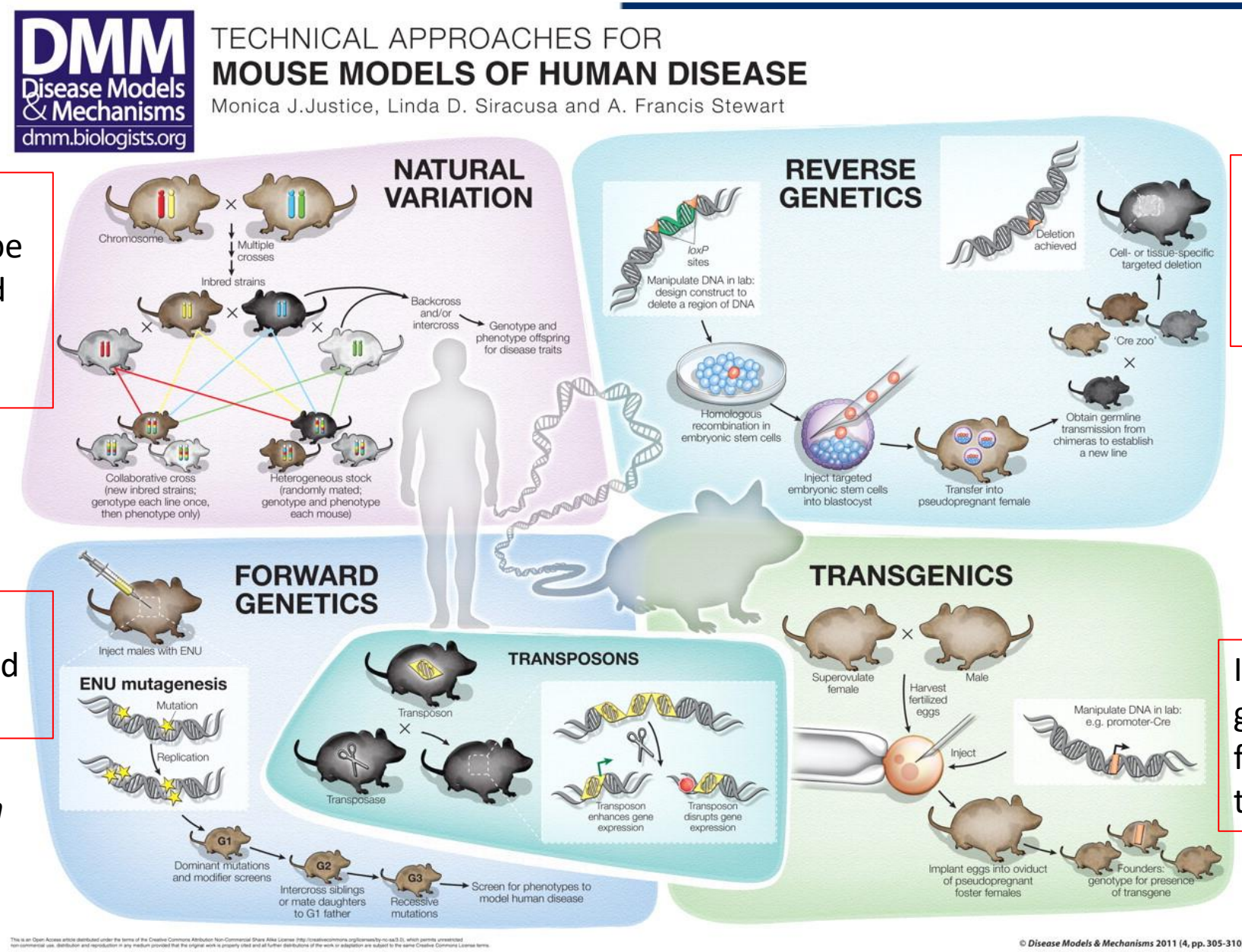


Patient Derived Xenografts are models of cancer where the tissue or cells from a patient's tumor are implanted into an immunodeficient or humanized mouse

A humanized mouse model: xenotransplanted with human cells and/or engineered to express human gene products

Genetically engineered mouse models

Examples of mouse models



Accounts for the differences in phenotype exhibited among inbred strains (as well as between individuals)

Induces mutations in model organisms to study their role in disease

Embryonic Stem cells

Chemical Mutagenesis

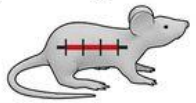
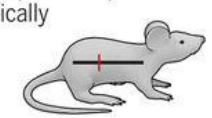
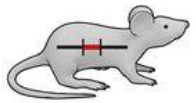
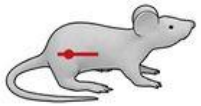
Zygotes

Identifies and links a mutation to an observed disease etiology

Insertion of a foreign gene into the genome followed by germ-line transmission

N-ethyl-N-nitrosourea is a highly potent *mutagen*

Examples of mouse models

Mouse model	Possible phenotypes	Some uses and questions
Transgenic Multiple copies of exogenous DNA 	•Phenotype severity often depends on transgene copy number; possible artifacts from overexpression and from insertion site of transgene	•Investigating later-stage disease mechanisms •Can late-stage disease be treated?
Knockin Replace mouse sequence with another sequence expressed physiologically 	•Phenotype may appear in mid- and late-life; may be mild	•Investigating earlier-stage disease mechanisms •When does disease start? •Can we treat prodromal/presymptomatic disease? •Can we develop biomarkers?
Genomically humanized Replace mouse sequence with the orthologous human genomic region 	•Phenotype may appear in mid- and late-life; may be mild	•Investigating earlier-stage disease mechanisms •When does disease start? •Can we treat prodromal/presymptomatic disease? •Can we develop biomarkers? •Do human proteins behave differently from mouse proteins?
Chromosome engineered aneuploidy A chromosomal region is duplicated or deleted 	•Phenotype may be mild	•Investigating dose-sensitive genes/mechanisms •Dose-sensitive gene mapping •Investigating disease mechanisms and biomarkers
Transchromosomal model Human chromosome added to the mouse genome 	•Phenotype may be mild •Expressing mouse and human genes	•Investigating dose-sensitive genes/mechanisms •Dose-sensitive gene mapping •Investigating disease mechanisms and biomarkers •Investigating human genomic DNA function in a mouse cellular environment
Chimeras, mouse-mouse, and human-mouse Mice consisting of two different cell lines, e.g., mouse-mouse or mouse-human 	•Phenotype may be mild but depends on the cell lines used, which could be wildtype or genetically manipulated	•How does pathology spread? •Is disease cell autonomous? •Do human cells behave differently from mouse cells?
Inducible and conditional Temporal or spatial control of gene expression 	•Phenotypes vary depending on the genetic manipulation	•Investigating protein function •Investigating temporal/spatial specificity in pathogenesis •Which cell types are key to pathogenesis? •Is disease cell autonomous? •Is disease reversible?
Knockout Functionally delete a gene 	•Phenotypes vary but often are severe in null animals	•Investigating loss of function •What are loss-of-function effects?

Mouse models of Human Disease

Why Use Transgenic Mouse Models?

- Model specific aspects of a human disease
- Test drugs or other treatments in a controlled system

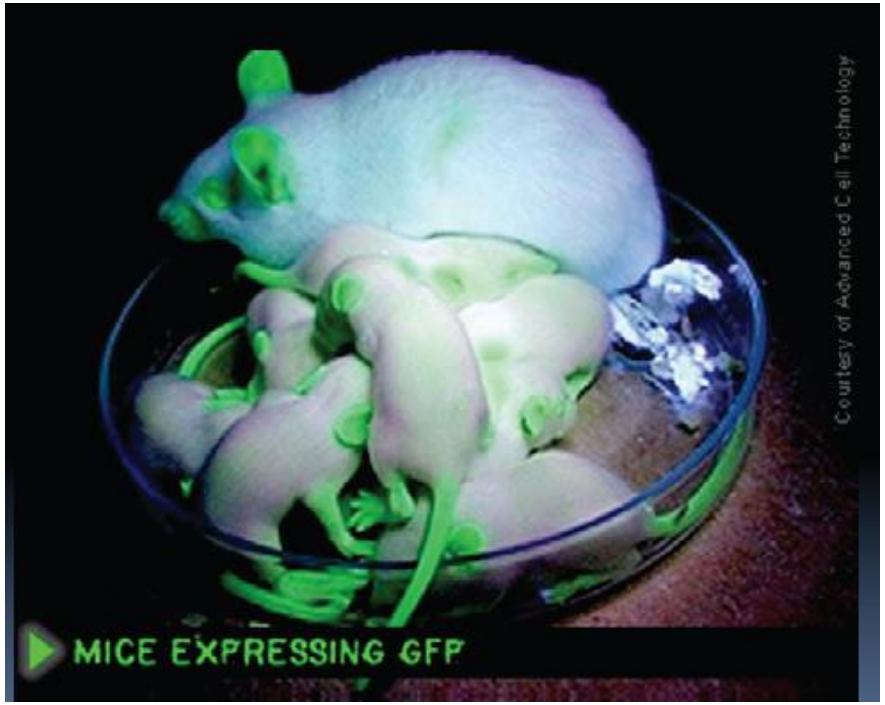
- Highlight the function of your gene of interest
- Cross to different lines to expand experimental possibilities

ingenious targeting laboratory

Transgenic and knockout mice

A transgenic animal is much more complex than working with cultured cells

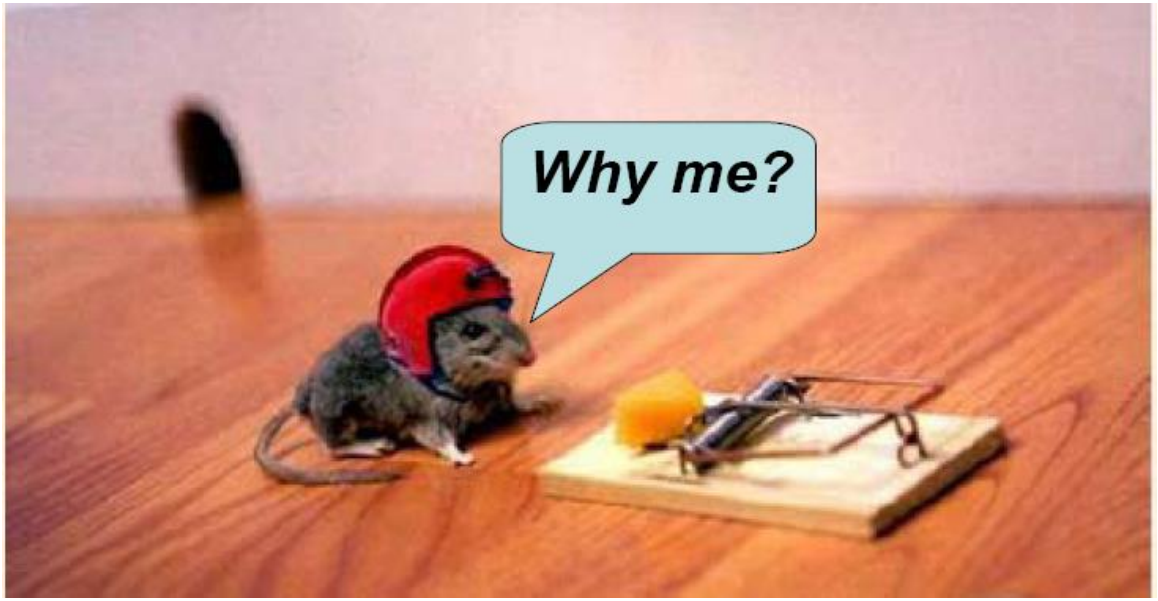
One of the goals of mouse transgenesis, is to use molecular genetic approaches to create better mouse models of human diseases that are based on known genetic lesions.

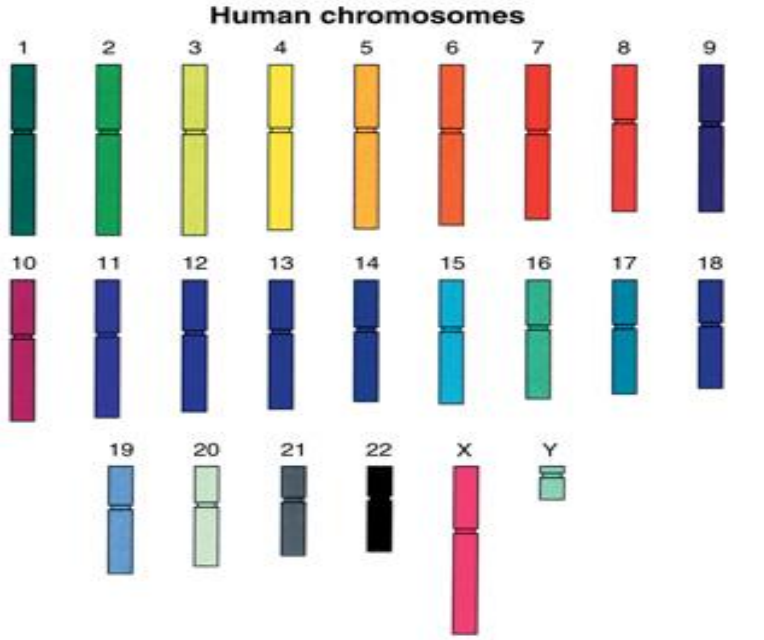


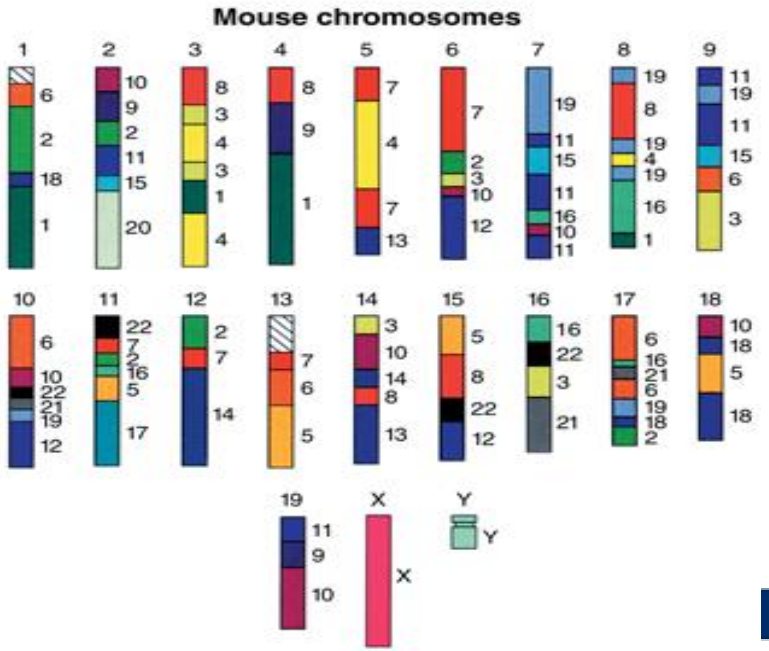
Transgenic and knockout mice

Mice have 19 autosomal chromosomes and two sex-chromosomes (X and Y), many of which contain large segments of DNA that are highly conserved between mouse and humans.

The mouse is a reliable stand-in for humans in medical research, thanks to a genome that is 85 percent identical.







Transgenic and knockout mice

Synteny: The co-localization of genes on chromosomes of related species.

Homolog: The situation where nucleic acid or protein sequences are similar because they have a common evolutionary origin. Often used loosely to indicate that sequences are very similar.

Ortholog – gene sequences are similar between species.

Paralog – gene sequences are similar within a species.

Adapted from the Department of Energy; genomics.energy.gov

Transgenic and knockout mice

Disease Mode	Gene
Cystic Fibrosis	CFTR
Atherosclerosis	Apo E, apo (a), Apo A-II
anti-Atherosclerosis Gene Therapy	Apo AI, Ape E, LDLR
B-Thalassemia	ß -globin
Sickle Cell Anemia	$ß^S$ (and variants)
Inflammatory Bowel Disease	Interleukine-2, Interleukin-10 and T-cell Receptor ,ß ; MHC II
Severe Combined Immunodeficiency Disease	Rag-1, Rag-2
Muscluar dystrophy Gene Theraphy	Dystrophin
Alzheimers disease	ß -amyloid
Amyotrophic lateral sclerosis (ALS)	neurofilament heavy chain
Insulin Dependent Diabetes Mellitus	interferon-
Cancer	many oncogenes and tumor supressor genes

Transgenic and knockout mice

- **Spontaneous Mutants**

– Occurs as the result of spontaneous mutation

– Examples:

- db/db (Diabetic mouse)
- nu/nu (Nude mouse)

- **Induced Mutants**

– Transgenics

- Overexpression
- Inducible / conditional

– Gene targeting

- Knock-outs
- Knock-ins

– Mutagenic Mice:

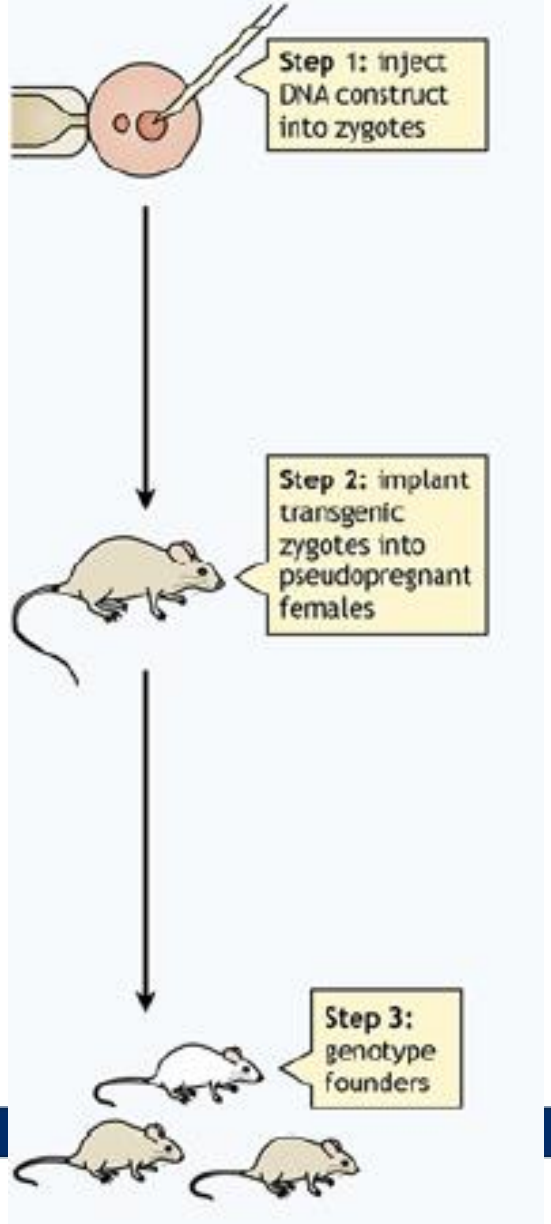
- Chemical mutagenesis of Embryonic Stem cells
- Chemical mutagenesis / Irradiation of Mice

Transgenic and knockout mice

- Example: Transgenic Mice (hair gene removed)
- Used to help burn patients and others by making human facial parts (ears, nose lips, etc.)

