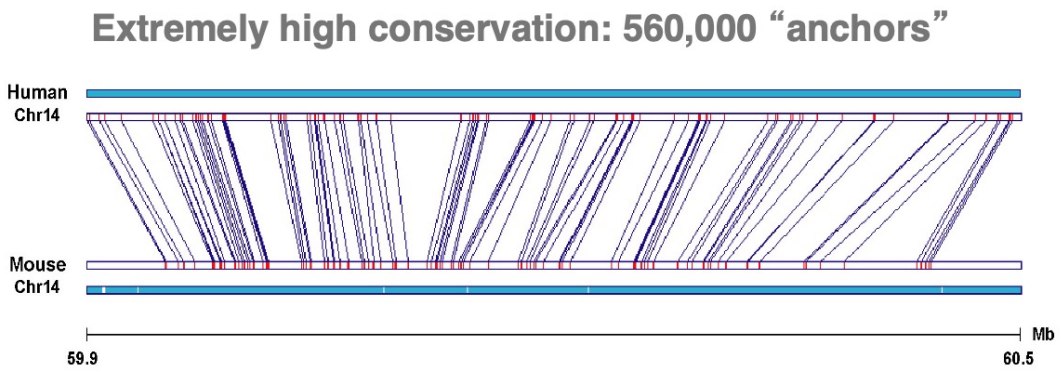
Mus musculus

ADVANTAGES

- Small
- Lots of pups
- Mutant availability
- Sequenced genome
- Genomic manipulation
- It's a MAMMAL!
- We are separeted by only 60 milion years

Characteristics



Mouse-Human Comparison
both genomes 2.5-3 billion bp long
> 99% of genes have homologs
> 95% of genome “syntenic”

Genome:

- Chromosomes: 19 autosomes, and XX/XY
- Number of genes: ~20.000

Biology:

- Gestation period: 20 days
- Weaning: 3 weeks
- Sexual maturity: 7 weeks
- Average lifespan in lab: 1.5–2.5 years
- Litter size (Offspring): 6–9
- Litters per female: 4–8

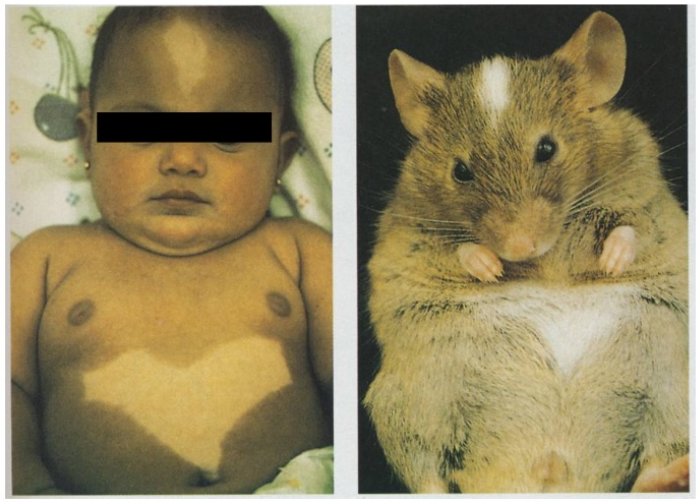
Mouse Genetics

- **"Forward Genetics" approaches** seek to understand which genes contribute to a phenotype in an unbiased manner:
  - Natural variation
  - Insertional mutagenesis (e.g., transposons)
  - Chemical mutagenesis (e.g., ENU)

- **"Reverse Genetics" approaches** seek to understand what a candidate gene contributes to a phenotype:
  - Genetic engineering (e.g., CRISPR or homologous recombination in ES cells)

A model for human disease

ADVANTAGES



Kit mutation

DISADVANTAGES

- Differences in cognitive functions, behaviour, genic expression
- Big genome and Long gestation compared to other model systems
- **Requires animal facility**
- **Cost consuming**

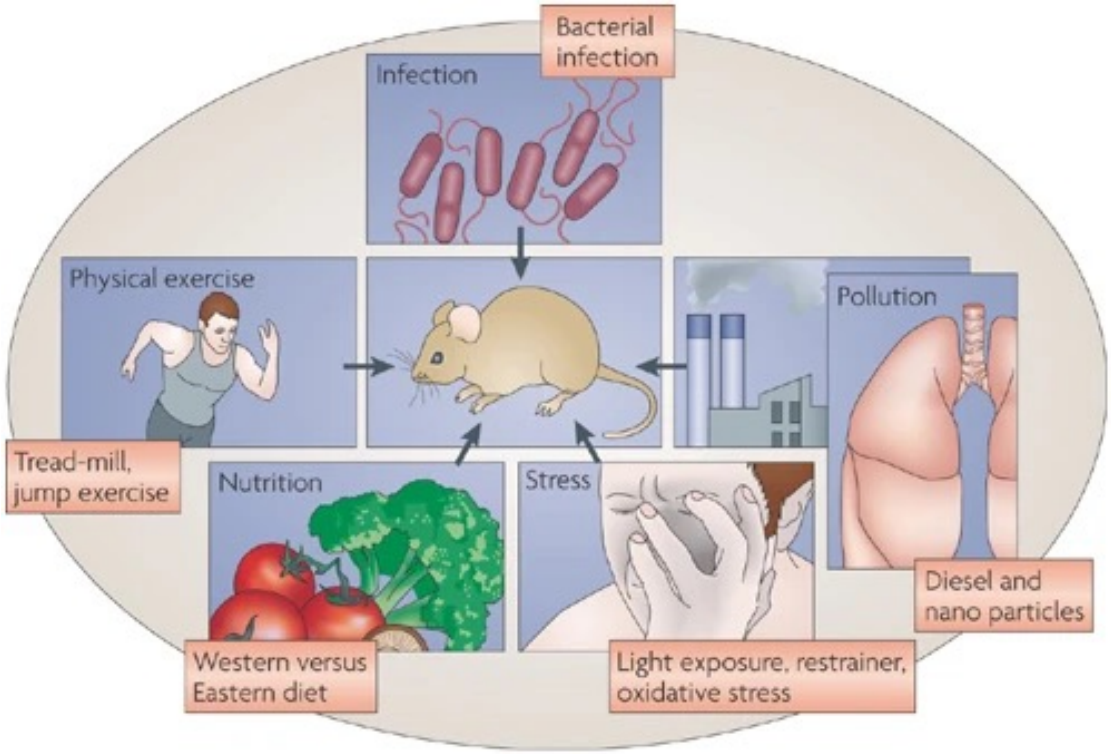
Why animal models?

- **Understanding the disease process**
  - Multiple and invasive samples can be obtained
  - The system can be manipulated to investigate disease mechanisms

- **Development of treatments**
  - Drug testing to modify the disease process
  - Testing of new therapies

Exploring the complex relationship between environment, genotype, and phenotype

By mimicking specific environmental exposures or lifestyles that have a strong impact on human health, researchers determine their effects on molecular networks and on the etiology and progression of disease. This reveals the physiological and molecular mechanisms of **genome-environment interactions**. "Challenge platforms" are currently being created that focus on the primary environmental risk factors for human health.



Nature Reviews | Genetics

Example: Diabetes-Related Mice Available for Research

- **Type I diabetes** (3 strains)
- **Type II diabetes** (3 strains)
- **Hyperglycemic** (27 strains)
- **Hyperinsulinemic** (25 strains)
- **Hypoglycemic** (1 strain)
- **Hypoinsulinemic** (5 strains)
- **Insulin resistant** (30 strains)
- **Impaired insulin processing** (7 strains)
- **Impaired wound healing** (13 strains)

Sources/Strains of laboratory mice

COMMON MOUSE SUPPLIERS

- The Jackson Laboratory
- Charles River
- Taconic Farms
- Harlan Sprague Dawley

COMMON MOUSE STRAINS

- C57BL/6J
- BALB/c
- C3H
- DBA/2

Inbred strains

Is defined as "*one that has been maintained for more than 20 generations of brother-sister matings and is essentially homozygous at all genetic loci, except for mutations arising spontaneously*"

Therefore, these strains are genetically identical in all other aspects except at the locus of interest - useful for studying cancer, inheritance of visible traits, histocompatibility etc…

The Jackson Laboratory is the major supplier of inbred strains

History

•Mice have been used for over 100 years to study mammalian inheritance and natural selection.

•Mice were originally kept by "fanciers" (hobbyists) who established unique lines selected for coat color or other external characteristics.

•Around 1900, a retired schoolteacher named Abbie Lathrop began breeding and selling mice from her home in Granby, MA.

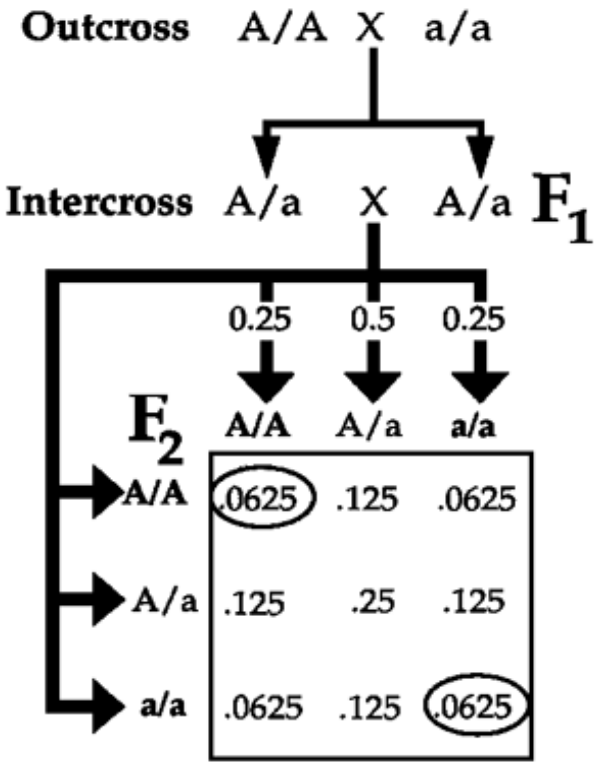
•Among her clients was William Castle of Harvard University, who established some of the first popularized inbred mouse strains.

•Abbie Lathrop also conducted her own experimental breeding program, giving rise to strains such as C57BL/6 and C57BL/10.

•In 1929, The Jackson Laboratory was founded to serve as a repository for inbred strains and mutant lines for experimental purposes.

Inbreeding

Repeated mating between brothers and sisters leads to a completely homozygous genome: no variation!



- **Crossing to obtain F1**
- **F1 x F1** – brother/sister matings (cross-mated)
- **F2 x F2** – F2 chosen at random, etc....
- **F19 x F19** – defined as **inbred**
- essentially (**98.7%**) homozygous at all loci

Inbred vs outbred

Inbred strains are genetically stable, provide simple genetic comparisons, but can lack the range of heterogeneity found in human populations

More outbred populations capture more heterogeneity, but are "one of a kind" (and can be challenging to work with!)

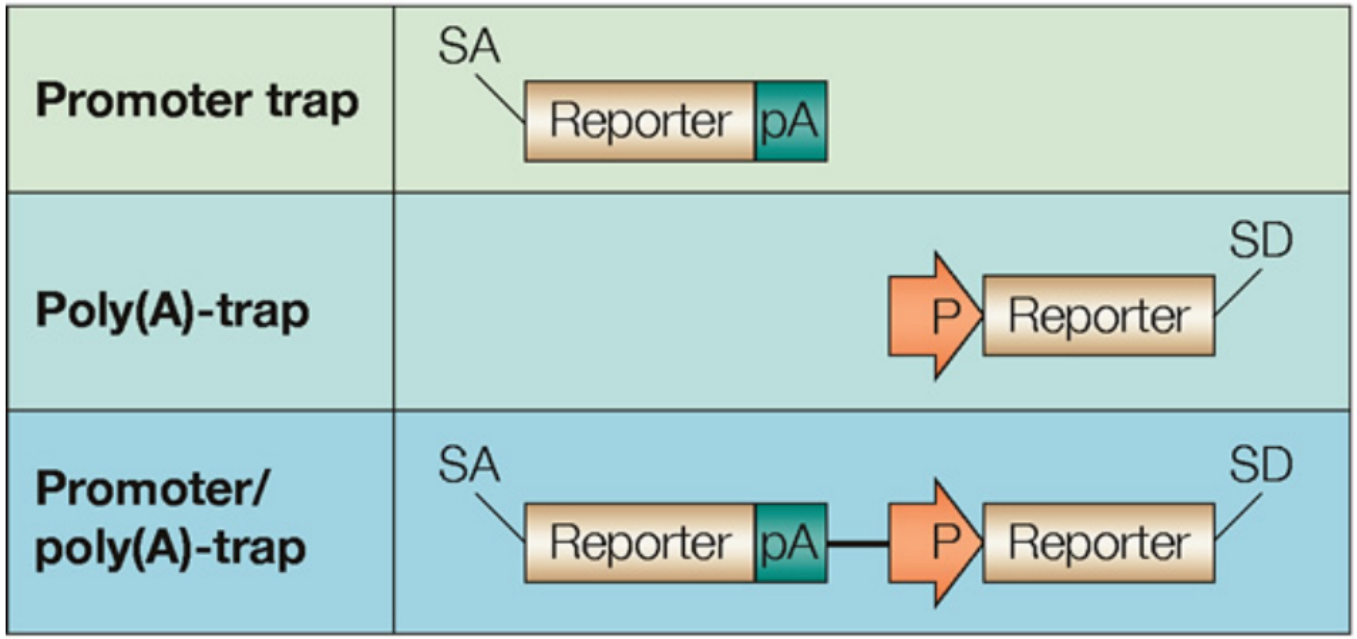
Insertional mutagenesis in mouse

- Random mutagenesis with retrovirus
- Vectors for genomic sequence replacement (Knock-out)
- Vectors for genomic sequence insertion (Knock-in)
- Inducible systems (cre-LoxP, tet, ER/Tam, ecd)
- Gene-trap
- Transposon mutagenesis

Gene trapping

- They are DNA elements capable of inactivating the gene into which they insert themselves.

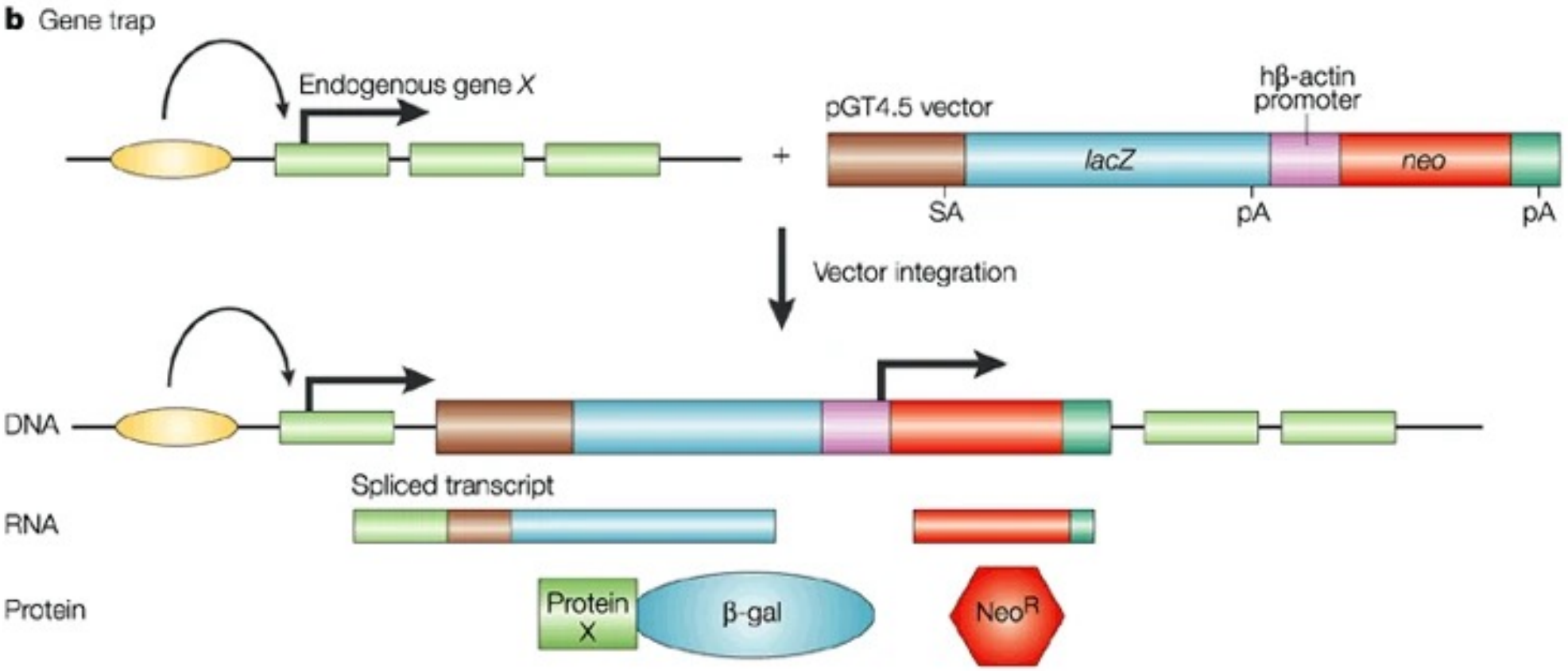
- They are carried by a plasmid or lentiviral vector and integrated randomly into the genome of ES (Embryonic Stem) cells.