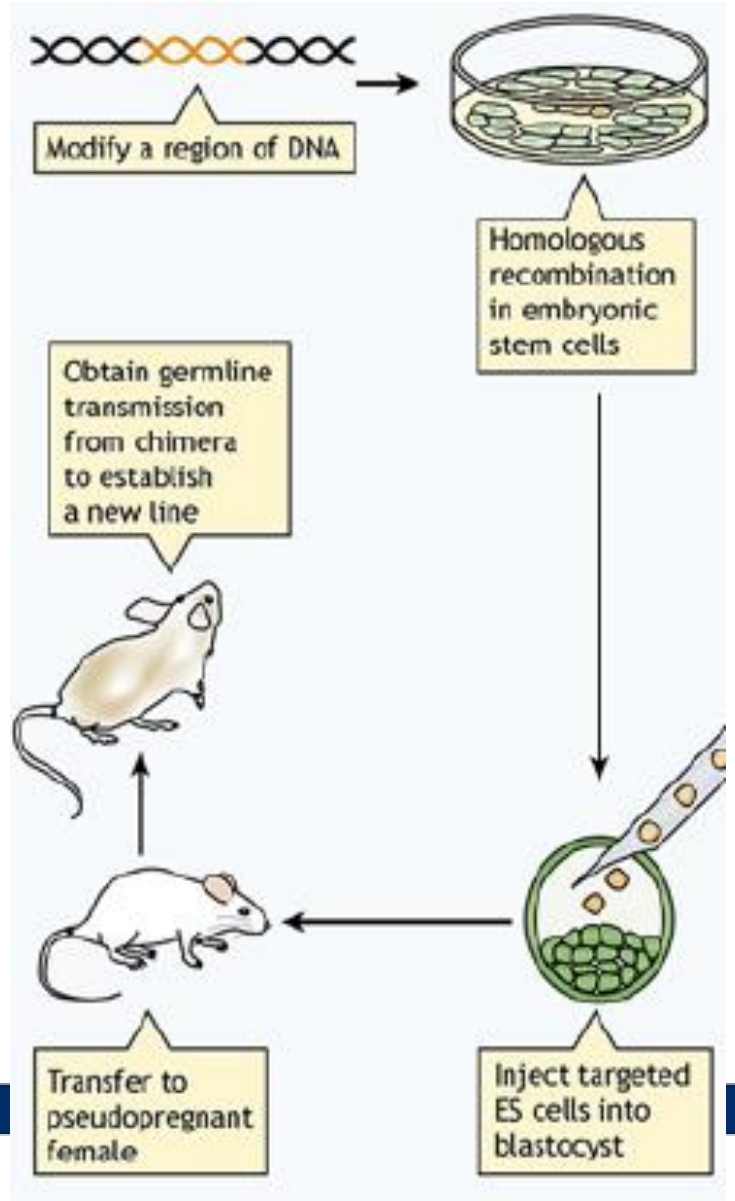
https://www.youtube.com/watch?v=kefoIXnLAN0

Producing Transgenic mice

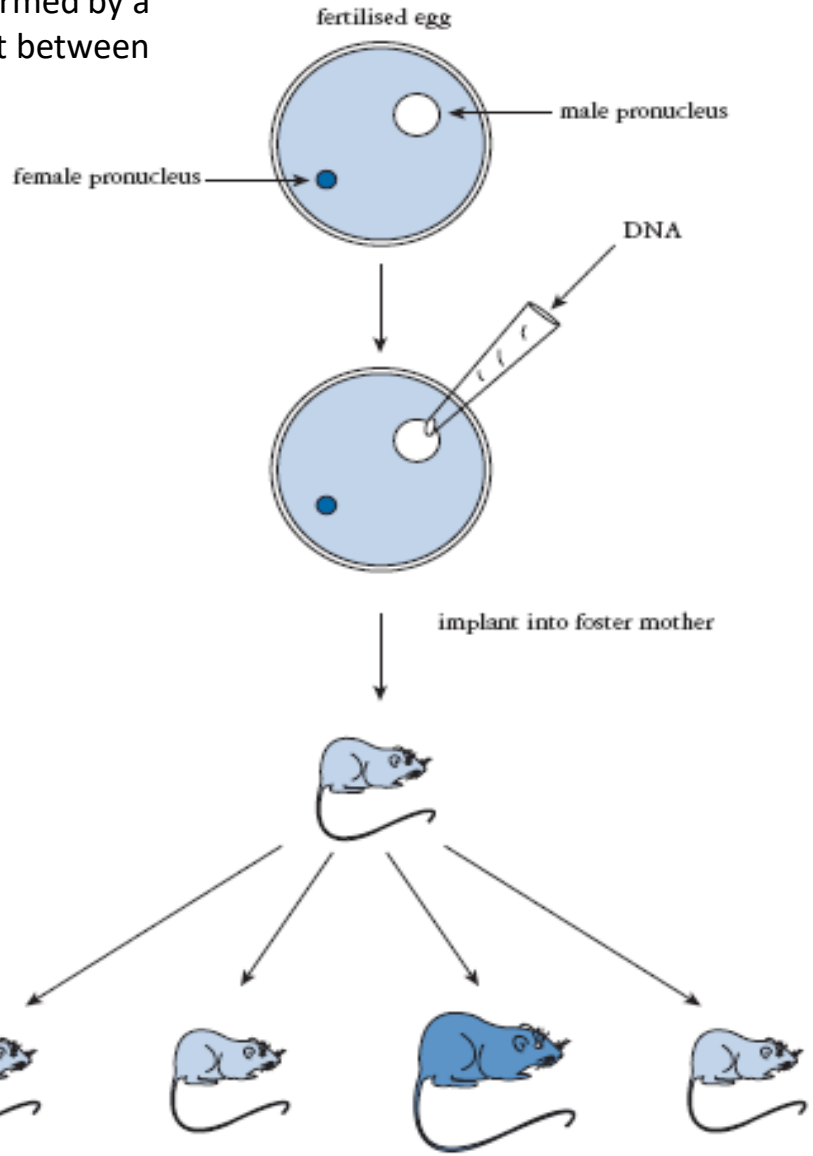


Pronuclear Injection

Gene Targeting in ES Cells



Zygote
eukaryotic cell formed by a fertilization event between two gametes.



Producing Transgenic mice

A **pronucleus** (plural: **pronuclei**) is the nucleus of a sperm or an egg cell during the process of fertilization. The sperm cell becomes a pronucleus after the sperm enters the ovum, but before the genetic material of the sperm and egg fuse.

Aim: to alter the germ line so that the genetic change is inherited in a stable pattern

Production of transgenic mice – the construct

Transgenes should include:

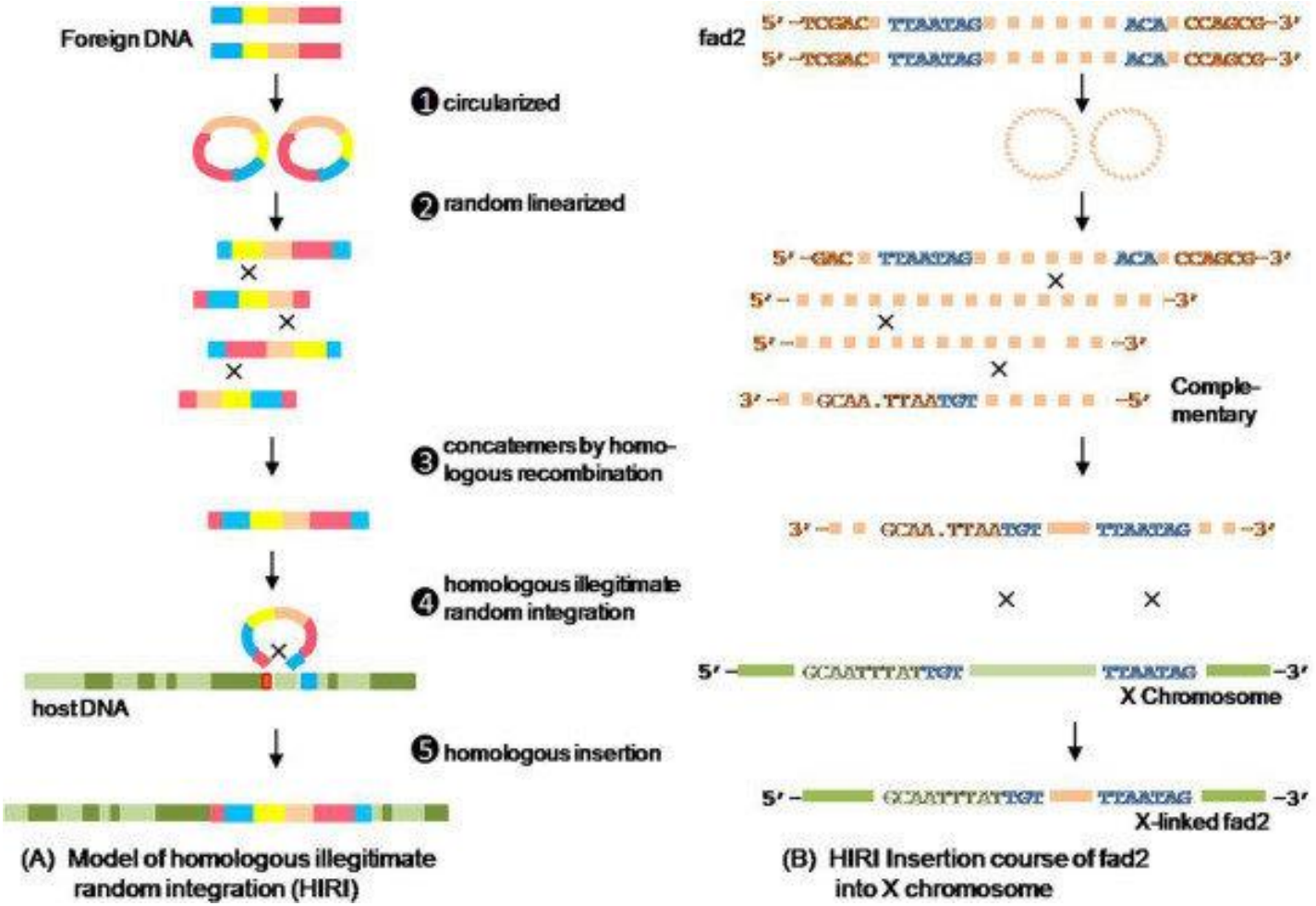
- Promoter, with or without enhancer
- Gene to be expressed, which may be a reporter gene
- Splice donor and acceptor sequences flanking an intron, these can be from another gene, e.g. beta-globin or SV40 t antigen
- Termination/polyadenylation sequences, these can be from another gene, e.g. beta-globin or SV40 t antigen

- **Transgenes must be excised from the bacterial plasmid sequences in order to be expressed in mice**
  - This is because the prokaryotic cloning vector sequences inhibit expression of eukaryotic genes introduced into the mouse genome.

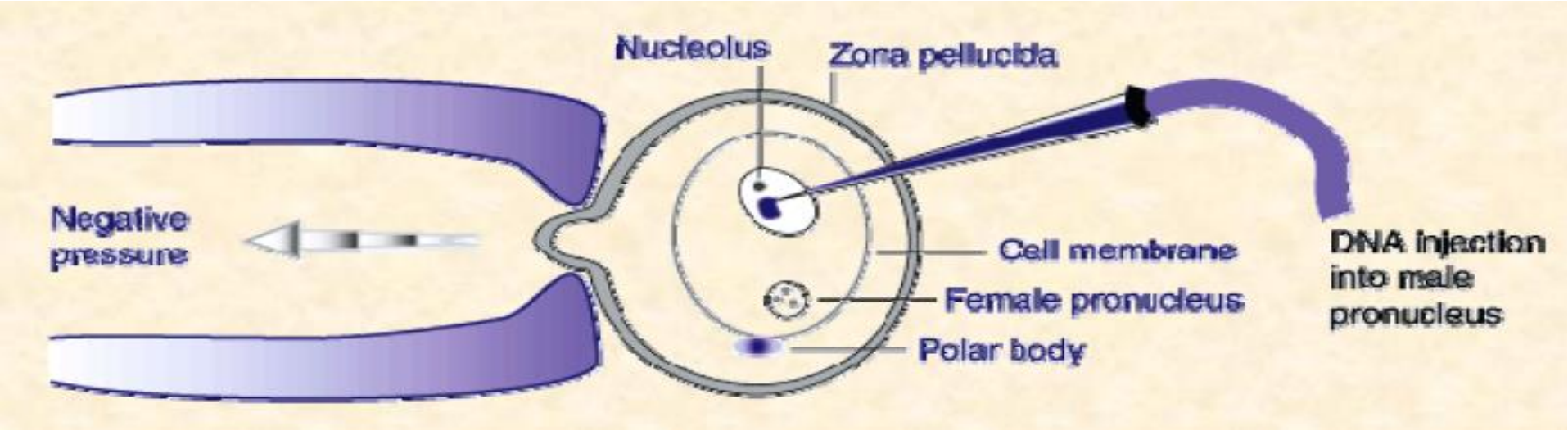
Random Integration!

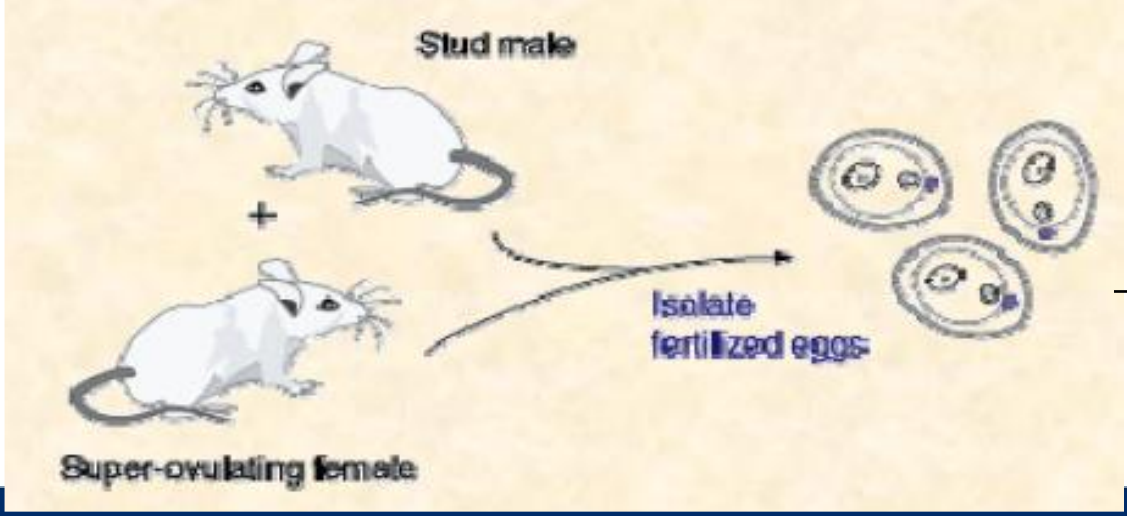
→ Sequences ensuring expression of the transgene

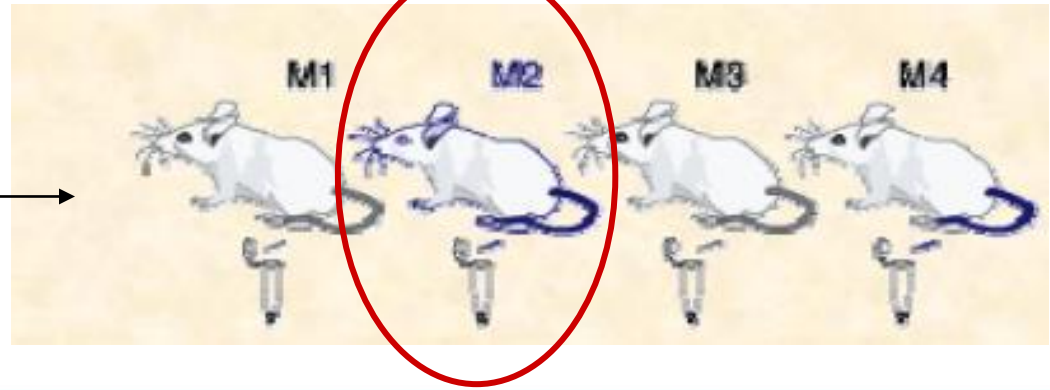
Random Integration!



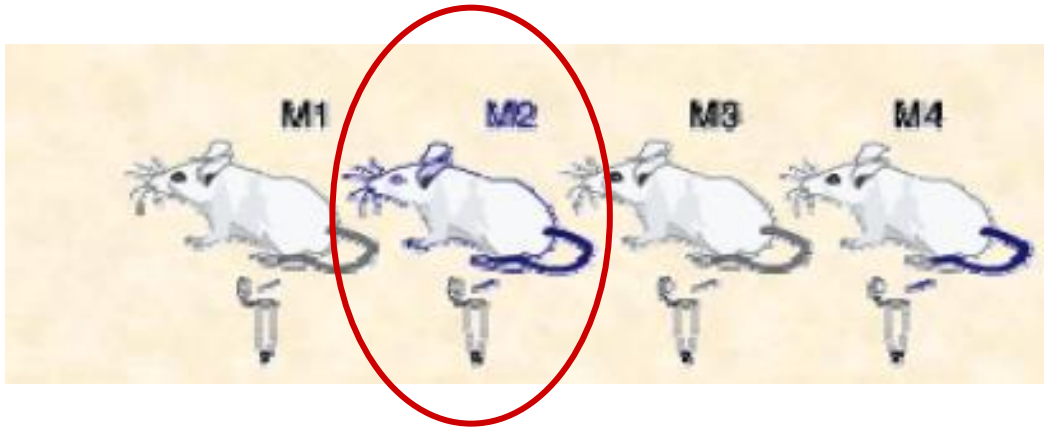
Production of transgenic mice - Introduction of genes into embryos



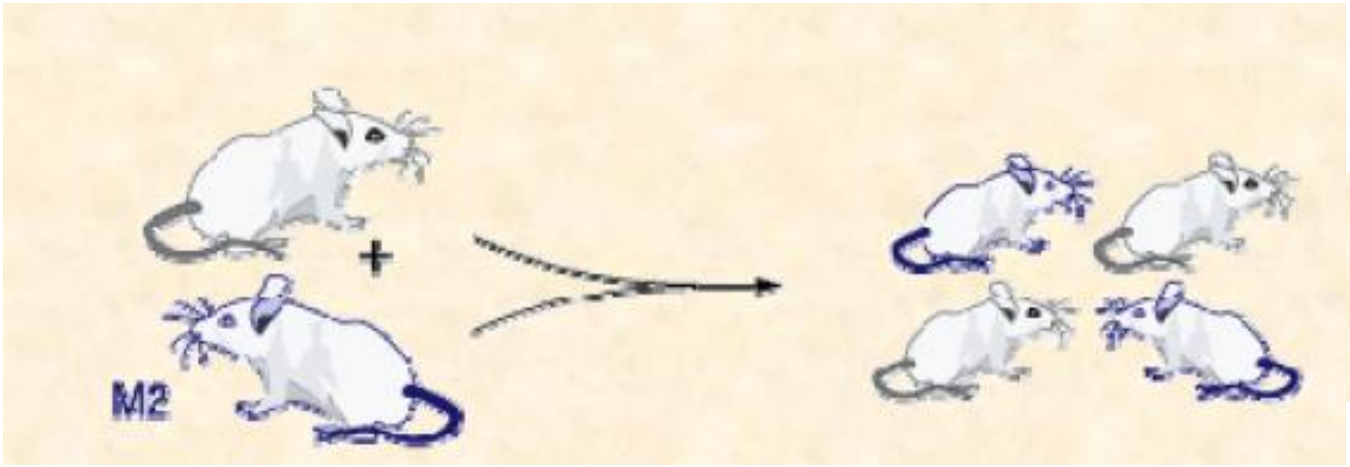




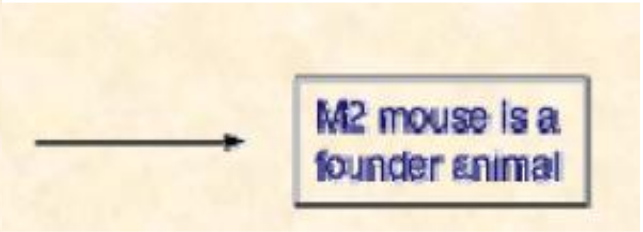
Producing Transgenic mice



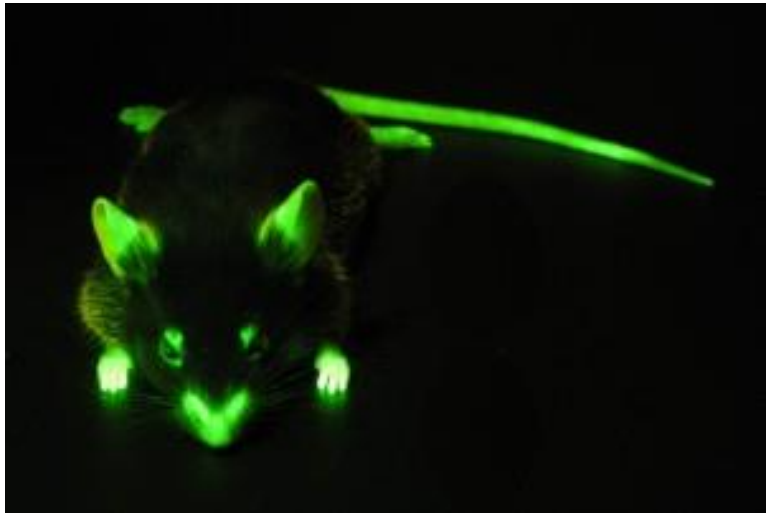
M2 is **backcrossed to non-transgenic mates** to identify **founder animals containing germline DNA** integration that results in a Mendelian inheritance pattern of the transgene.



Germline transmission



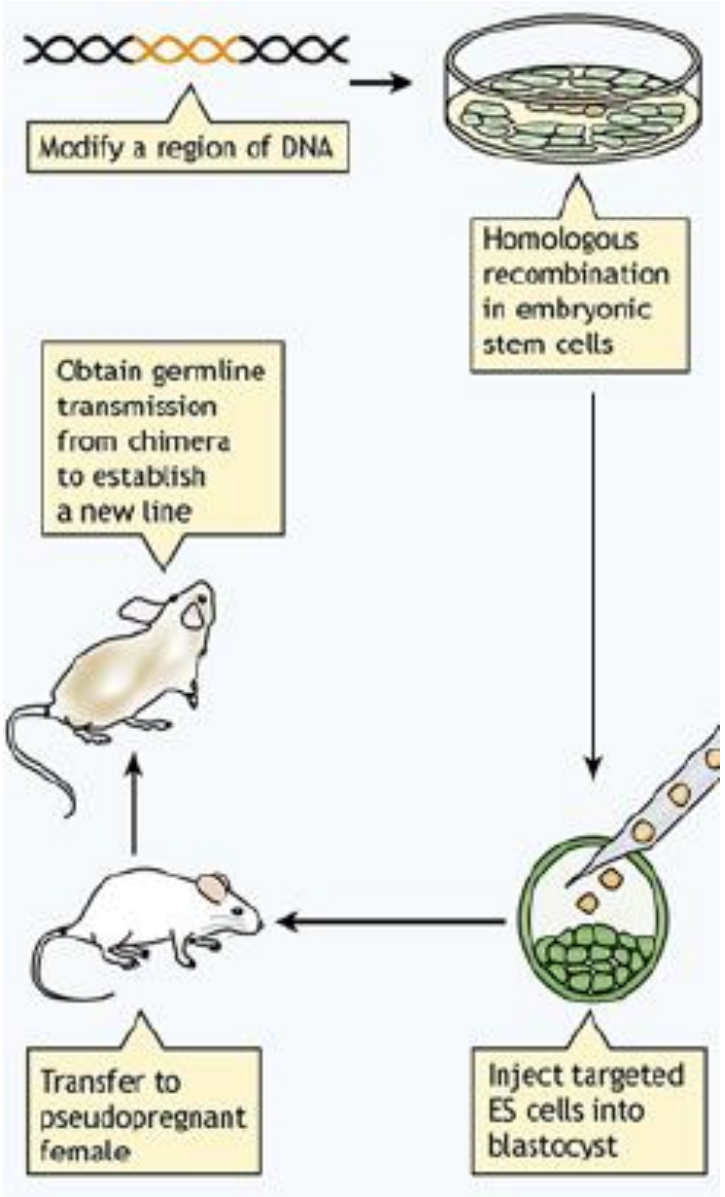
GFP Transgenic



GFP mouse

These UBC-GFP transgenic mice **express enhanced Green Fluorescent Protein under the direction of the human ubiqutin C promoter**. Mice homozygous for the transgene are viable, fertile, normal in size and do not display any gross physical or behavioral abnormalities. These mice express GFP in all tissues examined.

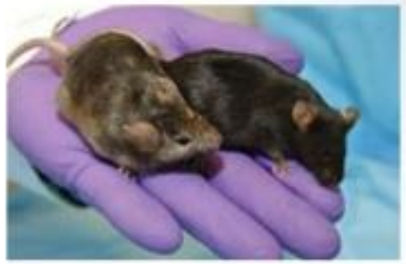
Gene Targeting in ES Cells



KO mouse

Gene Targeting in ES Cells

KNOCK OUT MICE



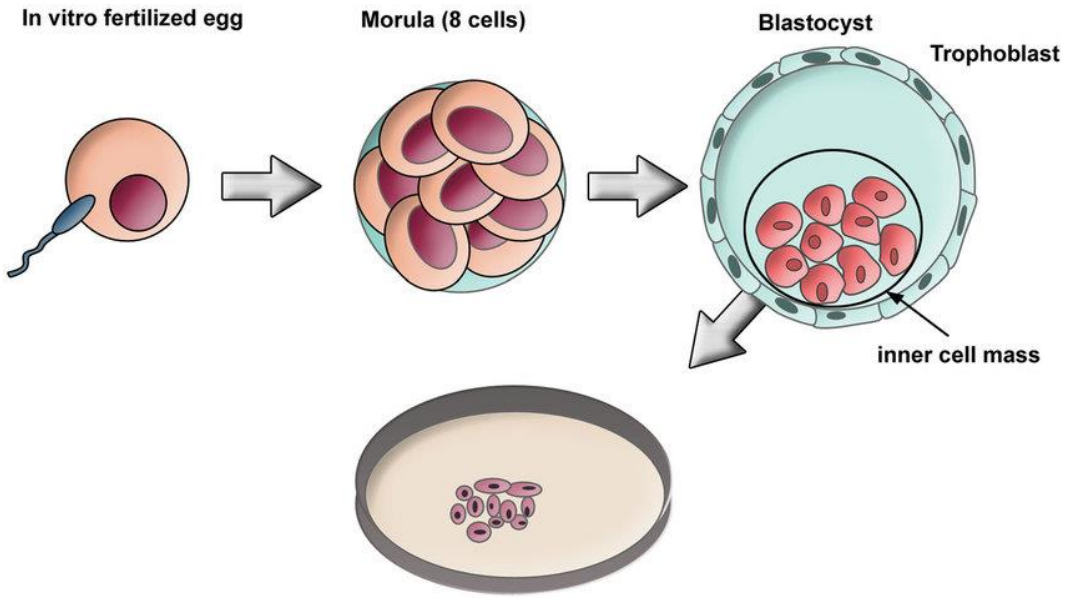
- a mouse in which a gene has been deleted/mutated (gene is inactivated)
- *specific* gene is targeted
- The loss of gene activity often causes changes in a mouse's phenotype and thus provides valuable information on the function of the gene.

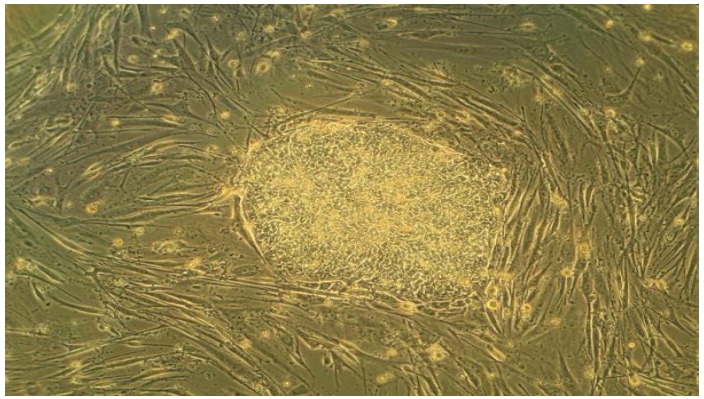
Production of transgenic mice using ES cell technology

Embryonic stem cells (ES cells) are stem cells derived from the inner cell mass of an early stage embryo known as a blastocyst.

embryos reach the blastocyst stage 4–5 days post fertilization ~they consist of 50–150 cells.

ES cells are pluripotent- are able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm.

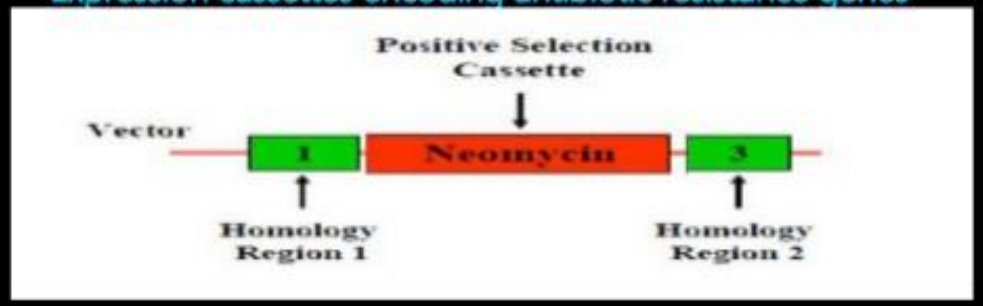


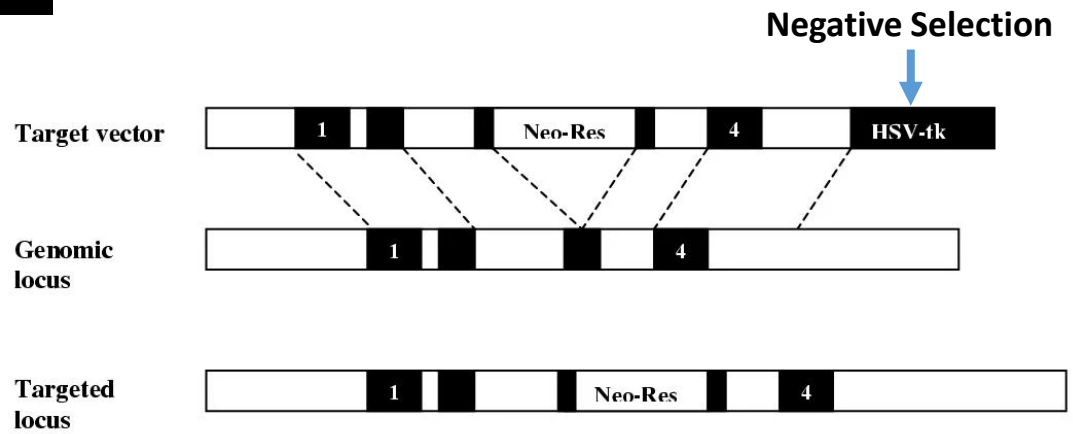


Gene Targeting in ES Cells

TARGETING CONSTRUCT FOR POSITIVE / NEGATIVE SELECTION

- To make targeting construct:
  - a positive selectable marker flanked by two "arms" of homologous sequence
  - a negative selectable marker outside one homologous arm
- **Positive selection markers**
  - Expression cassettes encoding antibiotic resistance genes

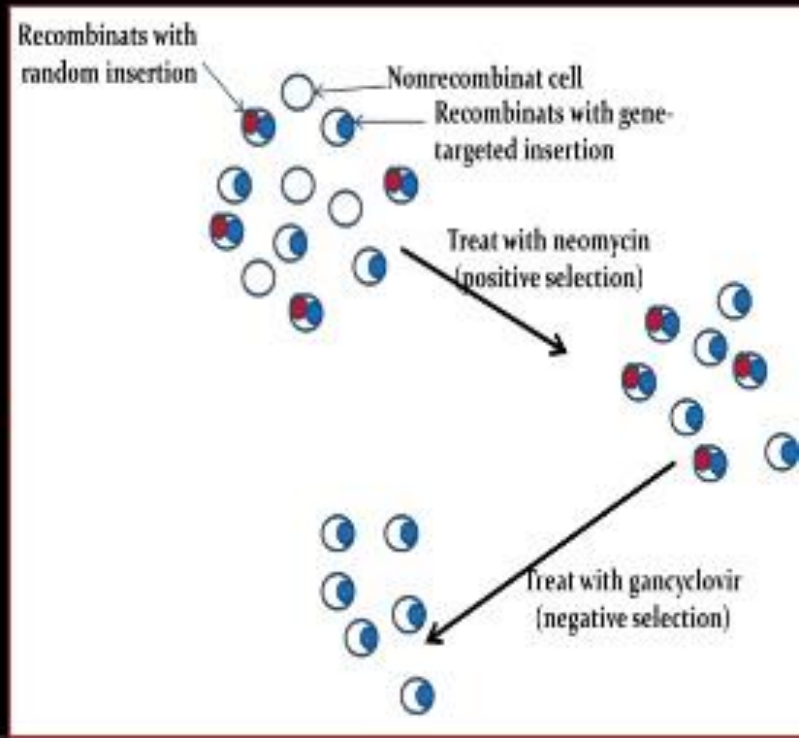




Gene Targeting in ES Cells

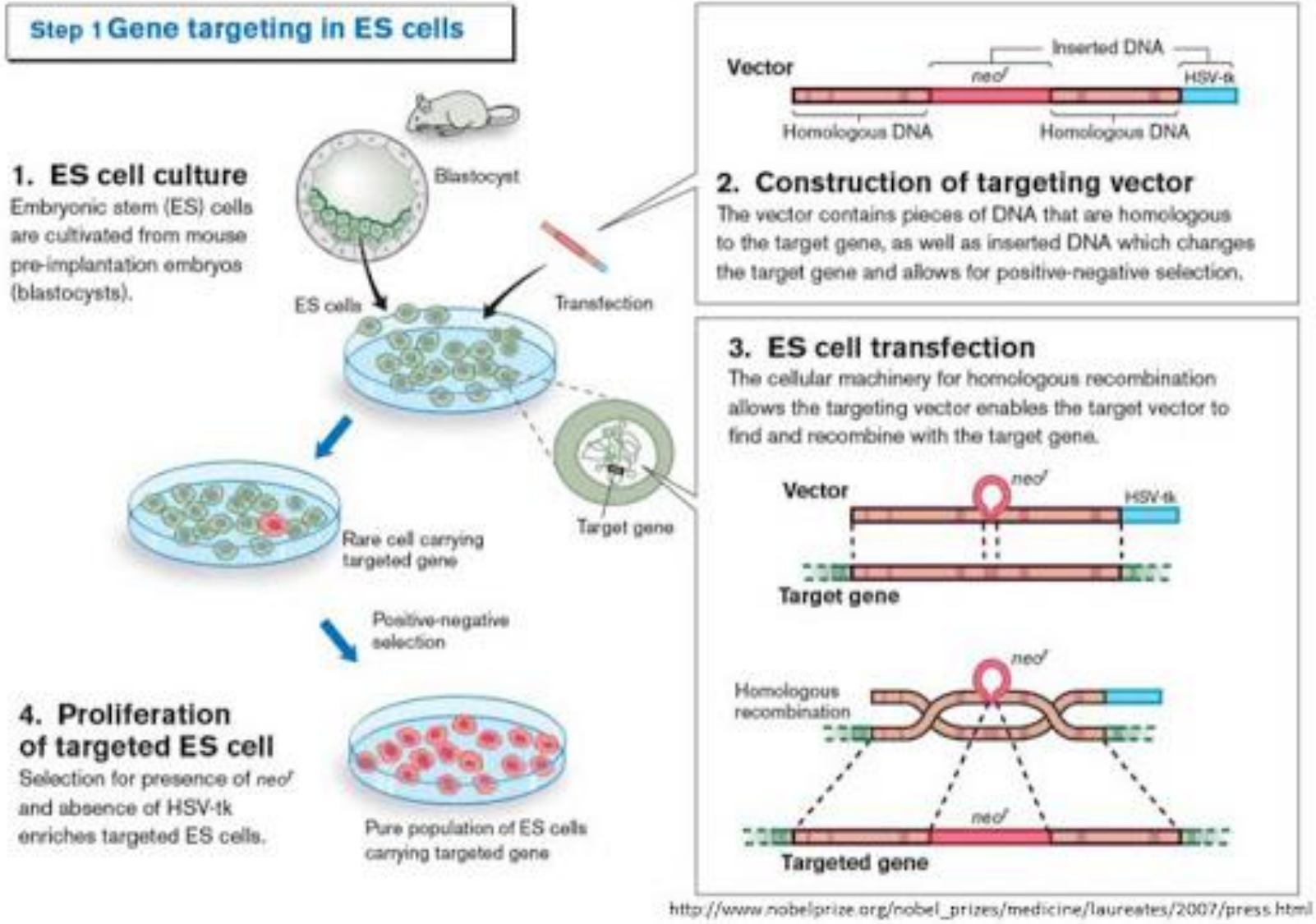
SELECTION STRATEGY

- **Positive Selection**
  - G418
  - Neomycin Resistance gene
  - confers resistance to G418
- **Negative Selection**
  - Gancyclovir
  - Herpes Simplex Virus Thymidine Kinase (HSV-TK) gene.
  - sensitive to gancyclovir
  - selects against random integrants



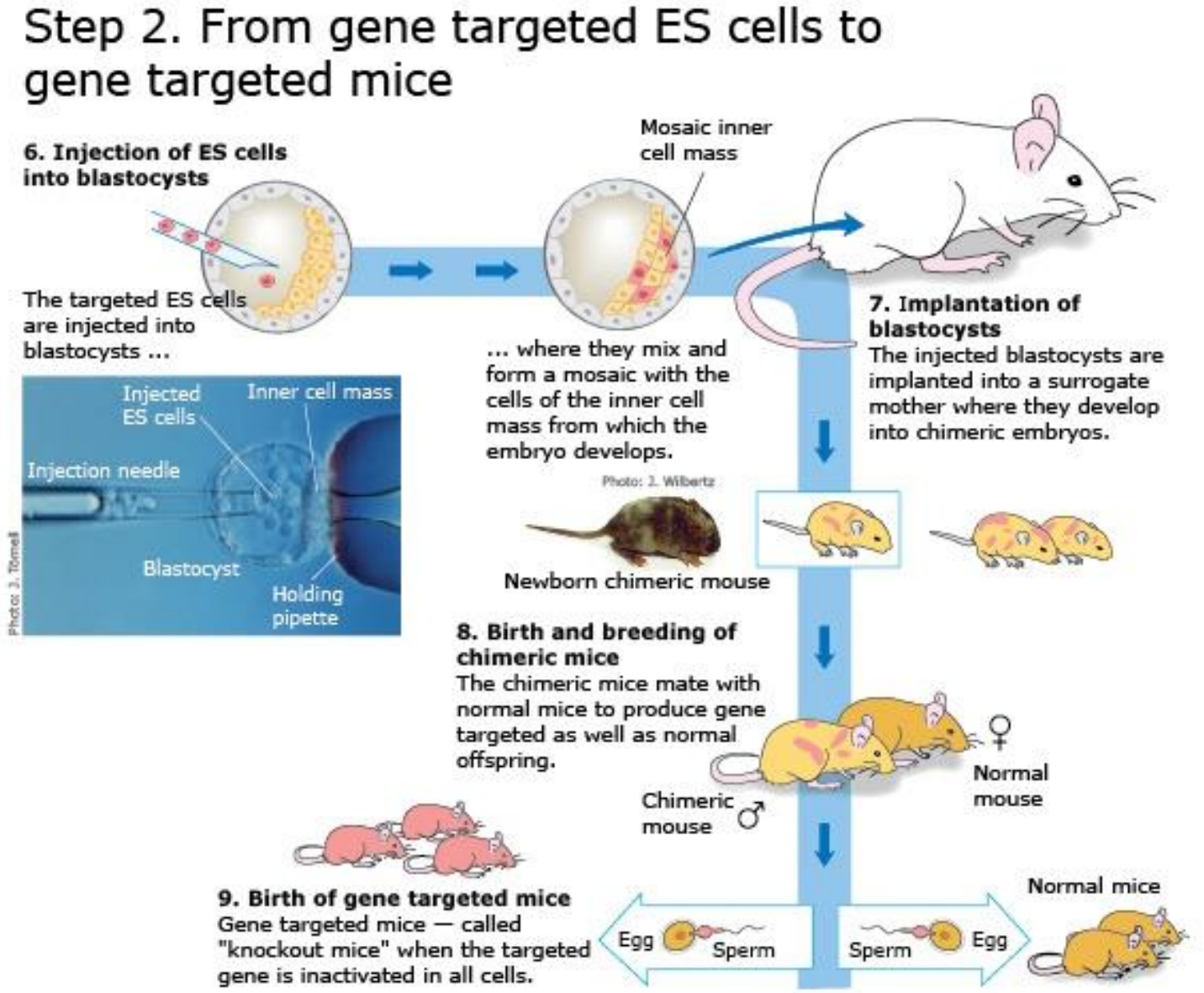
Gene Targeted Mice

--- Step 1. Gene Targeting in ES cells

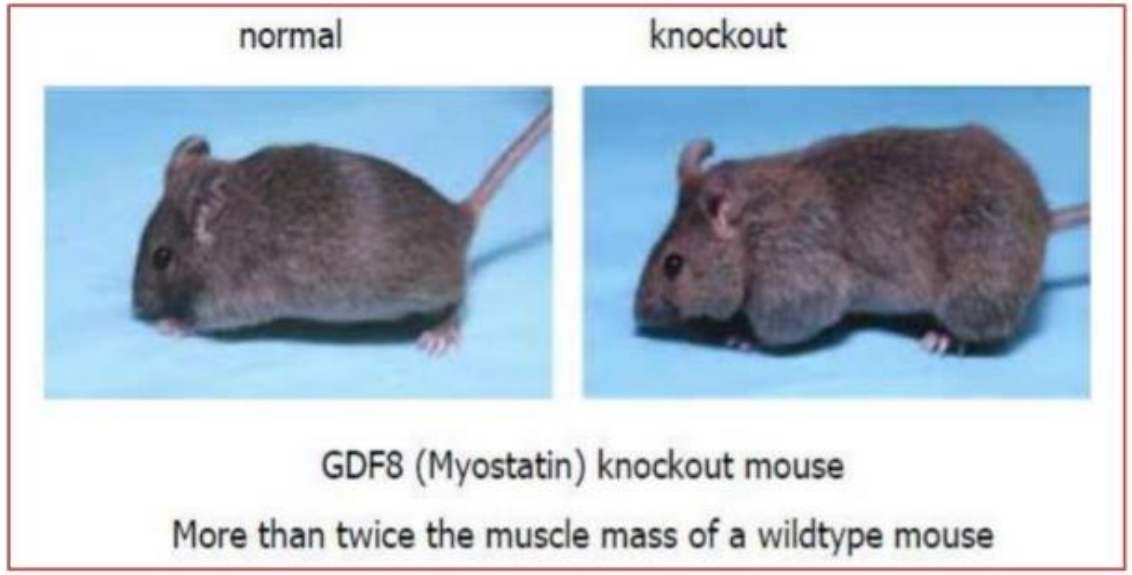


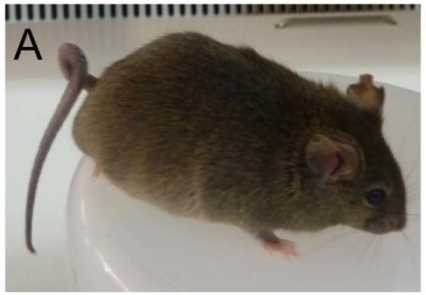
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2007/press.html

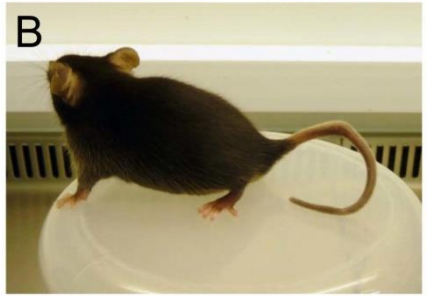
Gene Targeted Mice



Knockout mice







A chimeric mouse gene Targeted for the Agouti Color gene, with its offspring

Using mouse models in Progeria

Transgenic and knockout mice

- Knocking out expression of Nhlh2, a basic helix-loop helix transcription factor in mice results in adult onset obesity.