SA: splice acceptor

SD: splice donor

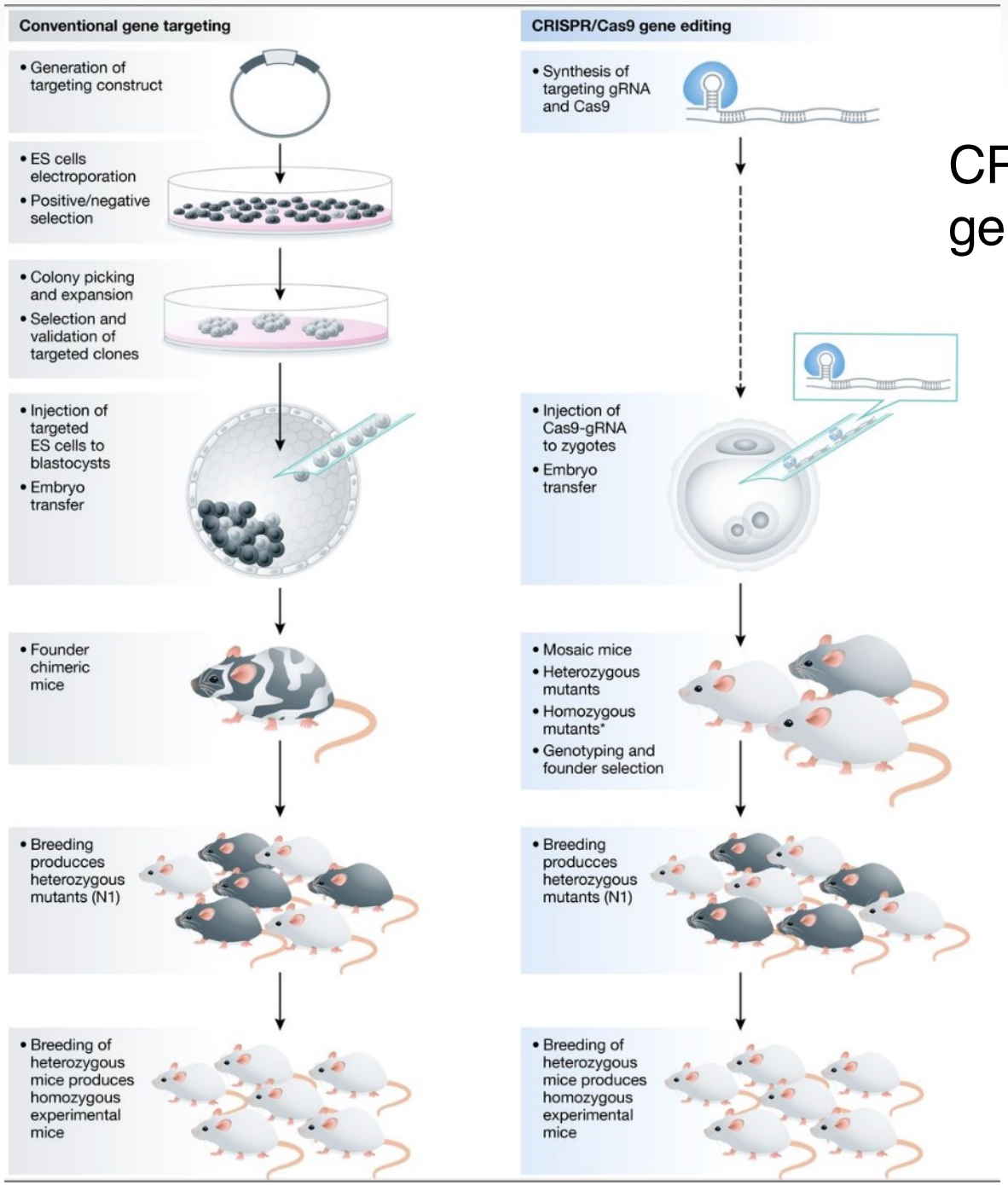
Gene trapping



Stanford et al, 2001, Nat. Revs. Genet.

How to modify the genome of mouse

Gene targeting (homologous recombination)



CRISPR/CAS9 gene editing

How to modify the genome of mouse

- Works only in ES cells

- The length of homology on both sides of the locus to be disrupted must be ~5-10 kb

- The source of the vector is important – it must be isogenic with the recipient cells (ES cells originate from 129 different genetic backgrounds)

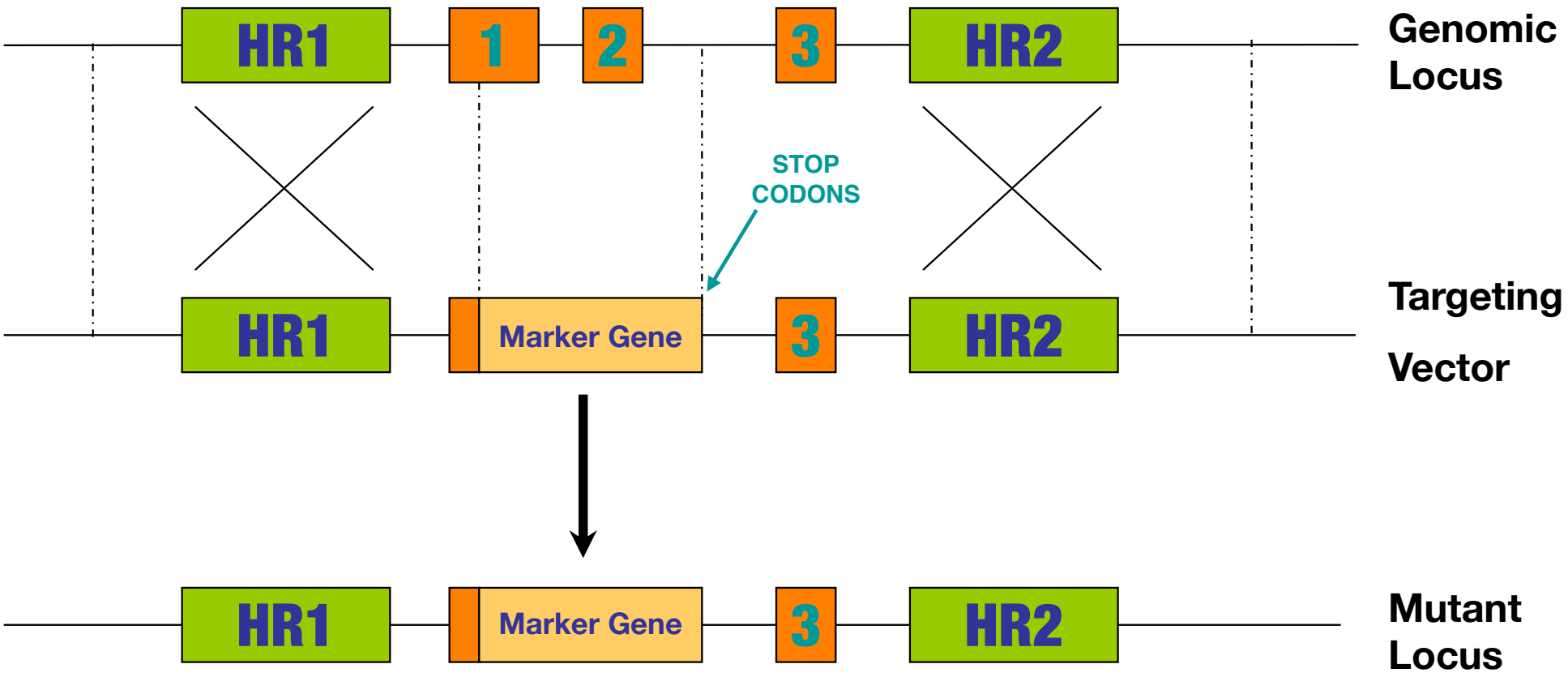
- Chromatin structure can influence targeting

- Clones must be analyzed using Southern blot and PCR

- It is necessary to verify whether the insertion is random or specific

- It is important to remove the selection marker

Vectors for genomic sequence replacement (Knock-out)



Positive selection

1) Drug Resistance

- Neomycin phosphotransferase (*neo*)
- Hygromycin B phosphoglycerate (*hyg*) They confer resistance to the neomycin analog, G418, and hygromycin.