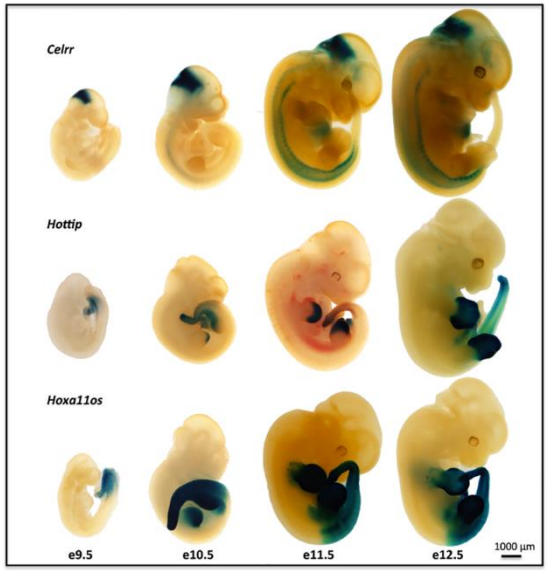
Nhlh2

Knockout systems – a variation on the theme

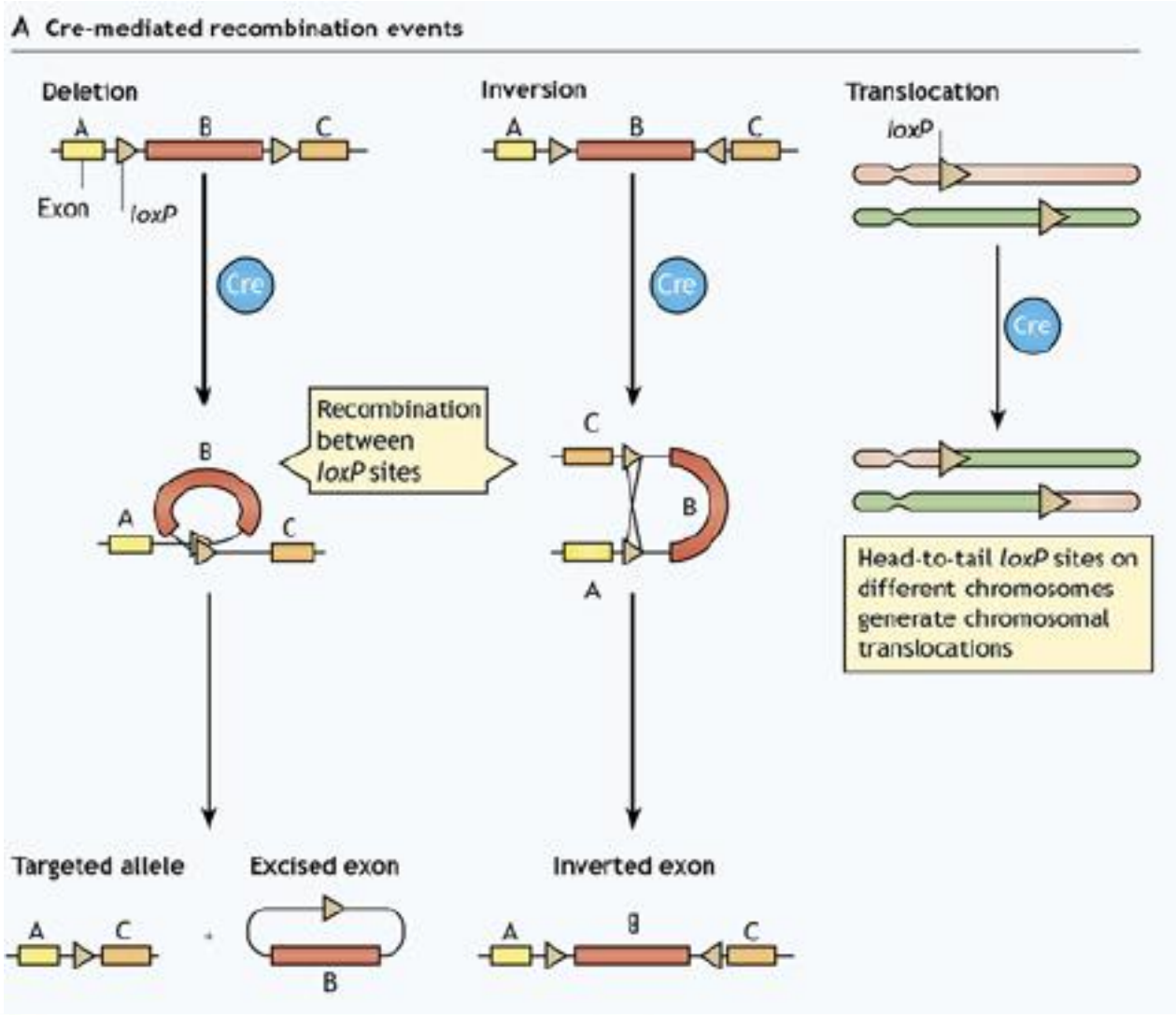
1. Conditional knockouts

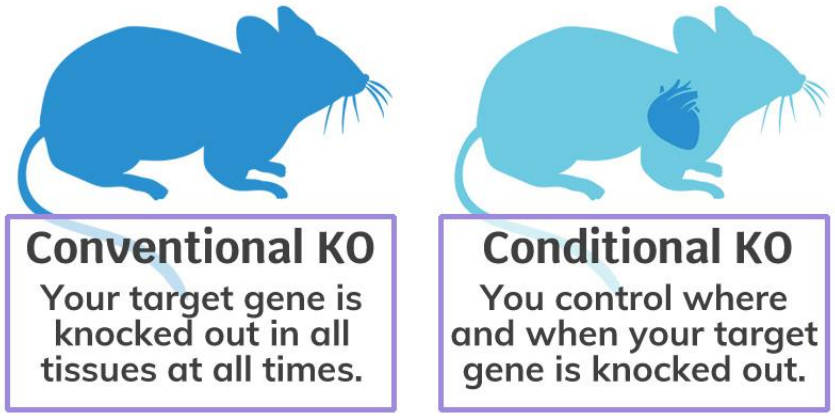
2. Knockin models

lacZ gene as a means of detecting tissue-specific gene expression



Conditional Mutagenesis





Cre-loxP system:
Consists of a single enzyme, Cre recombinase, that recombines a pair of short target sequences called the *Lox* sequences. This system can be implemented without inserting any extra supporting proteins or sequences. The Cre enzyme and the original *Lox* site called the *LoxP* sequence are derived from bacteriophage P1.

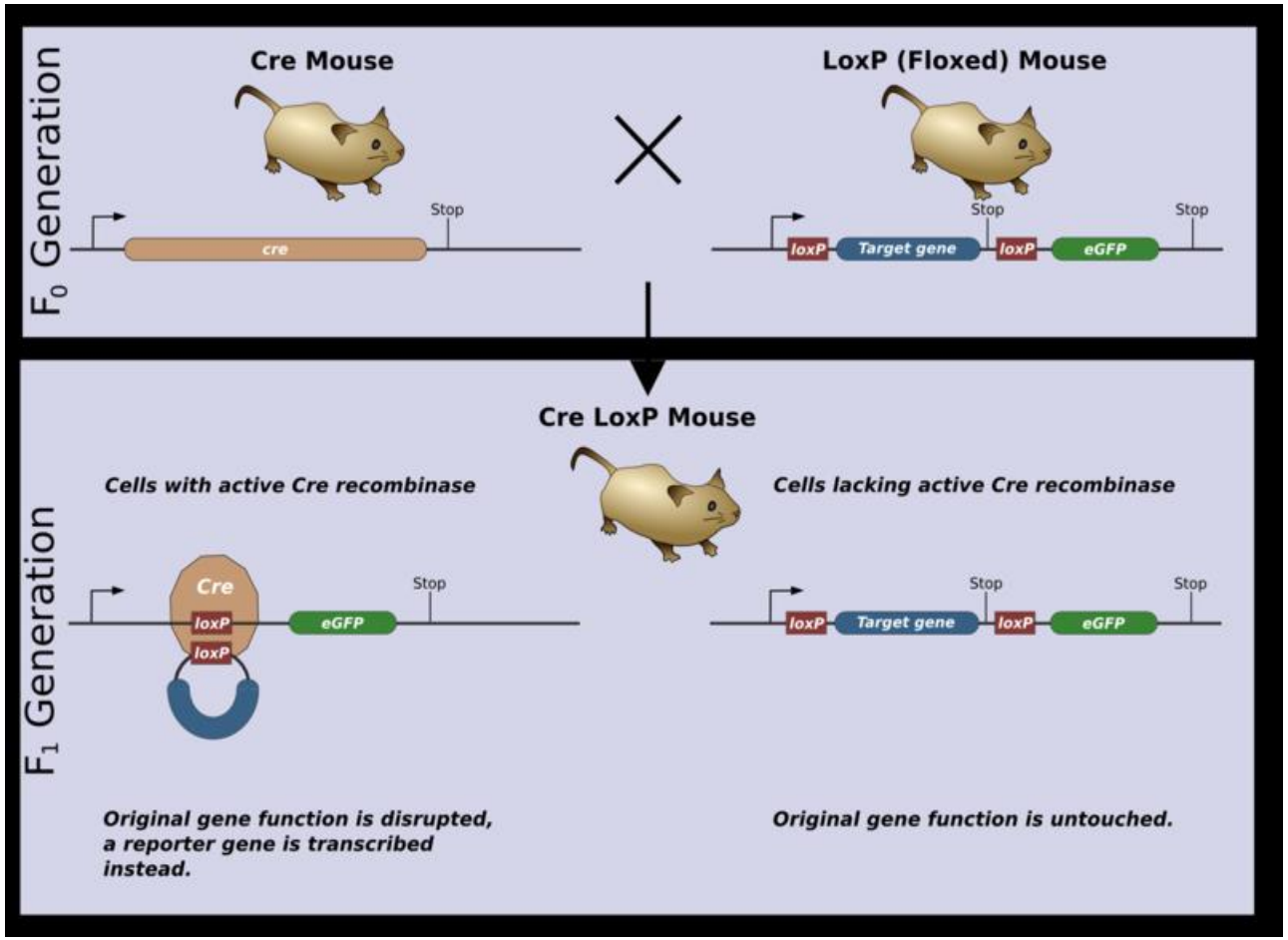
Knockout systems – the variation on the theme

Conditional gene targeting using the Cre/loxP system

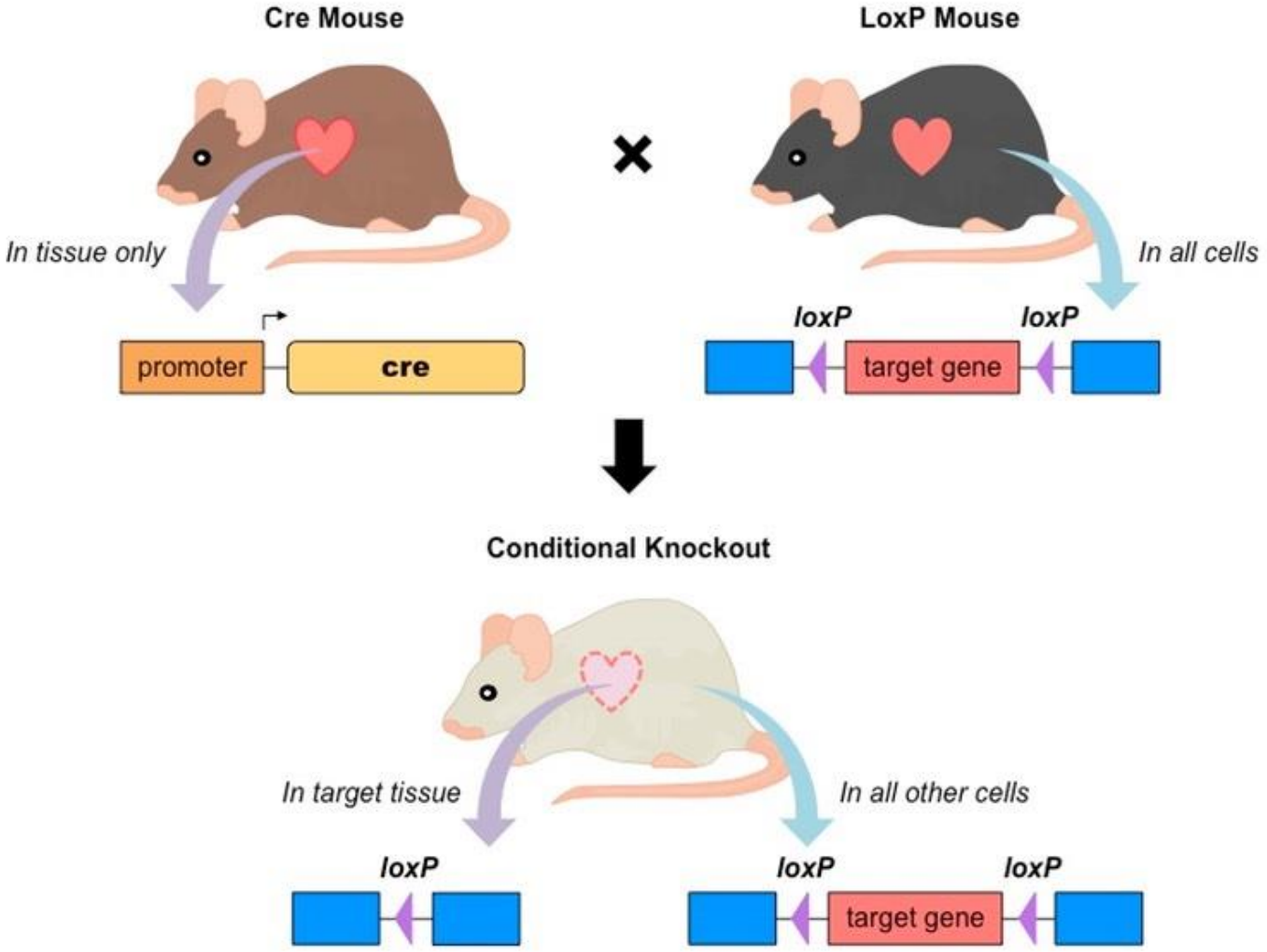
(Tissue specific knockouts/
tissue specific promoters)

Cre mouse:
a transgenic animal, which is finally bred to an animal line expressing Cre under either temporal or spatial control.

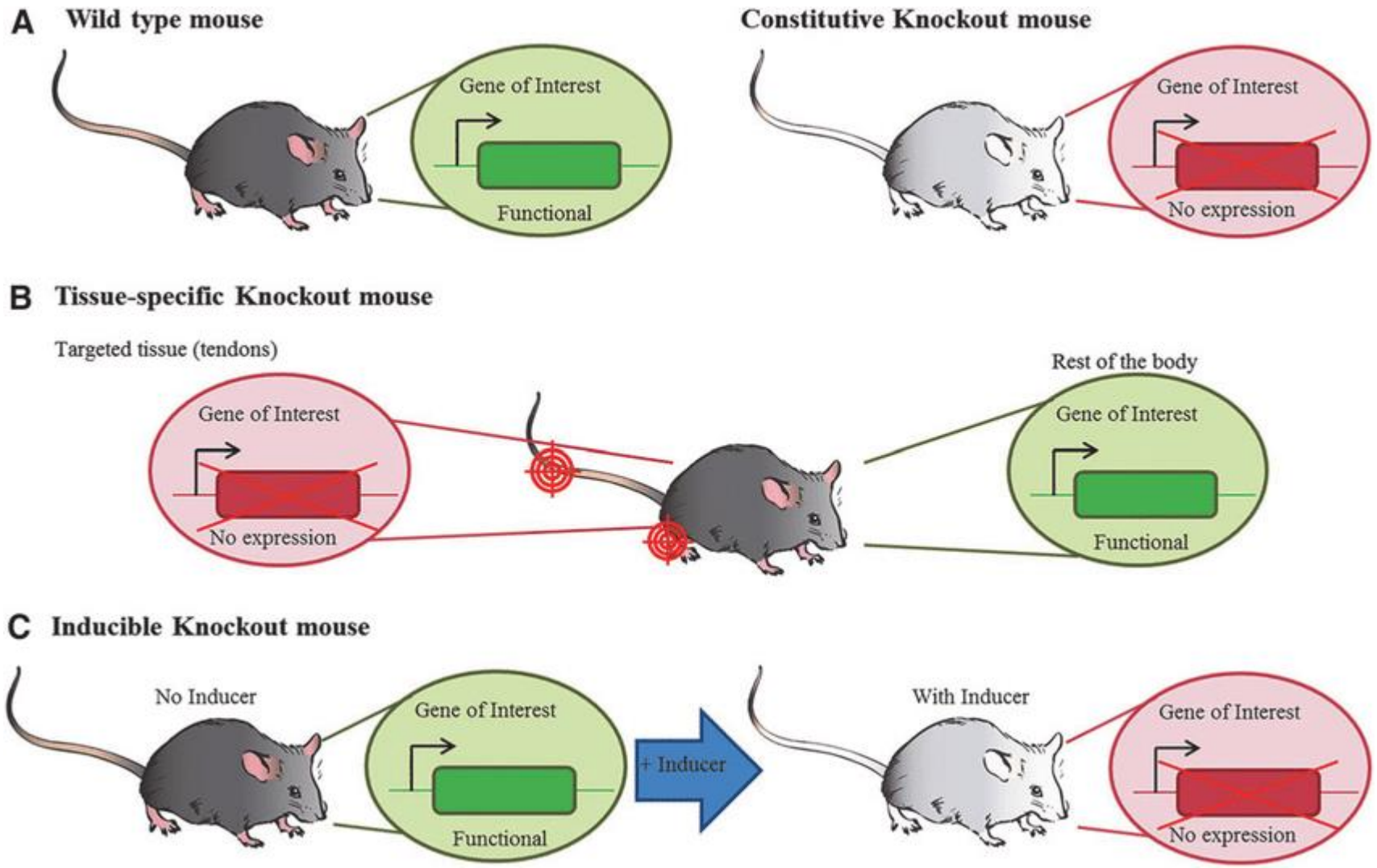
Cre can also be ubiquitously expressed to obtain a knockout in all tissues, including the germ line.



Conditional Mutagenesis



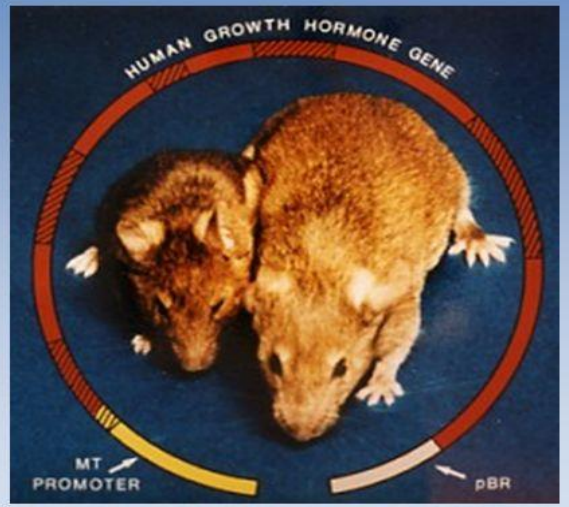
Conditional Mutagenesis



Transgenic and knockout mice

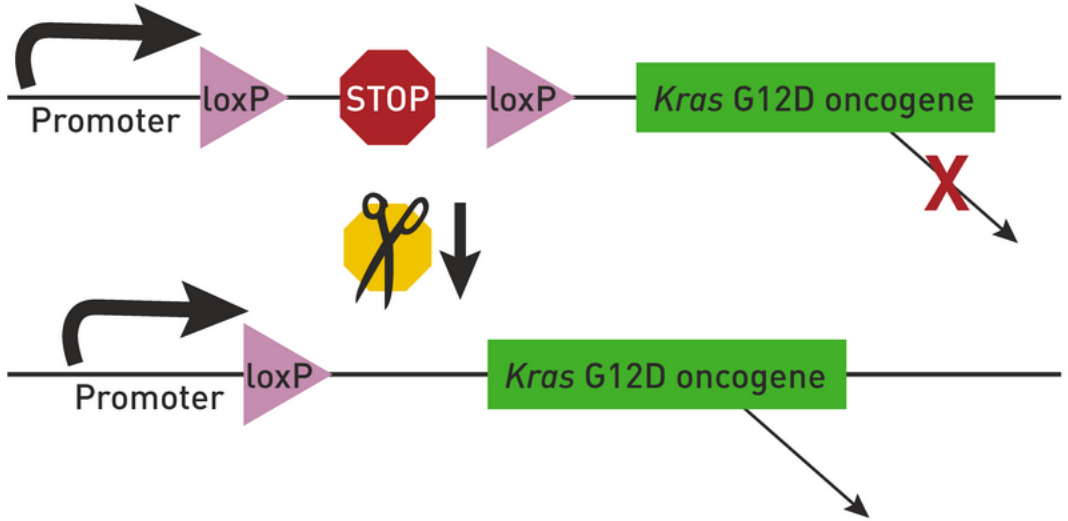
Example

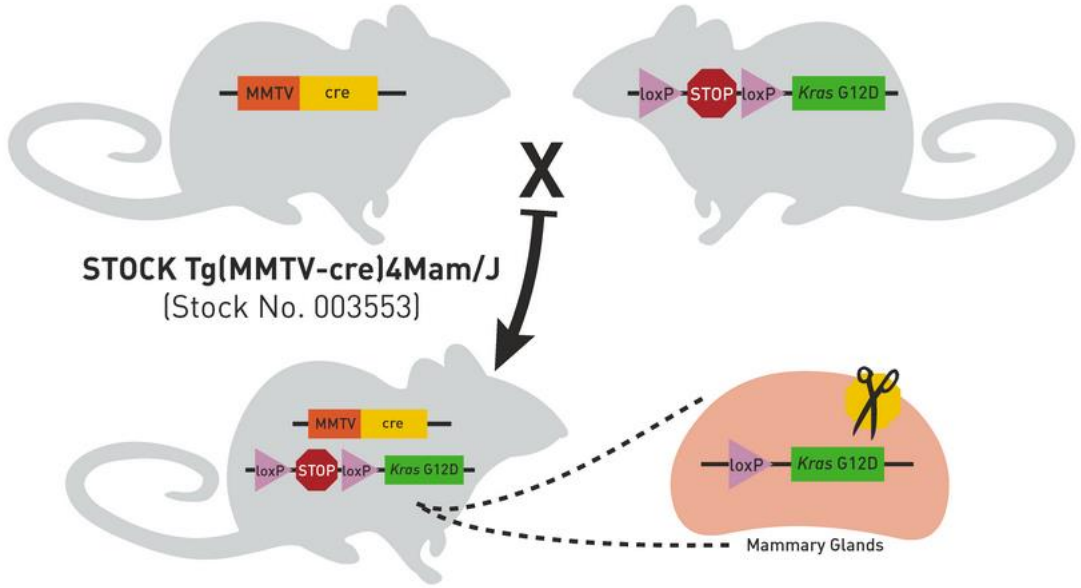
- Comparison between a transgenic mouse and a normal mouse
- The giant mouse developed from a fertilized egg transformed with a recombinant DNA molecule containing the Human Growth Hormone



hGH

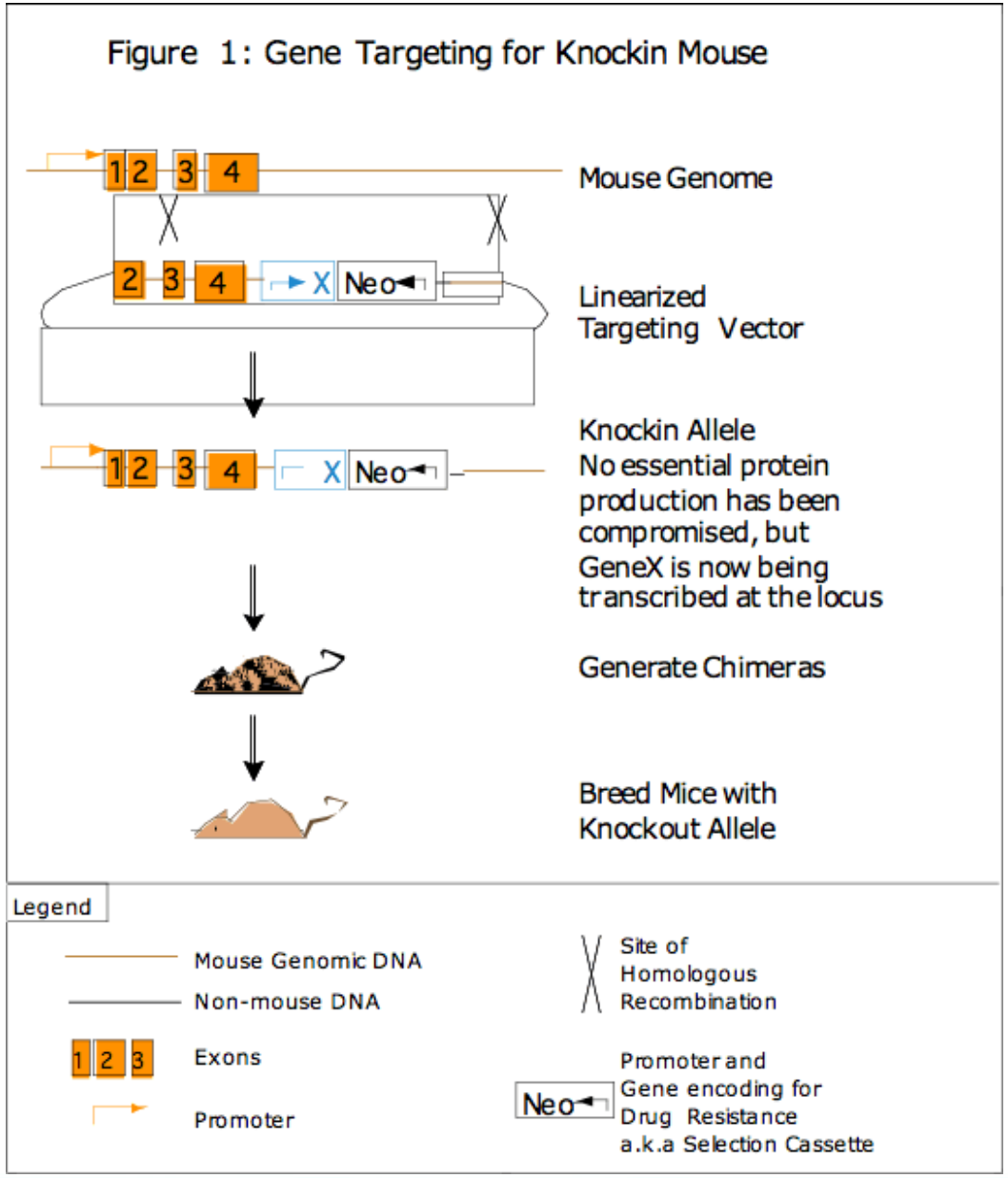
Conditional Mutagenesis

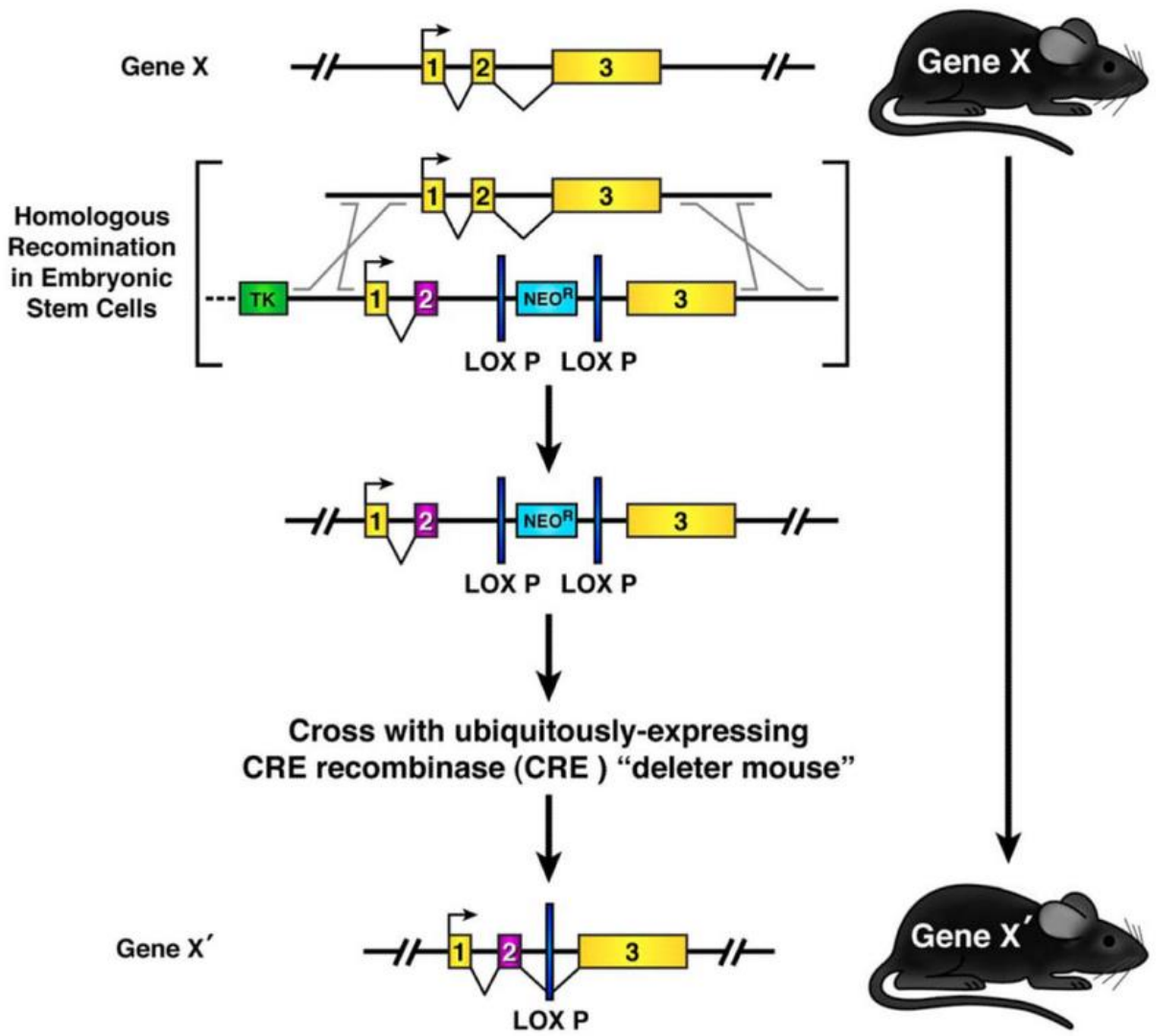




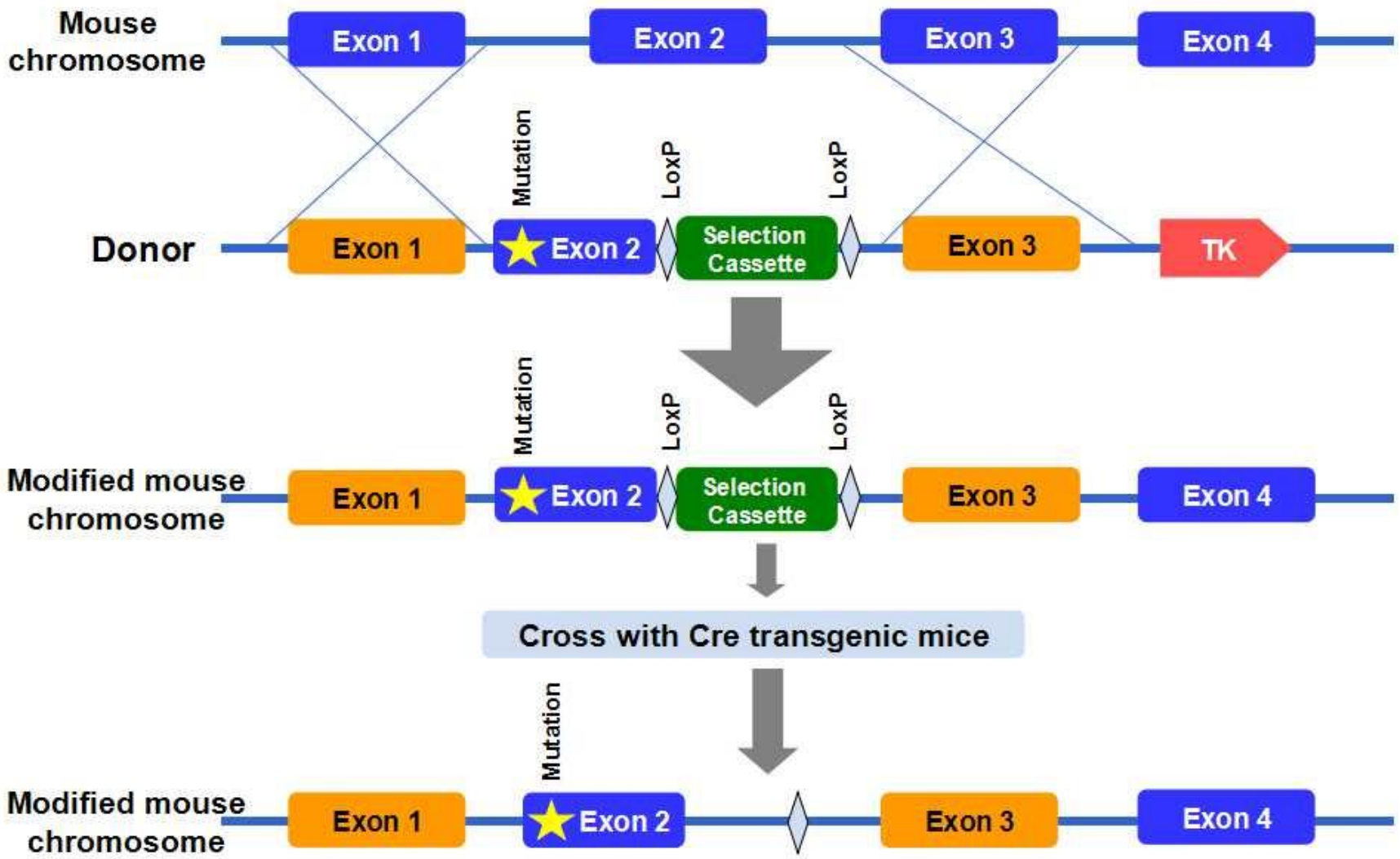
The Cre/lox system can also be used to produce strains in which a transgene is either inducible or expressed only in certain tissues. For example, mating the transgenic strain in Figure 1 to a strain that expresses Cre recombinase in mammary tissue produces double transgenic offspring that express the *Kras* oncogene only in the mammary glands (Figure 5). Notice that only one generation of breeding is required (Hooray!).