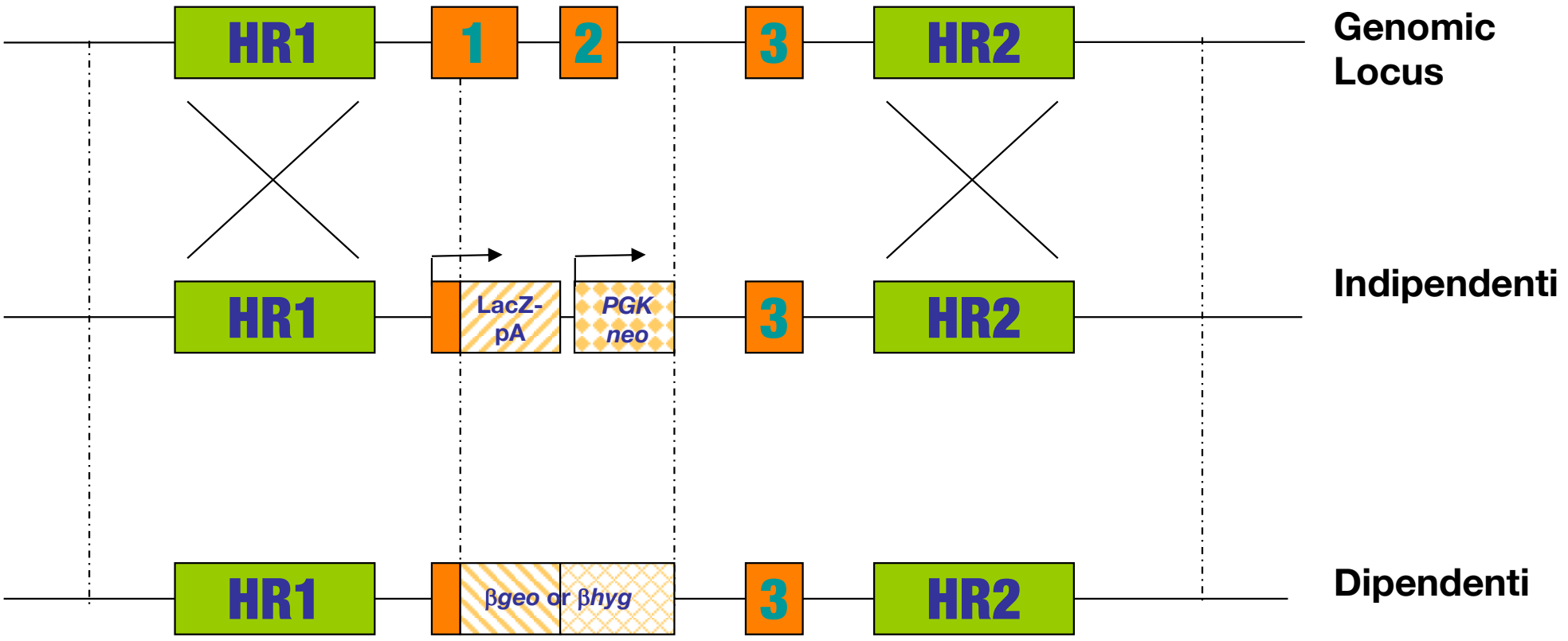
- INDEPENDENT: These include a cassette containing the phosphoglycerate kinase (PGK) promoter or the Herpes virus thymidine kinase (HSVtk) promoter.
- DEPENDENT: These do not require regulatory elements, but must be fused in-frame with the target gene.

2) Reporter Genes

- GFP, LacZ, and β-Geo

- N.B. Often, both are used together.

Positive selection

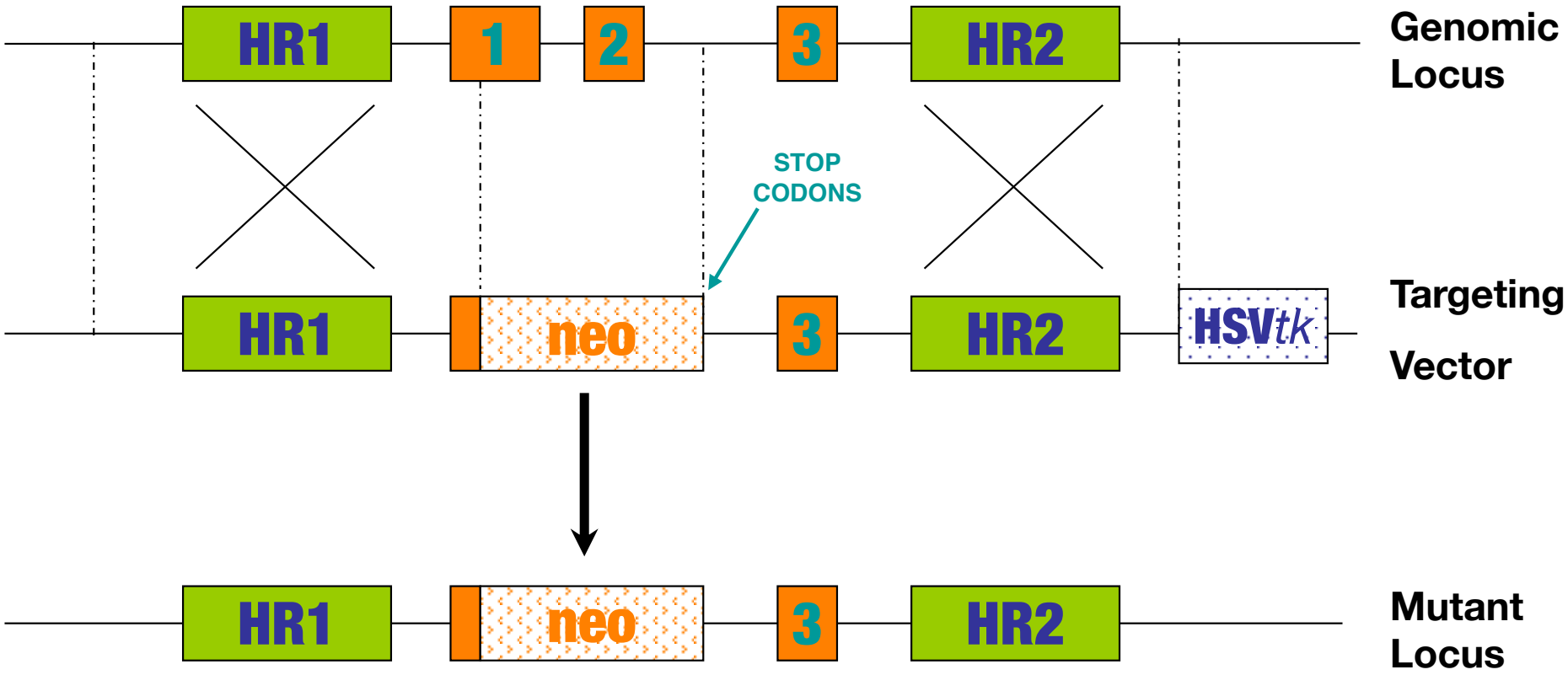


Positive-Negative selection

• A second marker for negative selection is placed at one end of the construct, outside the region of homology.
• **HSVtk** is the most commonly used and confers sensitivity to ganciclovir.
• It reduces the survival of [incorrectly targeted] clones by 5 to 10-fold.

Two possible integration scenarios:
1. RANDOM: Retention of both the positive and negative (tk) selection cassettes; results in lethality in the presence of ganciclovir.
2. HOMOLOGOUS: Integrates only the positive selection cassette, while the tk is lost during homologous recombination.

Positive-Negative selection



Critical points

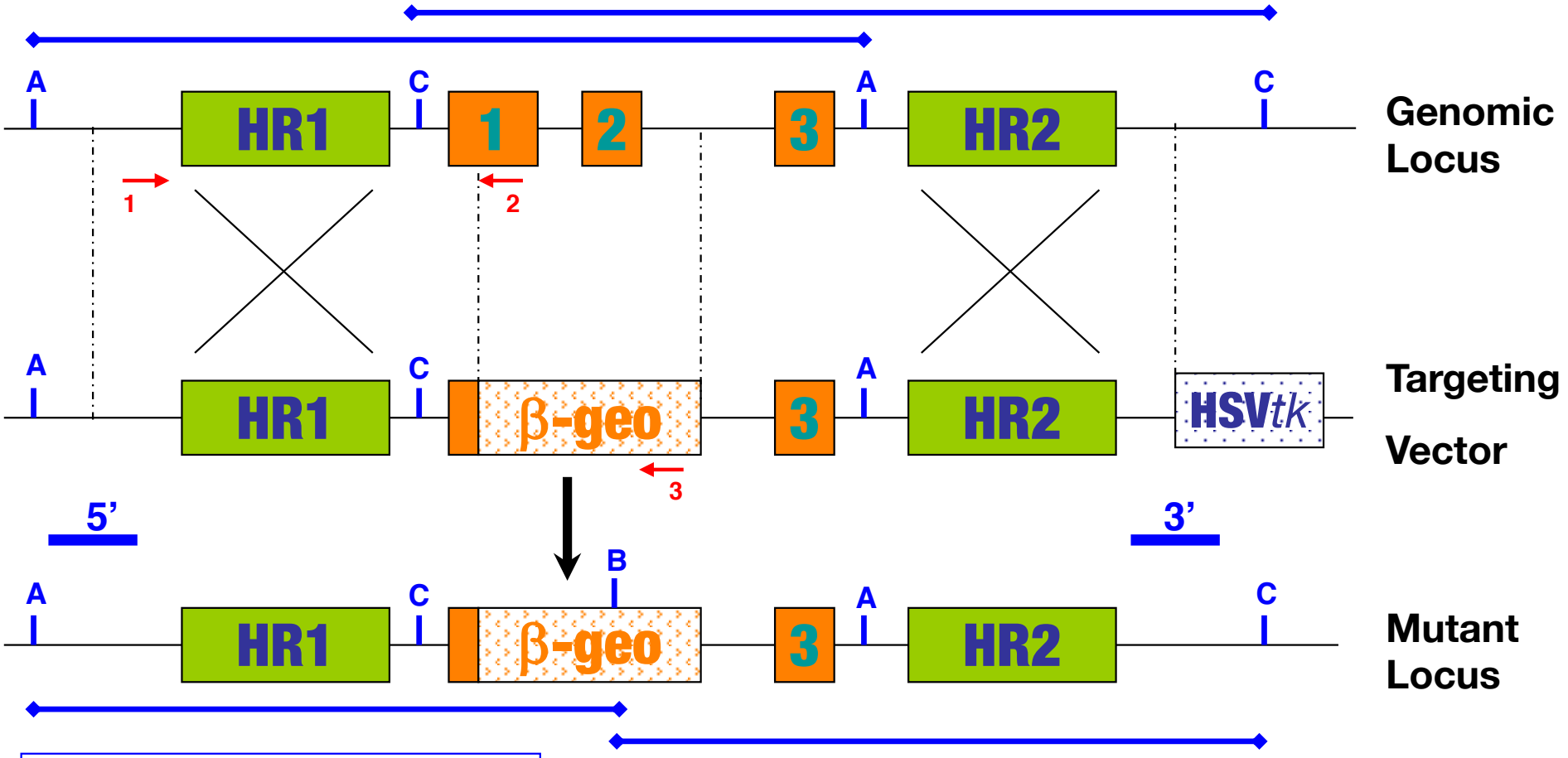
- **Isogenic DNA:** Important given the polymorphism of the various mouse strains. It increases targeting efficiency by as much as **25-fold**.