Knock In strategy

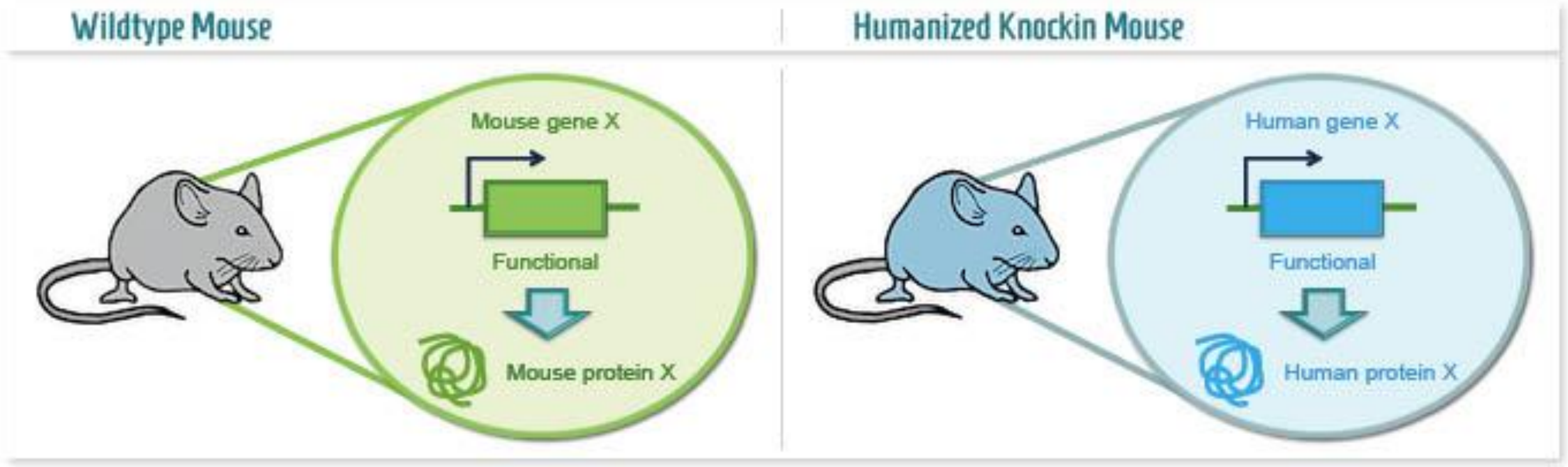




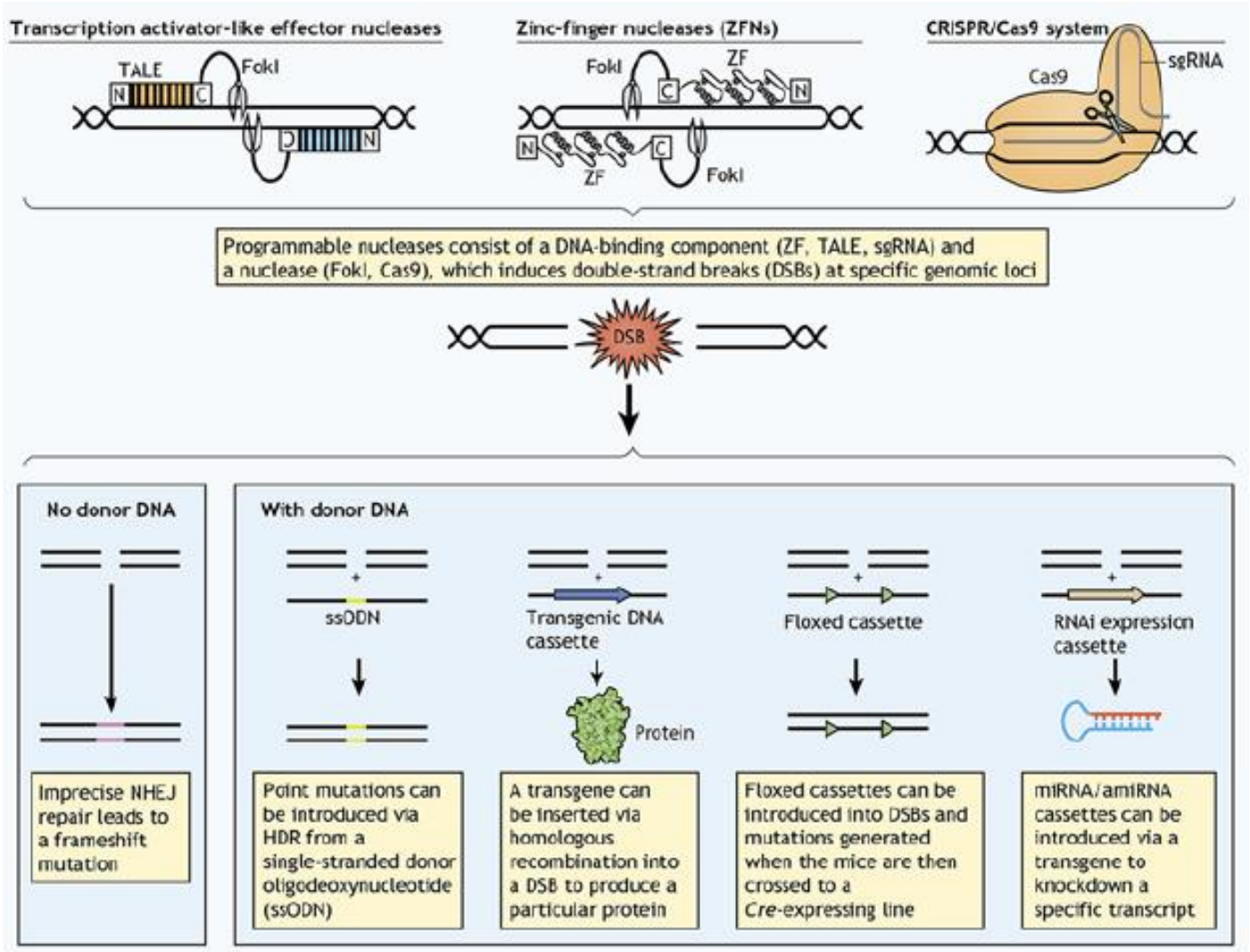
Knock In strategy: mutation



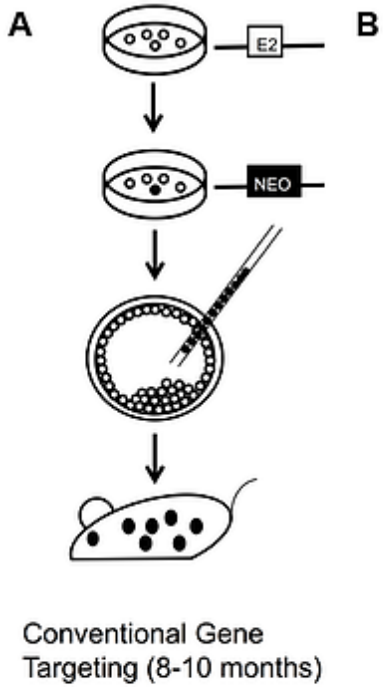
Humanized Knock In mouse



Genome Editing with Programmable Endonucleases

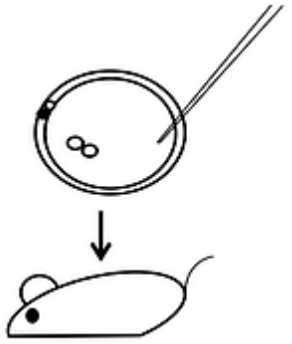


Gene Targeting in ES Cells



Conventional Gene Targeting (8-10 months)

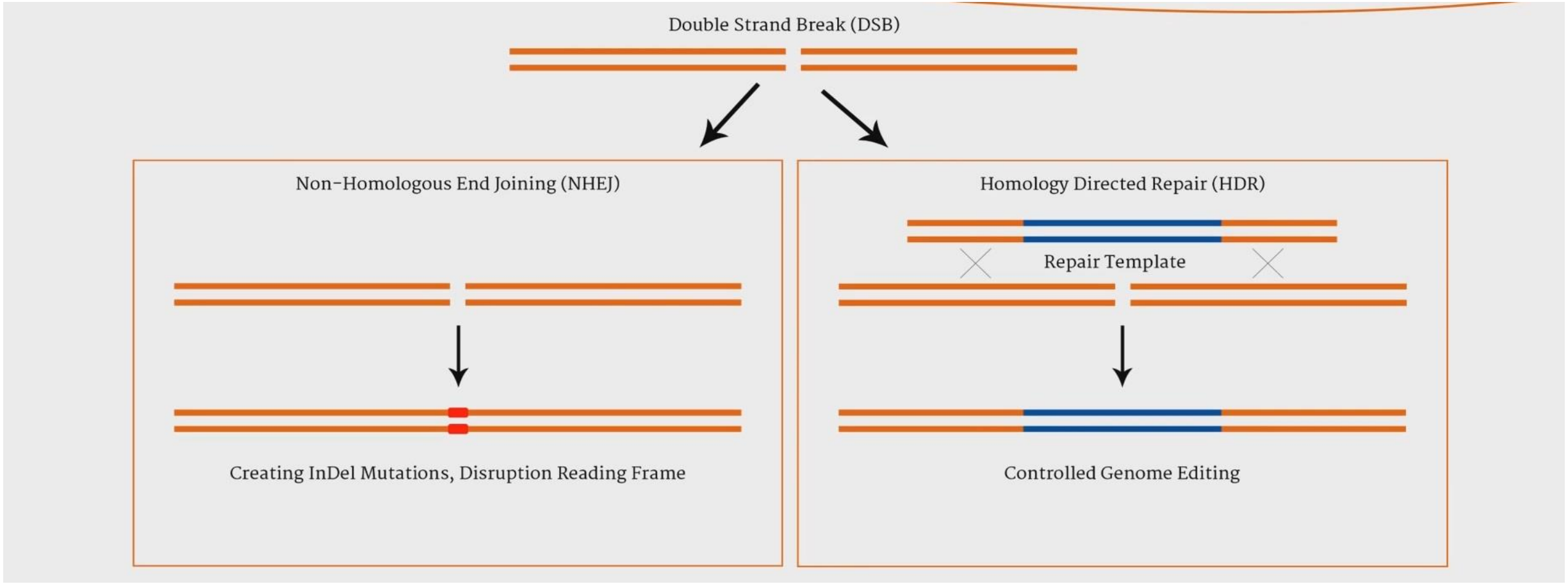
Pronuclear Injection



CRISPR Gene Editing (~3 months)

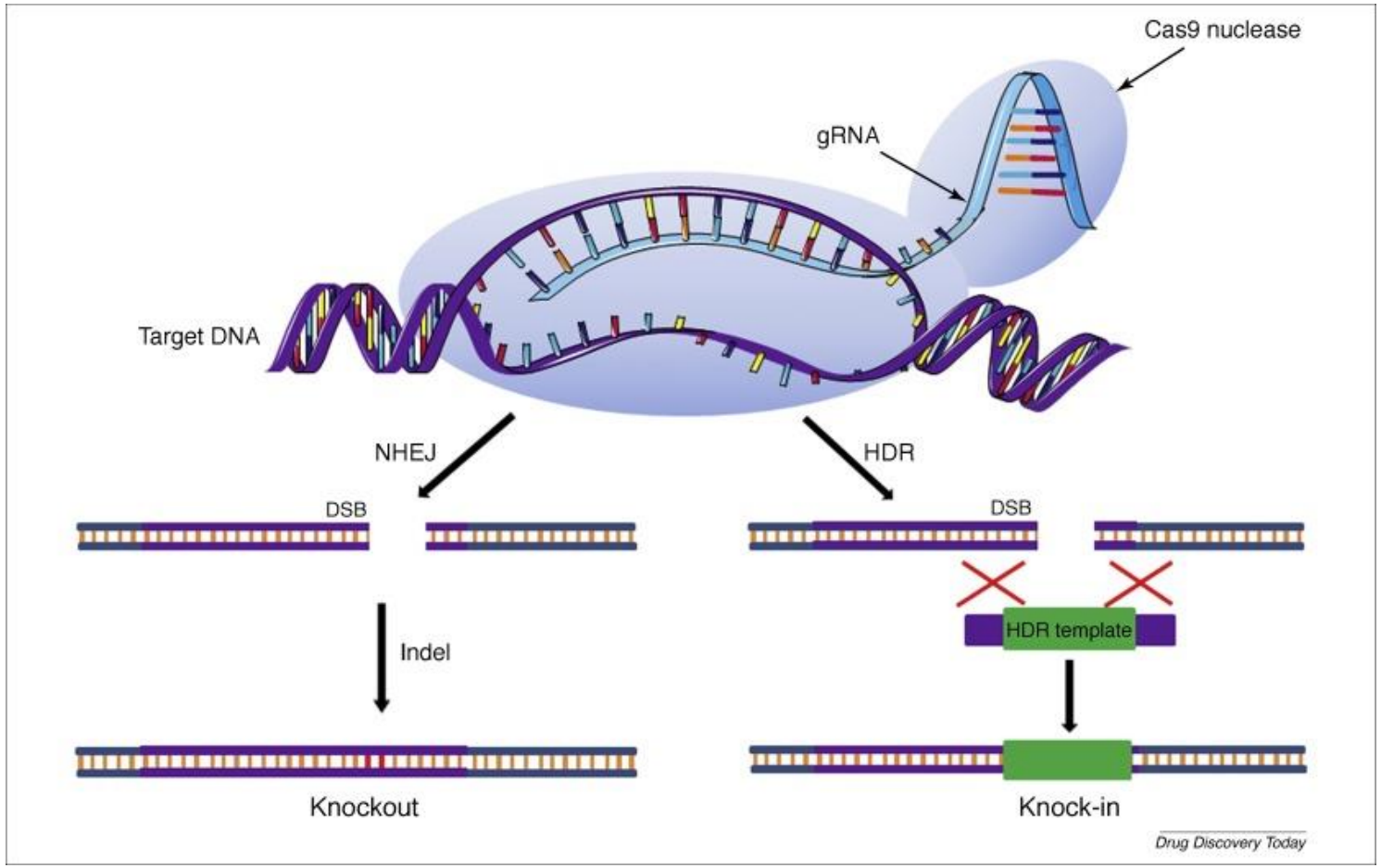
Figure 1: In conventional gene targeting (A), you must first generate your desired mutation in mouse ES cells, select for the mutation and inject the ES cells into a mouse embryo to create chimeric mice. This laborious process takes ~8-10 months. With CRISPR (B), you can inject CRISPR components directly into a zygote and obtain a knockout mouse in about 3 months). Image courtesy of Wenning Qin.

Double strand break repair

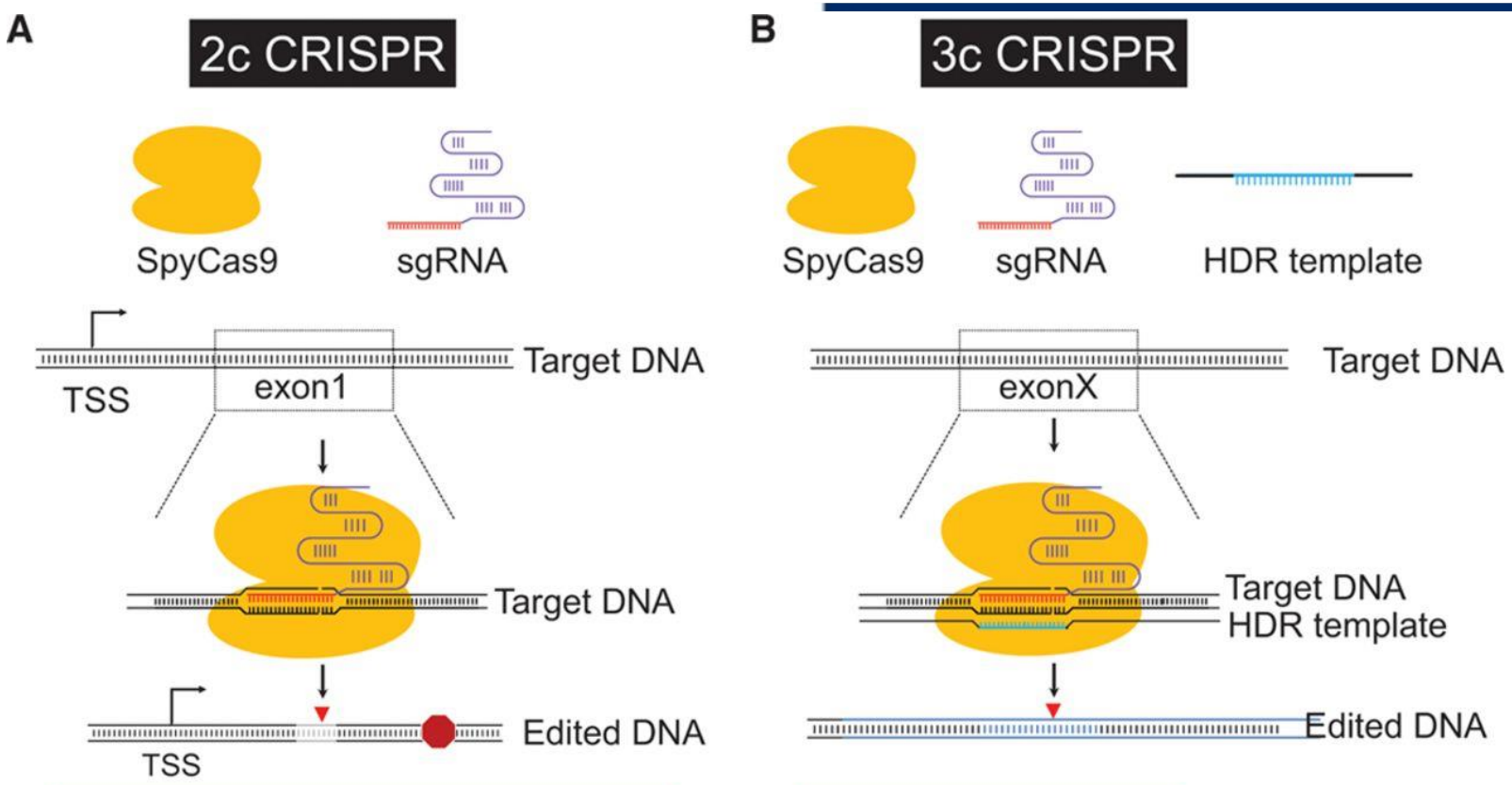


https://www.youtube.com/watch?v=1aJxXWkE3Ek

CRISPR CAS9-Mediated repair



CRISPR CAS9-Mediated genome editing



Random insertion/deletion/substitution

- Frameshift
- Premature stop
- Splice disruption
- Regulatory element deletion (Promoter/enhancer/lncRNA)

Precise genome editing

- Gene correction
- SNP modeling
- Conditional allele
- Epitope tagging
- Fine mapping regulatory element

Production of transgenic mice using CRISPR CAS9

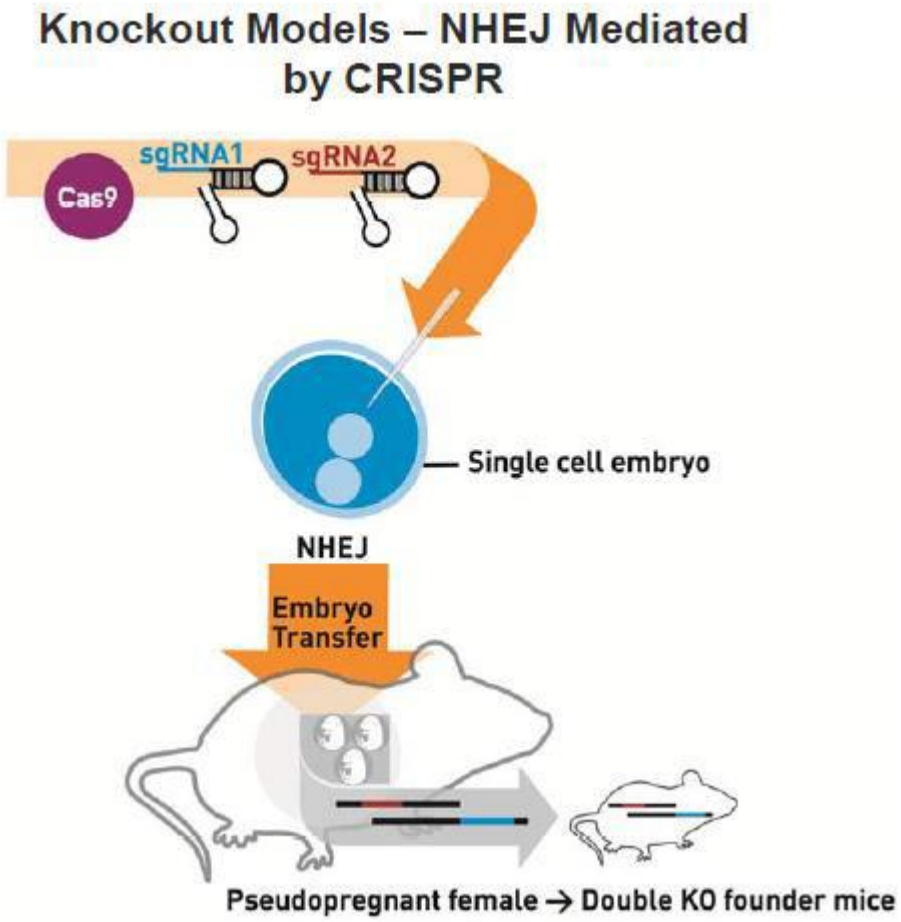
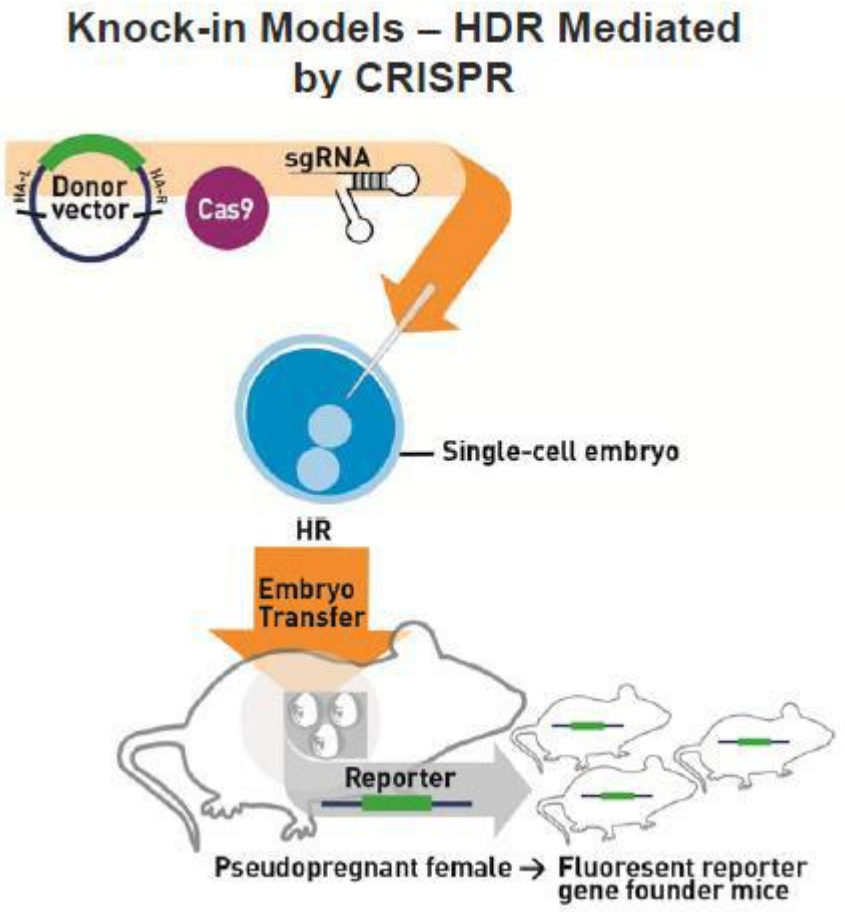


Figure 1. Generating knockout (KO) mouse models using a Non-Homologous End Joining (NHEJ) approach utilizes optimized sgRNAs co-injected with Cas9 mRNA, resulting in mice with frame-shifting insertion/deletion (indel) mutations and gene disruption.