- **Length of homologies:** An increase in homology from **1.3 to 6.8 kb** increases the frequency of homologous recombination **250-fold**.

- **Construct size:** Can be up to **20 kb**.

- **Screening:** Use multiple methodologies to screen for positive clones.

Screening



SOUTHERN BLOT SCREENING:

A-A, C-C = both WT and MT, but different sizes, when probed with 5' and 3' probes, respectively

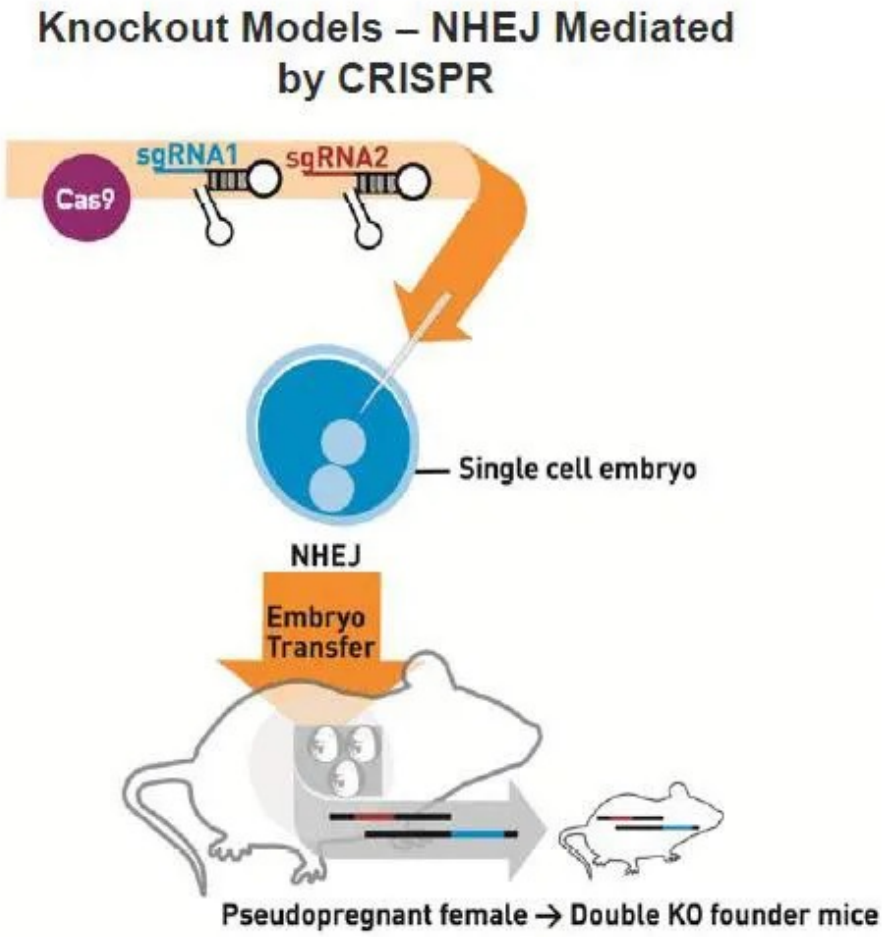
A-B, B-C = Specific to MT when probed with the same probes

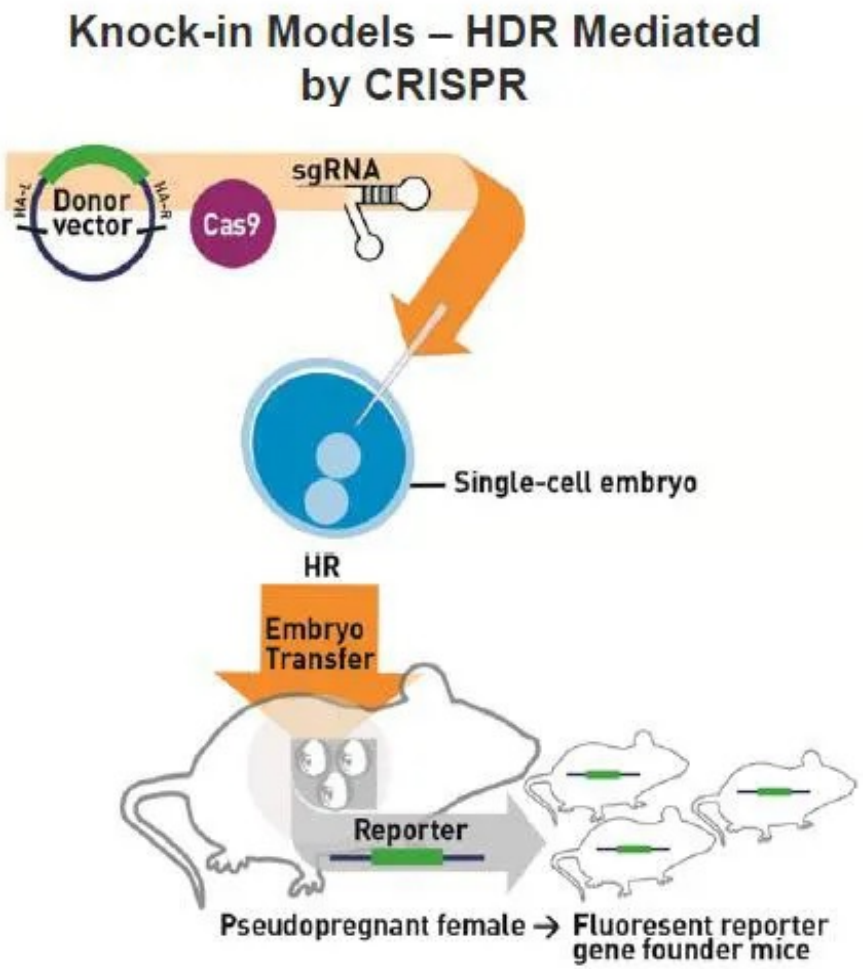
PCR SCREENING:

1-2 = WT locus

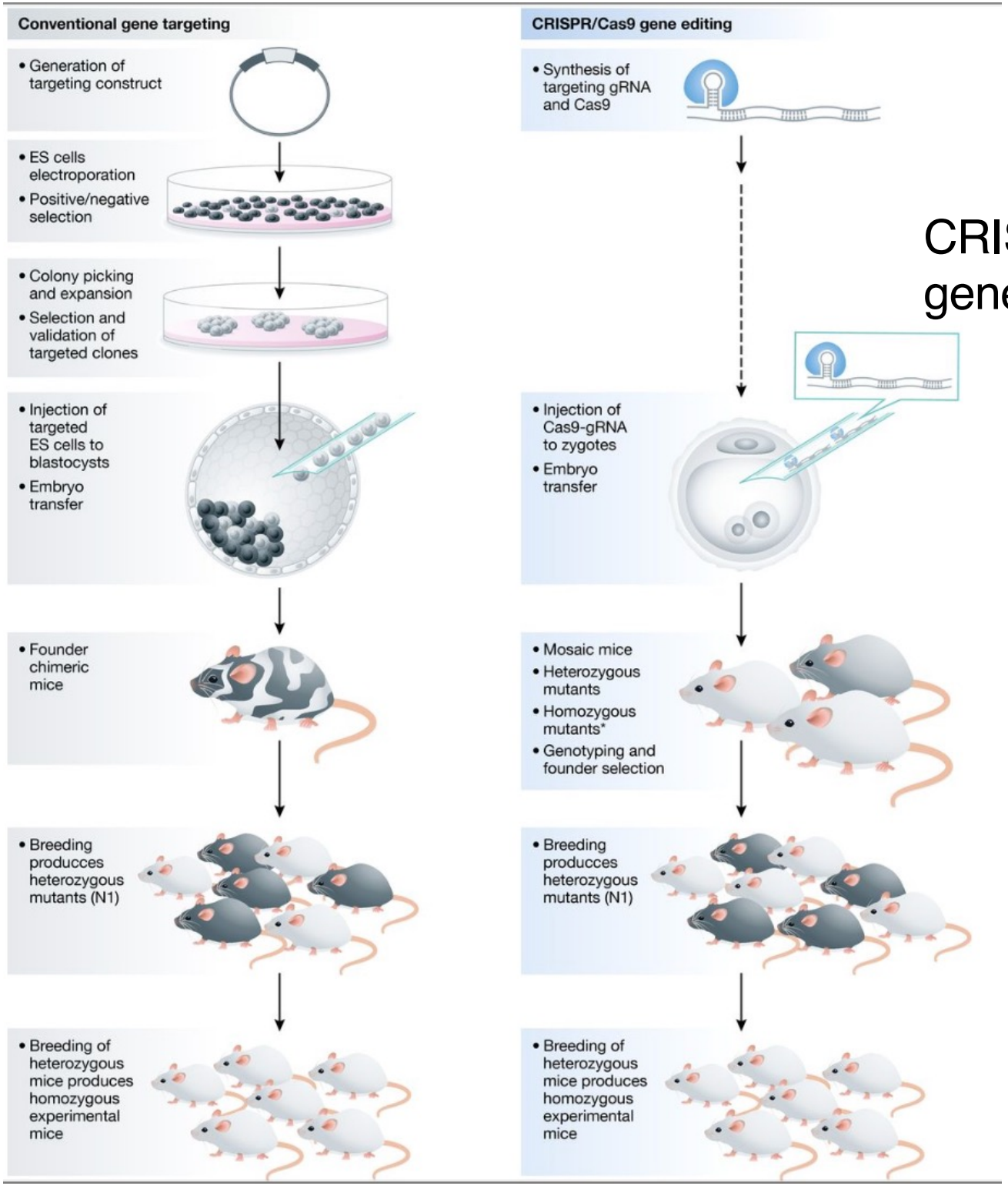
1-3 = MT locus

CRISPR/Cas9 gene editing in mouse





Gene targeting (homologous recombination)



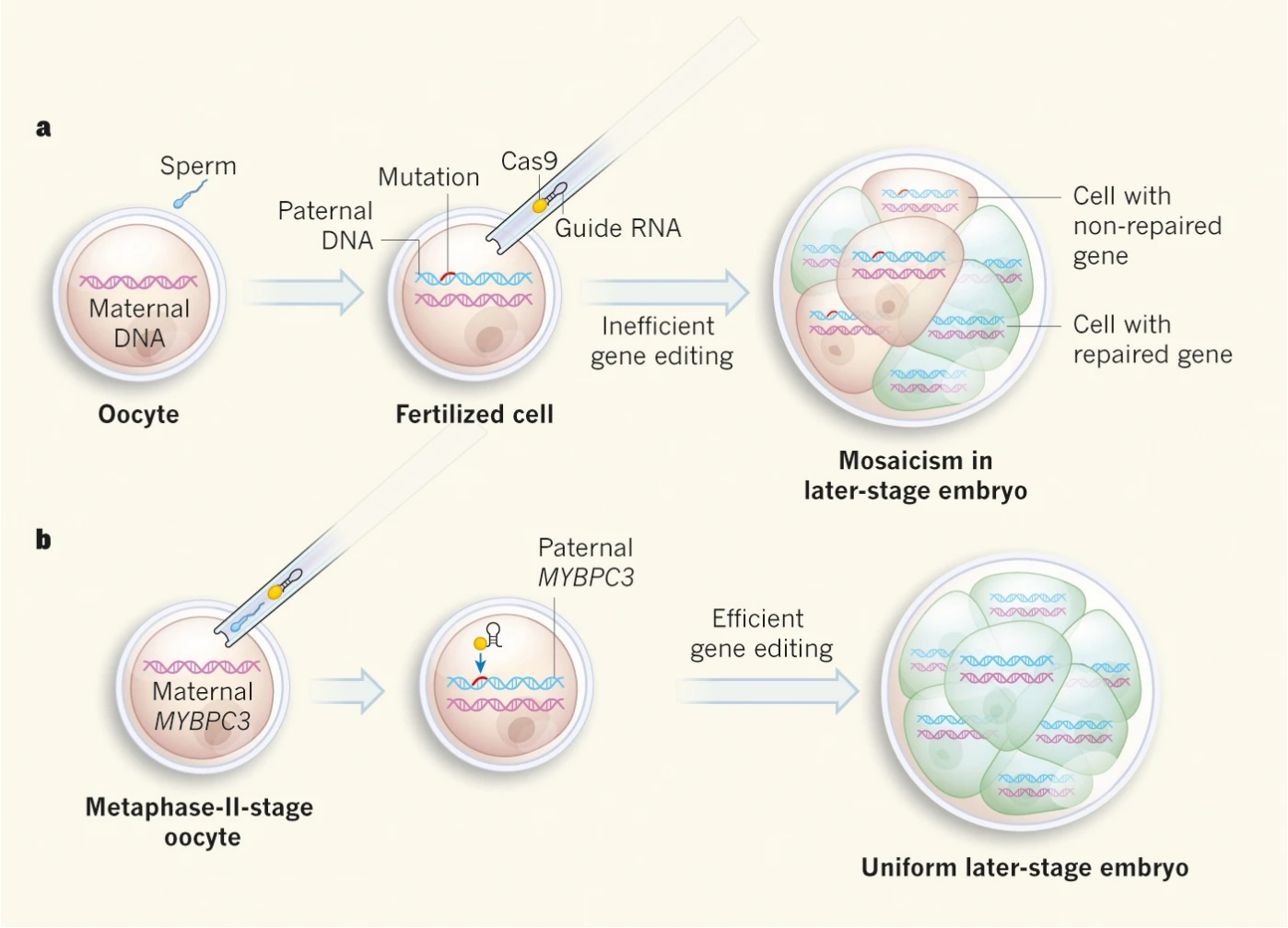
CRISPR/CAS9 gene editing

Success rate 50%-55%

Success rate 85%-95%

Mosaic Work-Around

CRISPR injected after fertilization operates after the first division and results in a subset of cells being fixed. When an organism has a mixture of edited and unedited cells, it is called a "mosaic». Injecting the CRISPR-Cas9 complex at the same time as the sperm prevented mosaicism almost completely .



CRISPR-mediated Genome Editing: Faster Timeline, Lower Cost, More Flexibility

	Conventional Gene Targeting	CRISPR-based Gene Knockout	CRISPR-based Gene Knock in
Timeline	**40 weeks**	**6 weeks**	**8 weeks**
Cost – Model Creation	$15,000	$3,000	$7,000
Cost - Breeding to GLT/NEO Excision	$20,000	$4,000	$4,000
Flexibility	**129**	**any strain**	**any strain**

Cre-Lox system

- Cre is a 38 kDa recombinase from the P1 bacteriophage.

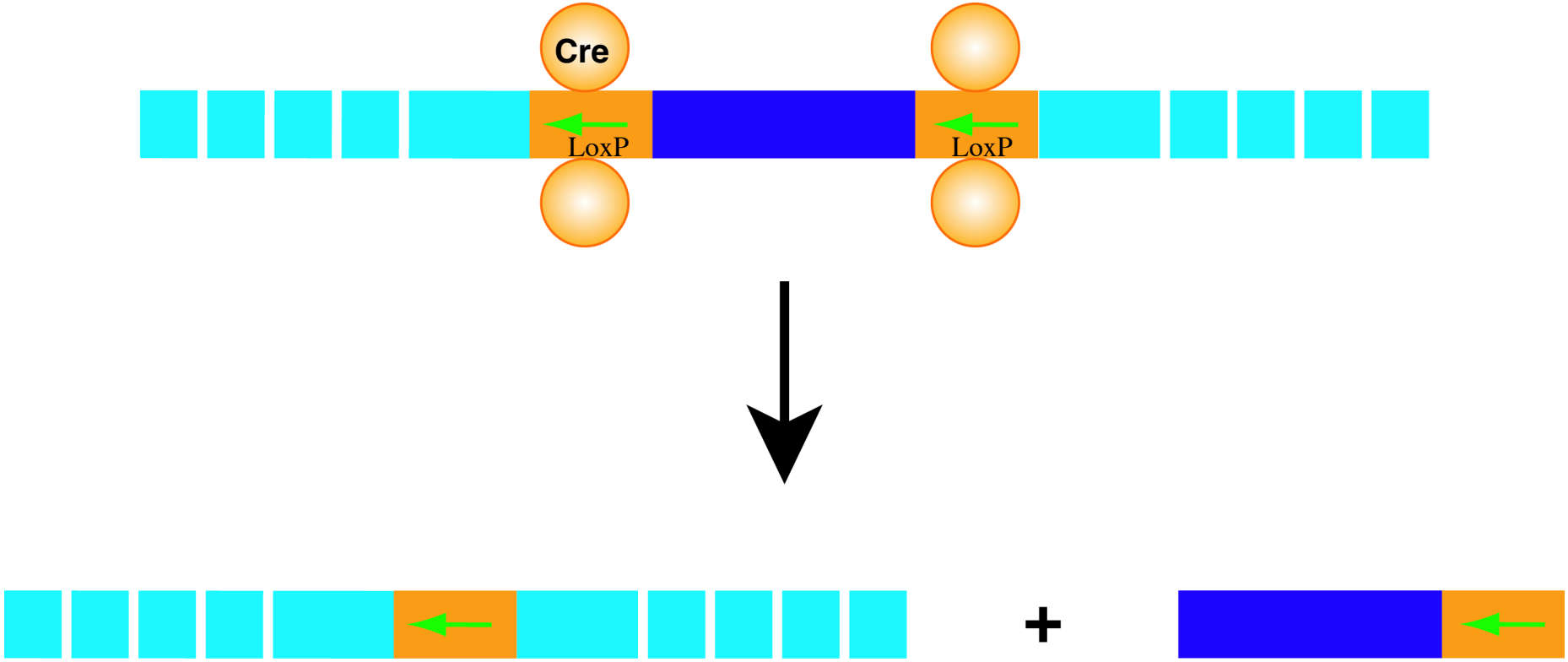
- Cre recognizes a 34 bp site called LoxP, inducing reciprocal and conservative recombination between two LoxP sites.