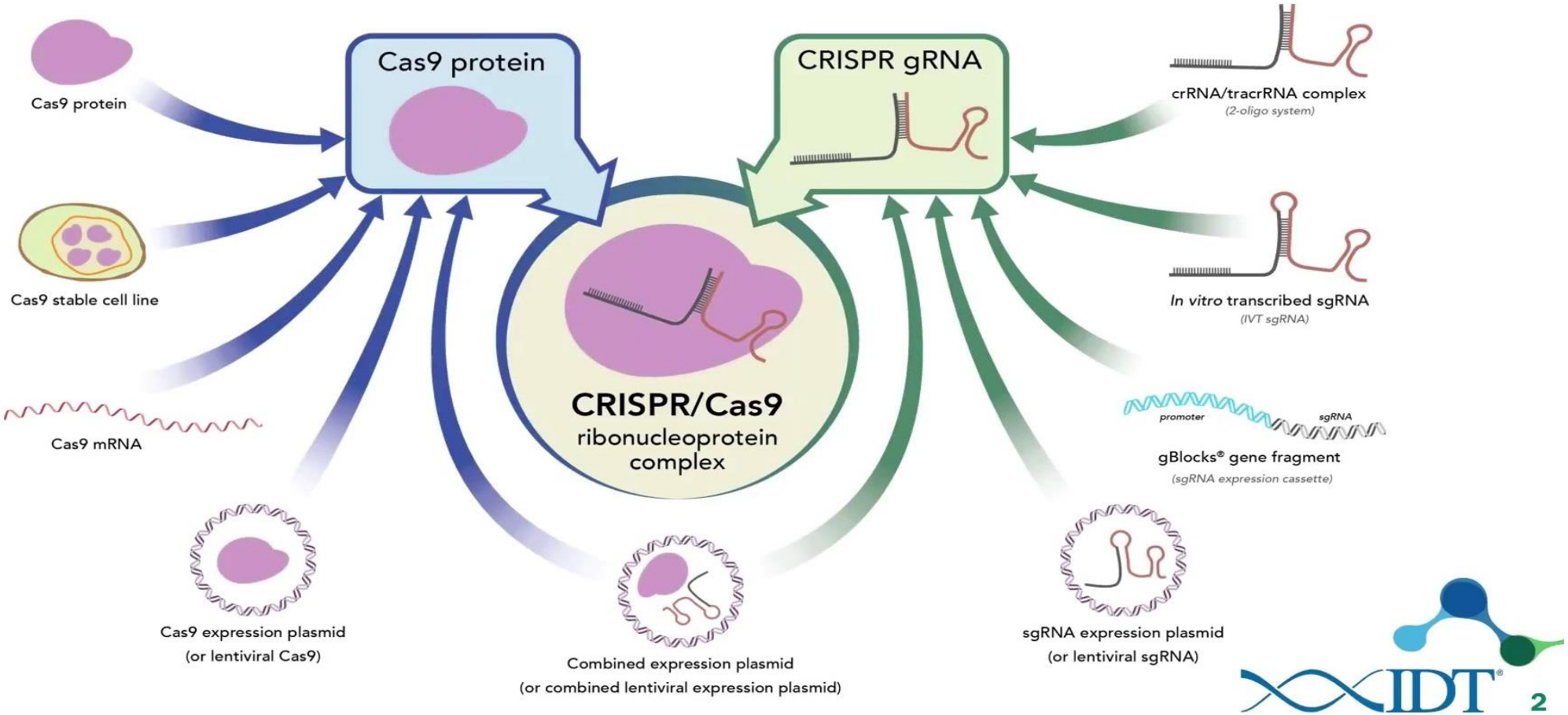
Use of Cas9-expressing mice in this manner reduces the time and resources it would take to design traditional gene-targeted mutants and to cross these multiple mutants together to create mice with the necessary complex genotypes. Mice that express Cas9 systemically can be used for similar studies. With these mice, only injections with a sgRNA- expressing virus is required.

Production of transgenic mice using CRISPR CAS9

Figure 2. Generating knock-in (KI) mouse models using a Homology Directed Repair (HDR) use either a single-stranded oligonucleotide or plasmid donor template are co-injected with optimized sgRNAs and Cas9 mRNA.

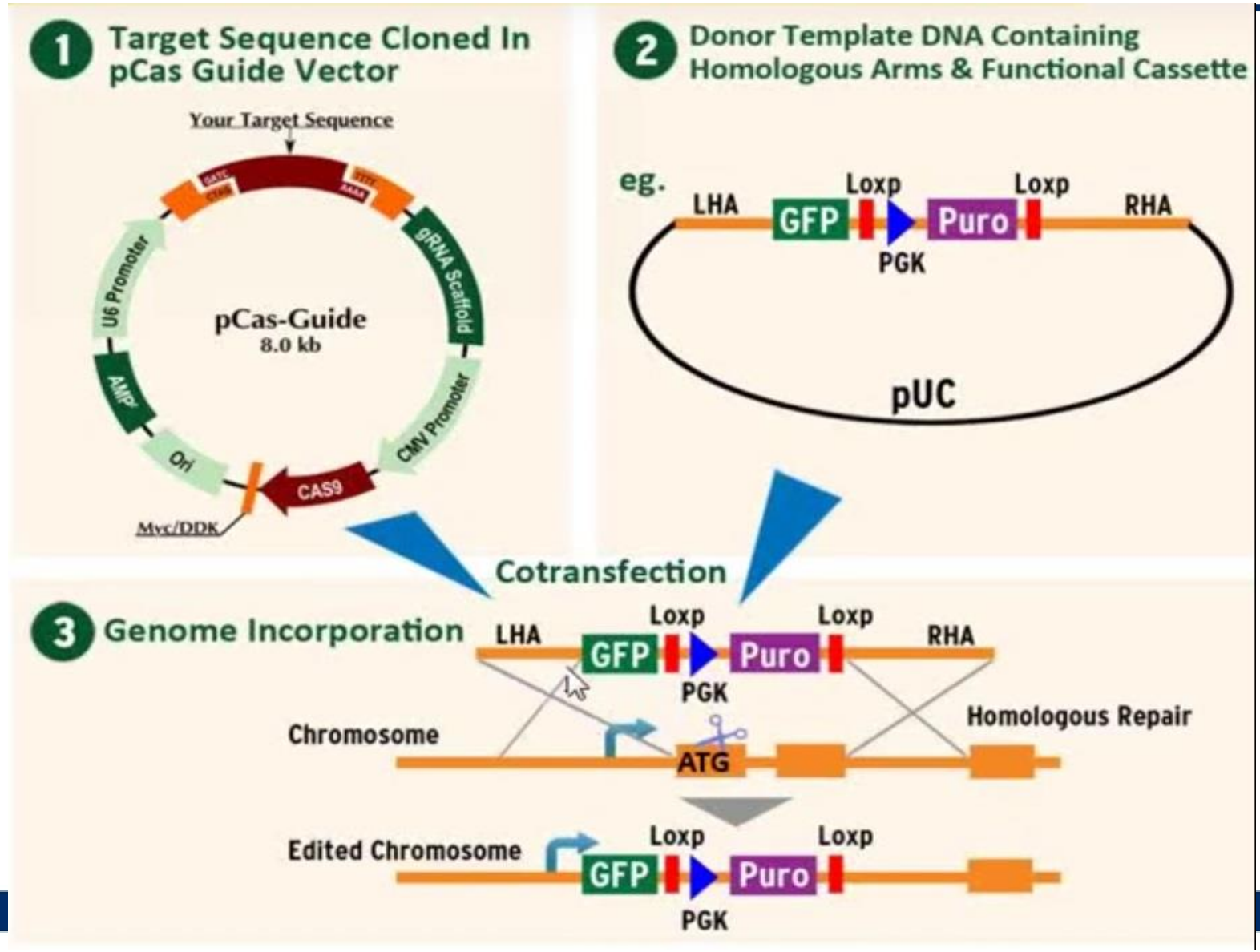


Available vector solutions

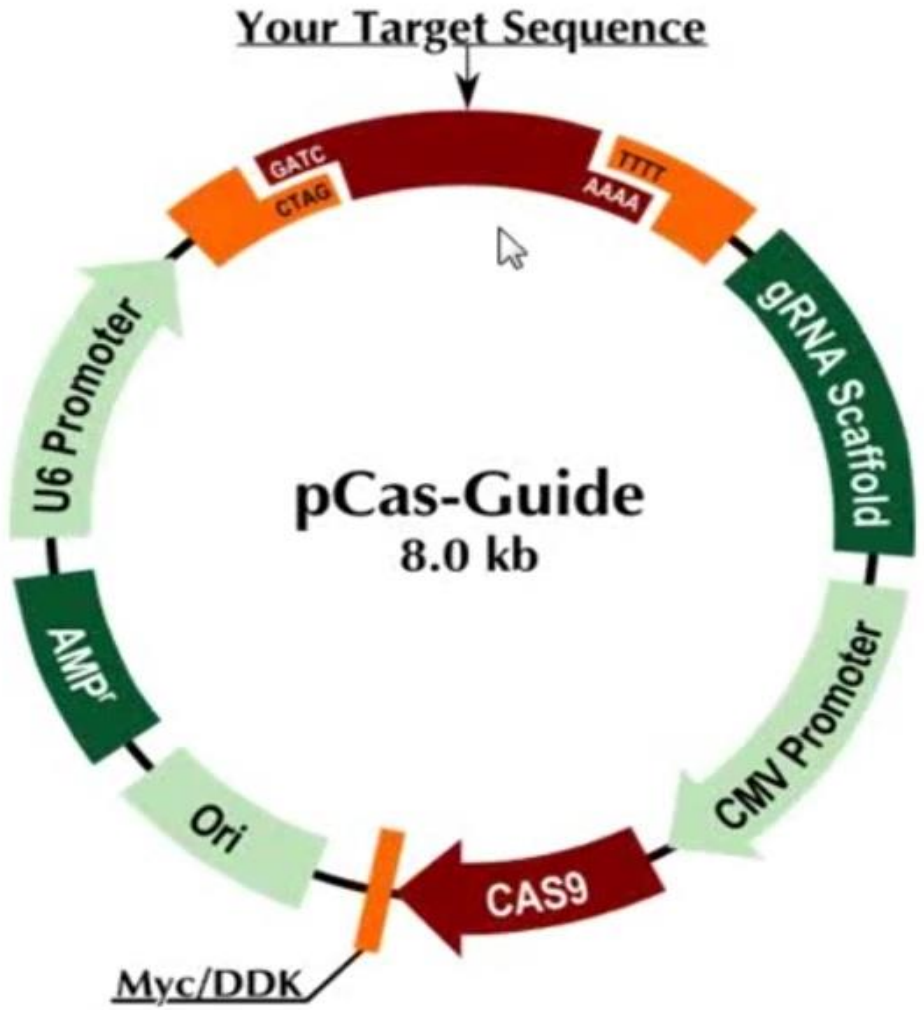


https://www.youtube.com/watch?v=HEAdu-JovLU

CRISPR vector systems - HR

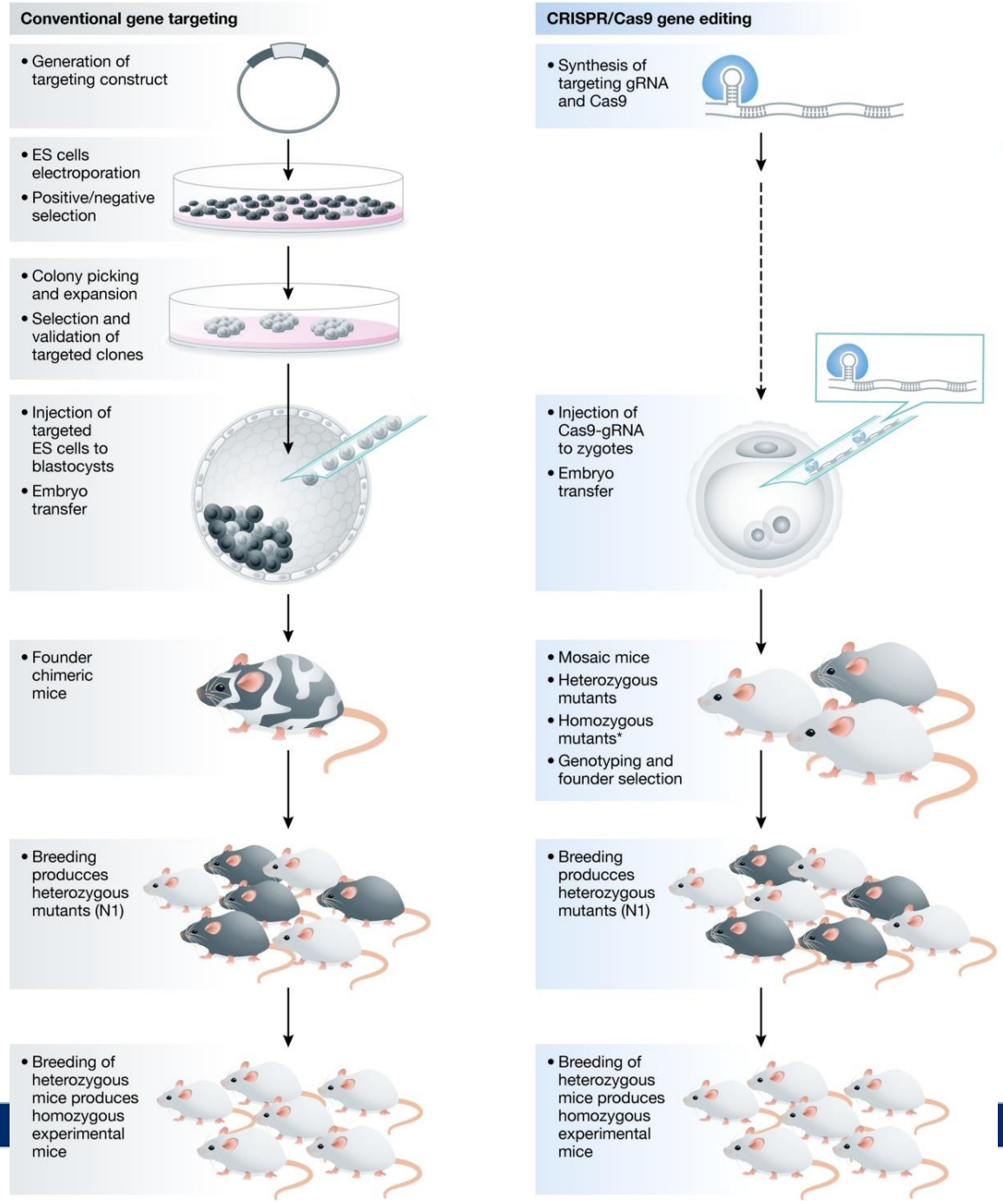


All-in vector



pCas-Guide

- Target sequence cloning
- Expresses Cas9

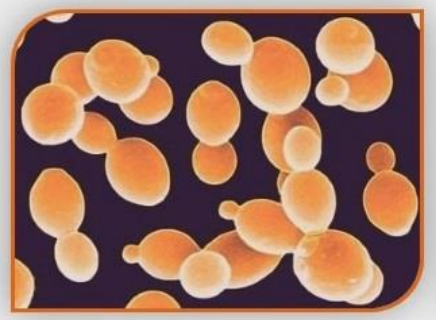


Production of transgenic mice using CRISPR CAS9

Faster!

CRISPR applications up to date

2012



Baker`s Yeast

Zebra Fish

Fruit Fly

Nematodes

Plants

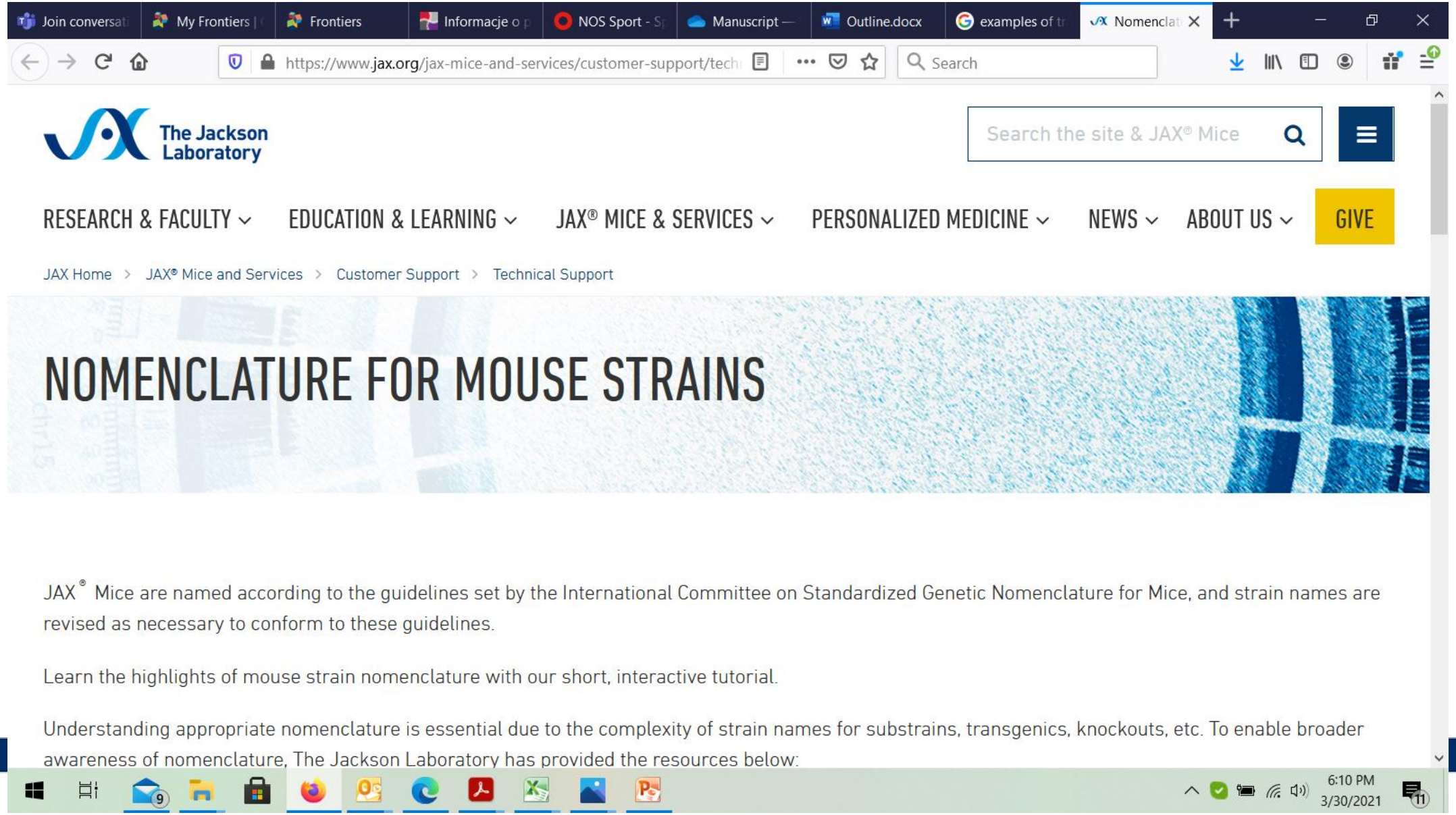
Mice

Mouse strains & mouse models

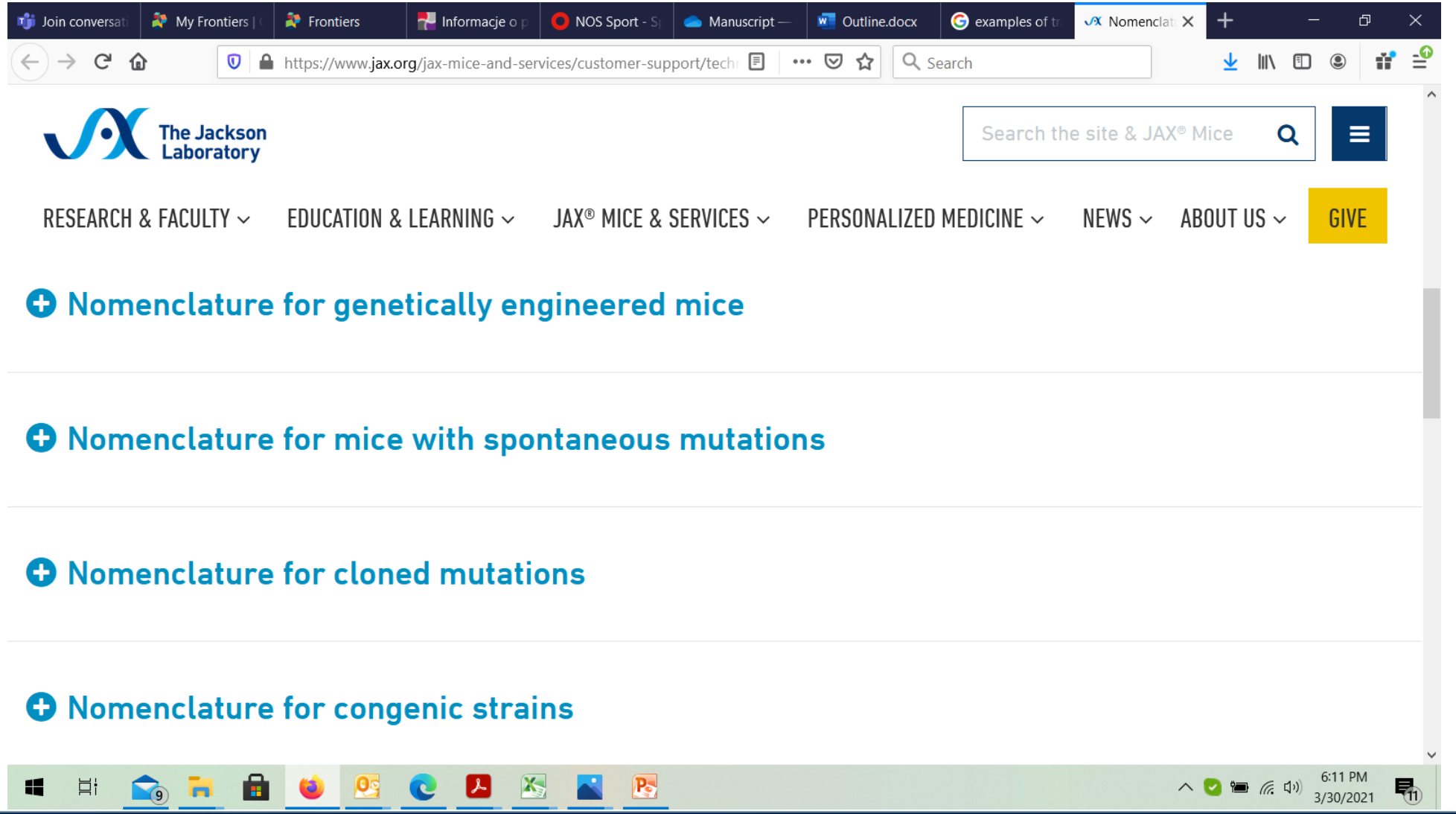
The survey results indicate that mouse is the overwhelmingly preferred laboratory animal;

The most widely used mouse strains are **C57BL/6 mice and BALB/c mice**. Other strains, such as A/J mice, **CD1 mice**, and ICR mice, were also used.

Mouse strains & mouse models



Mouse strains & mouse models



MOUSE NOMENCLATURE

Inbred and Hybrid

Inbred*

C57BL/6J = B6

129S1/SvImJ = 129S

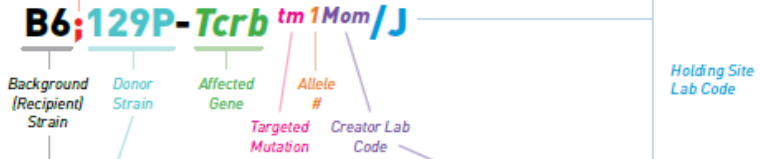
F1Hybrid*

B6129SF1/J ♀ ♂

*Standard strain abbreviations. Learn more at jax.org/nomenclature.

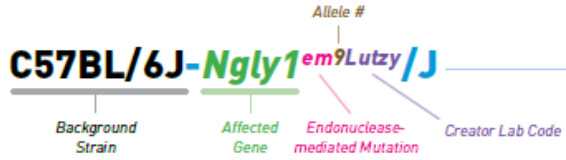
Spontaneous or Induced Mutation

Knock-out, Knock-in or Floxed



Transgenic (Tg)

Endonuclease-mediated



*Mixed Strain Background = semicolon (;)
Backcrossed to recipient inbred strain < 5 generations

** Congenic or Incipient Congenic = period (.)
Backcrossed to recipient inbred strain ≥ 5 generations

1-800-422-6423
(US, Canada & Puerto Rico)
1-207-288-5845
(from any location)
jax.org/nomenclature

The Jackson Laboratory

Mouse strains & mouse models

Mouse strains & mouse models

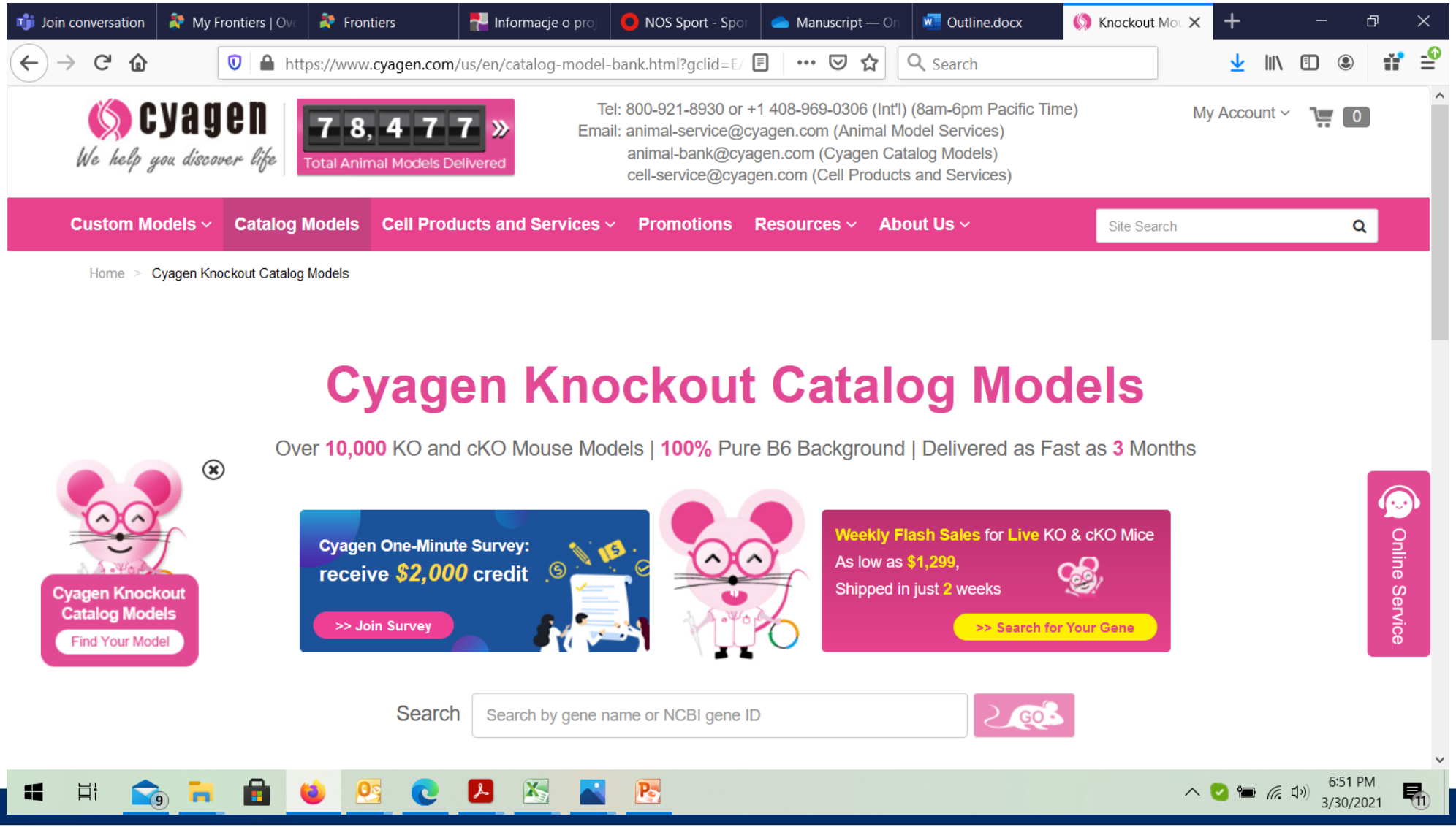
Data type	Count
Total mutant alleles (in ES cell lines and mice)	738,364
Mutant alleles in mice	24,339
Genes with mutant alleles	14,743
Genes with mutant alleles in mice	9,636
Mammalian phenotype ontology (MP) terms	8,744
Genes with phenotype annotations	8,903
Genotypes with phenotype annotations	43,335
Total MP annotations to genotypes	227,169
Human diseases with one or more genotypic mouse models	1,148
Mouse genotypes modeling human diseases	3,668
Quantitative trait loci (QTL)	4,696
Total recombinase (Cre)-expressing transgenes and alleles	1,739

[a] Data as of May 5, 2012, www.informatics.jax.org. New data are added to the MGI database daily; thus, actual counts will be higher than those shown here

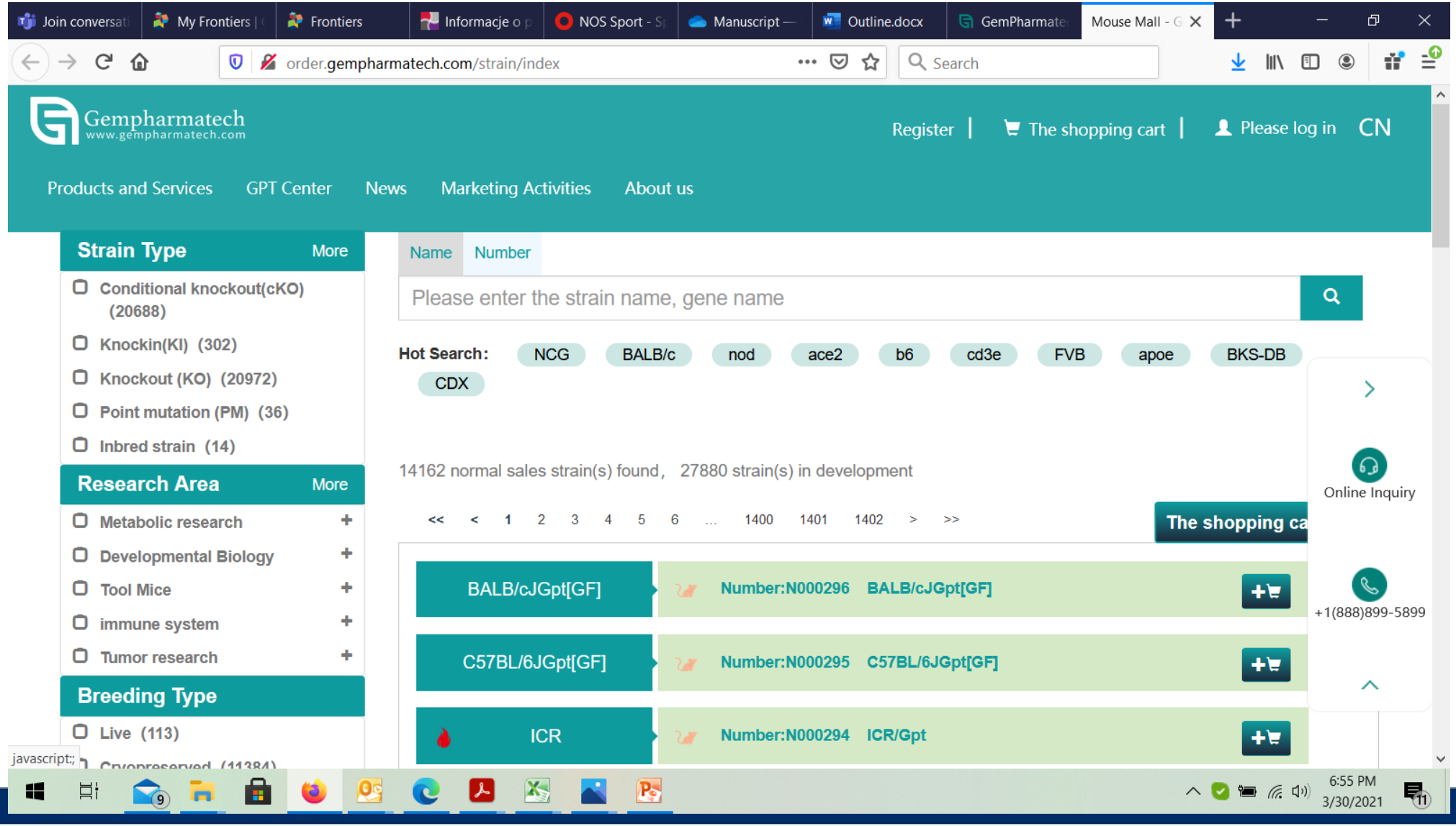
[b] Mutant allele counts include spontaneous, induced (e.g., by ENU), and genetically engineered alleles. Transgenes, which are not part of the normal mouse genome, are not included

[c] Mutants present only in ES cell lines versus those created in mice or made into mice from ES cells are distinguished in several table counts. All phenotype-related data refer to mutations present in mice

Mouse strains & mouse models



Mouse strains & mouse models



Mouse strains & mouse models

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The World's Largest Platform for Mouse Model Customization

- 20 years of experience
- Technical expertise in CRISPR/Cas9, TALEN, ZFN, ES cell-based HR, and Super TG
- Annual capacity of 5,000 new GEM lines
- 16,000 GEMs available for distribution and growing

Mouse strains & mouse models

◄ *Model Organisms for Biomedical Research*

Trans-NIH Mouse Initiatives

- *Deltagen and Lexicon Knockout Mice and Phenotypic Data* —New!
- *NIH Knockout Mouse Project (KOMP)* —New!
- *NIH Statement on Sharing and Distributing Mouse Resources*
- *NIH Plan for Mouse Genomics and Genetics Resources*
- *Courses & Scientific Meetings*
- *Funding Opportunities*
- *Reports & Publications*
- *Major Resources*
- *IC Contacts*

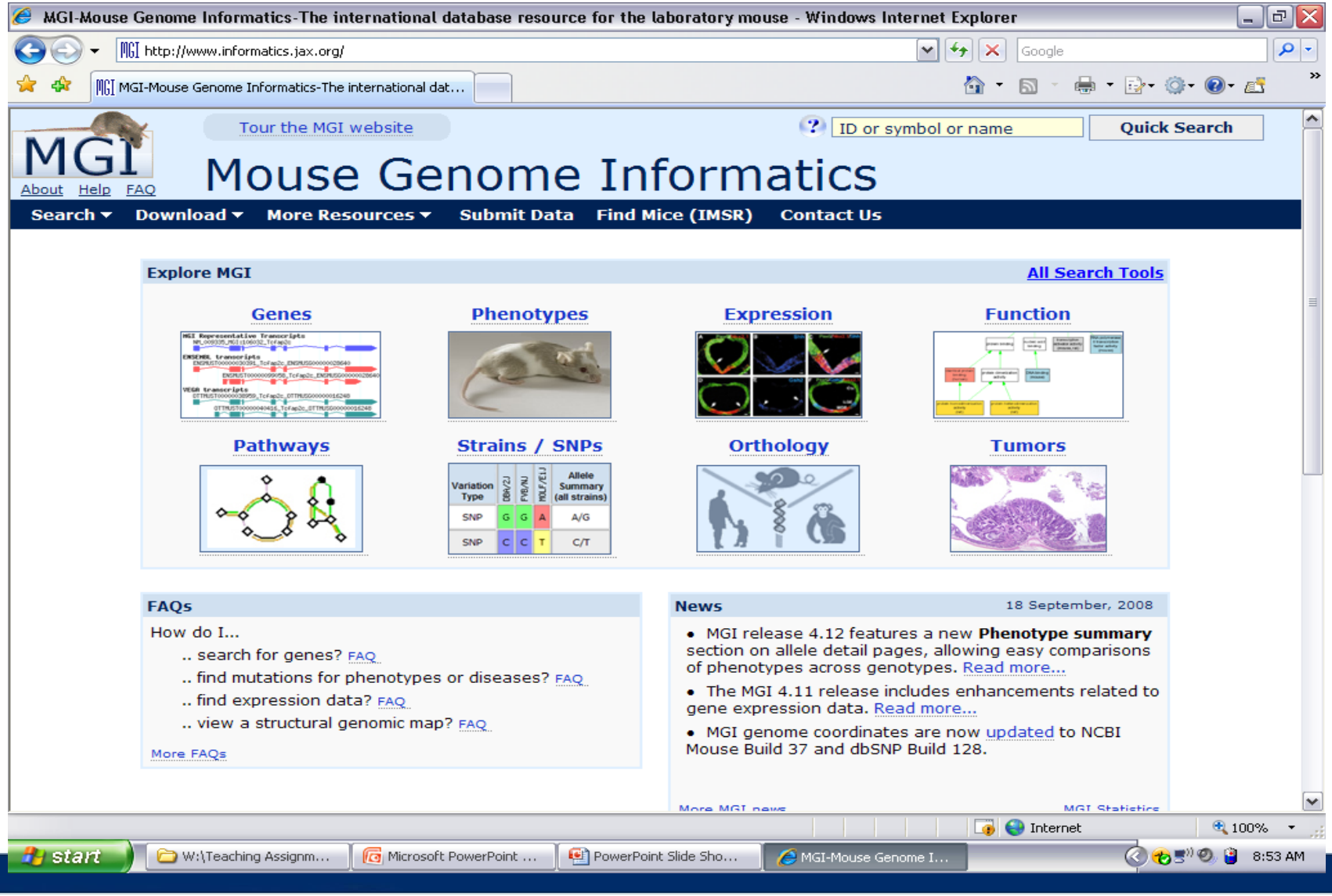
- *What's New*

Welcome to the NIH Mouse Initiatives Web site.

In March 1998, the NIH convened a group of scientists to develop priorities for mouse genomics and genetics resources. In response to the community's recommendations, the NIH has created a Trans-NIH Mouse Genomics and Genetics Resources Coordinating Group and a strategic implementation plan. For the convenience of all interested investigators, we have established this Web site as a central information resource. This site will provide information about funding opportunities; major mouse genomics and genetics resources; policies affecting resources; courses and scientific meetings related to the mouse initiative; and selected reports and publications. When appropriate, items not in response to the initiative, but which are deemed relevant to the initiative, will be posted. Posting decisions are made by a sub-committee of the Trans NIH Genomics Resources Working Group.

Suggestions for improving the Web page and for items to include are most

Mouse Genome Informatics



Mouse Genome Informatics

Mouse Genome Informatics

Mouse Genome Database

Gene Expression Database

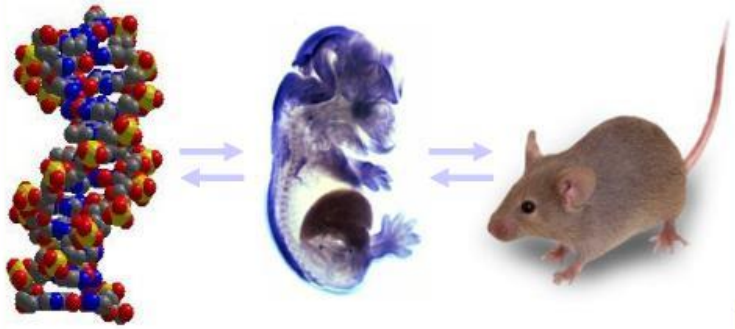
Mouse Genome Sequencing Project

Mouse Tumor Biology Database

Gene Ontology Consortium

www.informatics.jax.org

Genotype **Expression** **Phenotype**



Objective:

Facilitate the use of the mouse as a model for human biology by furthering our understanding of the relationship between genotype and phenotype.