- LoxP consists of two 13 bp inverted repeats separated by an 8 bp "core" region.

- This "core" region controls the directionality of the LoxP site.

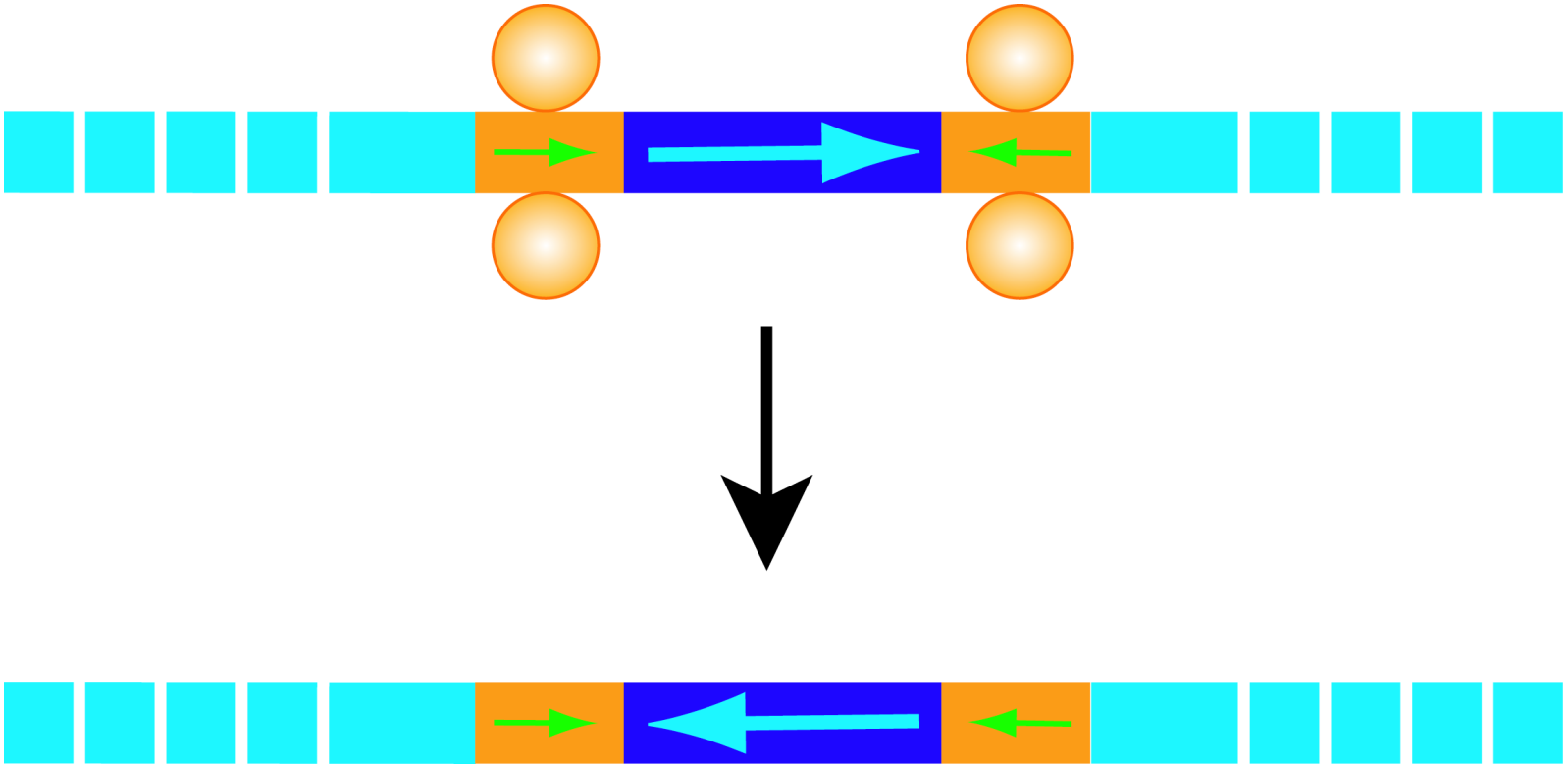
- Two Cre molecules bind to each LoxP site; therefore (in theory), 4 Cre molecules are required for each recombination event.

Tandem repetition



Risultato: Excisione della sequenza tra i LoxP (con ritenzione di 1 sito LoxP)

Inverted repetition



Risultato: Inversione della sequenza tra i LoxP

Applications of Cre-Lox system

- **Gene deletion** (Knockout)
- **Gene activation**, by removing the sequence [stop cassette] between the LoxP sites
- **Conditional Knockouts**, in combination with **TET** [Tetracycline-controlled systems], etc.
- **Point mutations**
- **Chromosomal alterations** (Translocations/Inversions)

Cre-Lox system

PROS

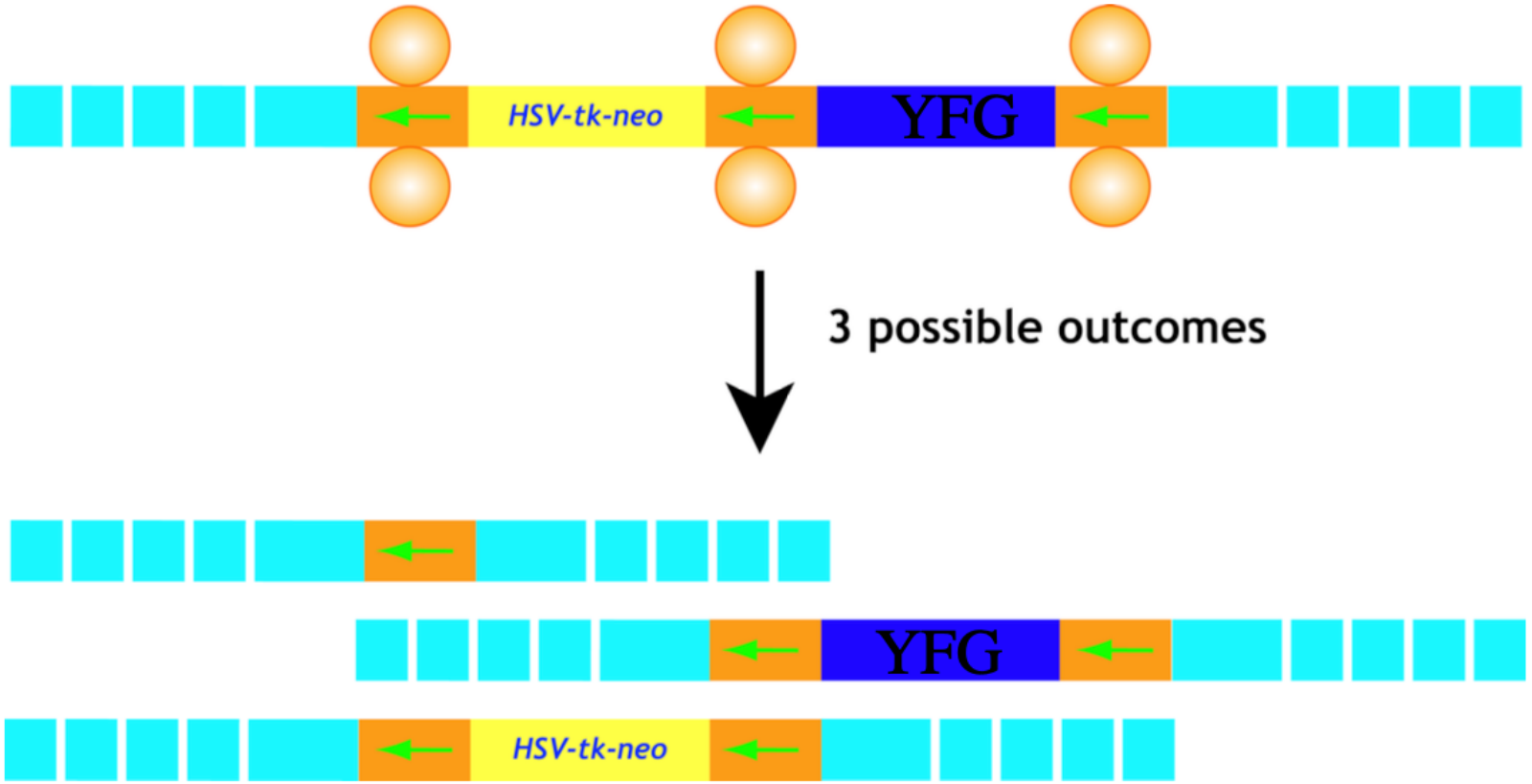
- **More effective** than other systems
- **Does not require external treatments** (unlike inducible systems that need drugs/triggers)

CONS

- **Mosaicism:** chromatin elements that influence Cre's activity (leading to incomplete recombination where some cells are edited and others are not)
- **Insert size limit** between the LoxP sites
- **High expression in mammals is toxic** (excessive Cre can cause unintended "off-target" DNA damage)

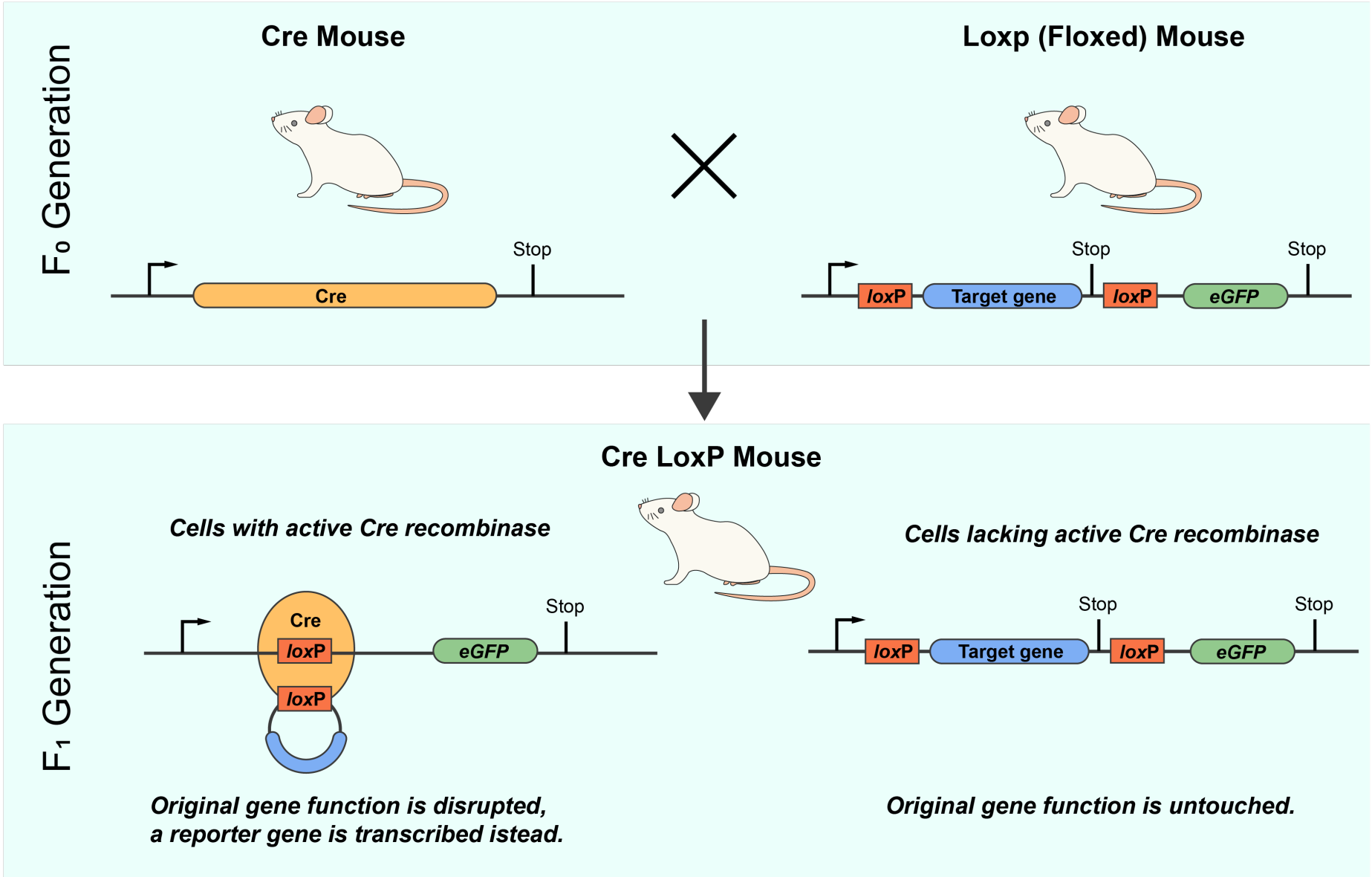
Applications of Cre-Lox system

Marker removal from genome



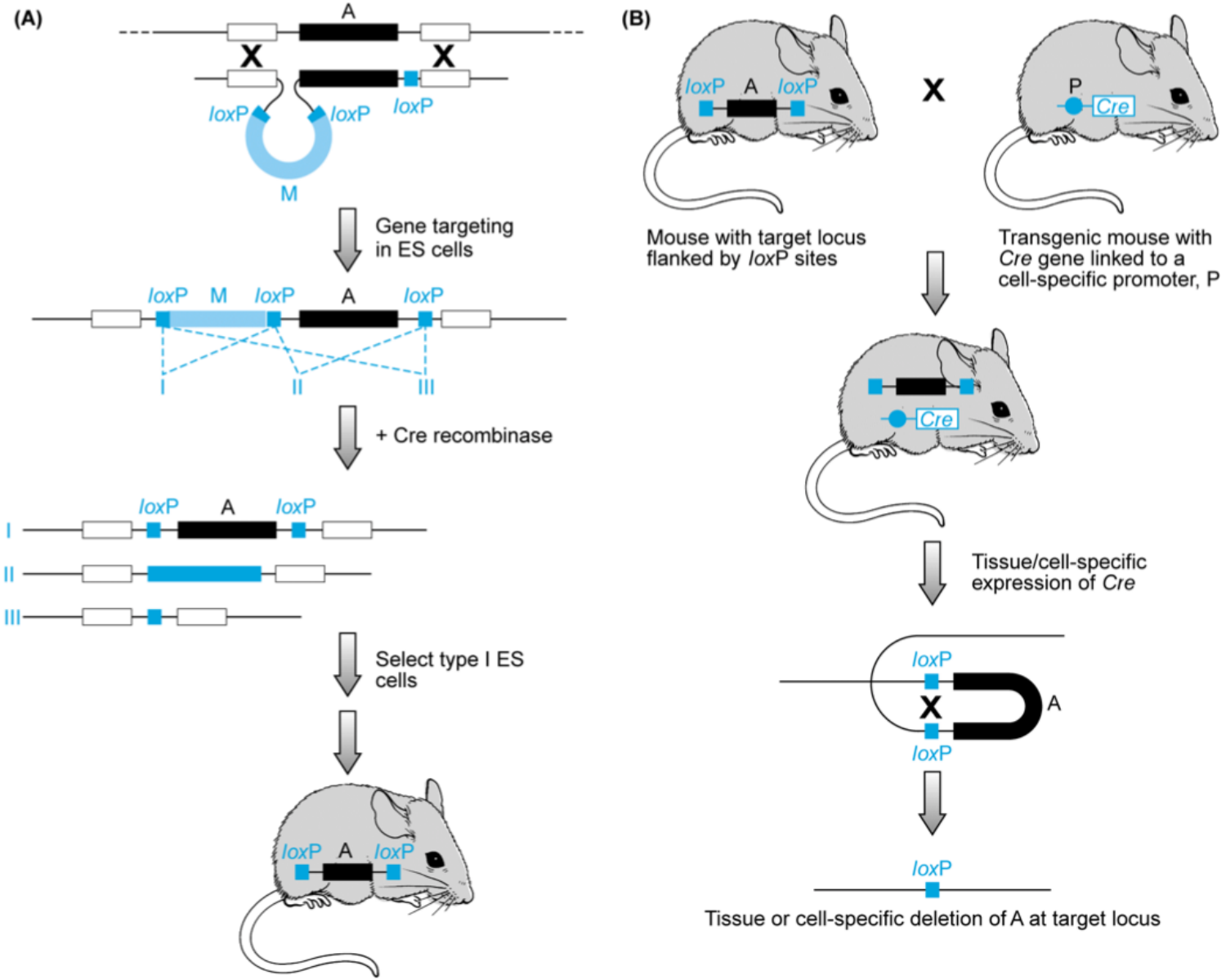
Cre-Lox strategies

Conditional KO



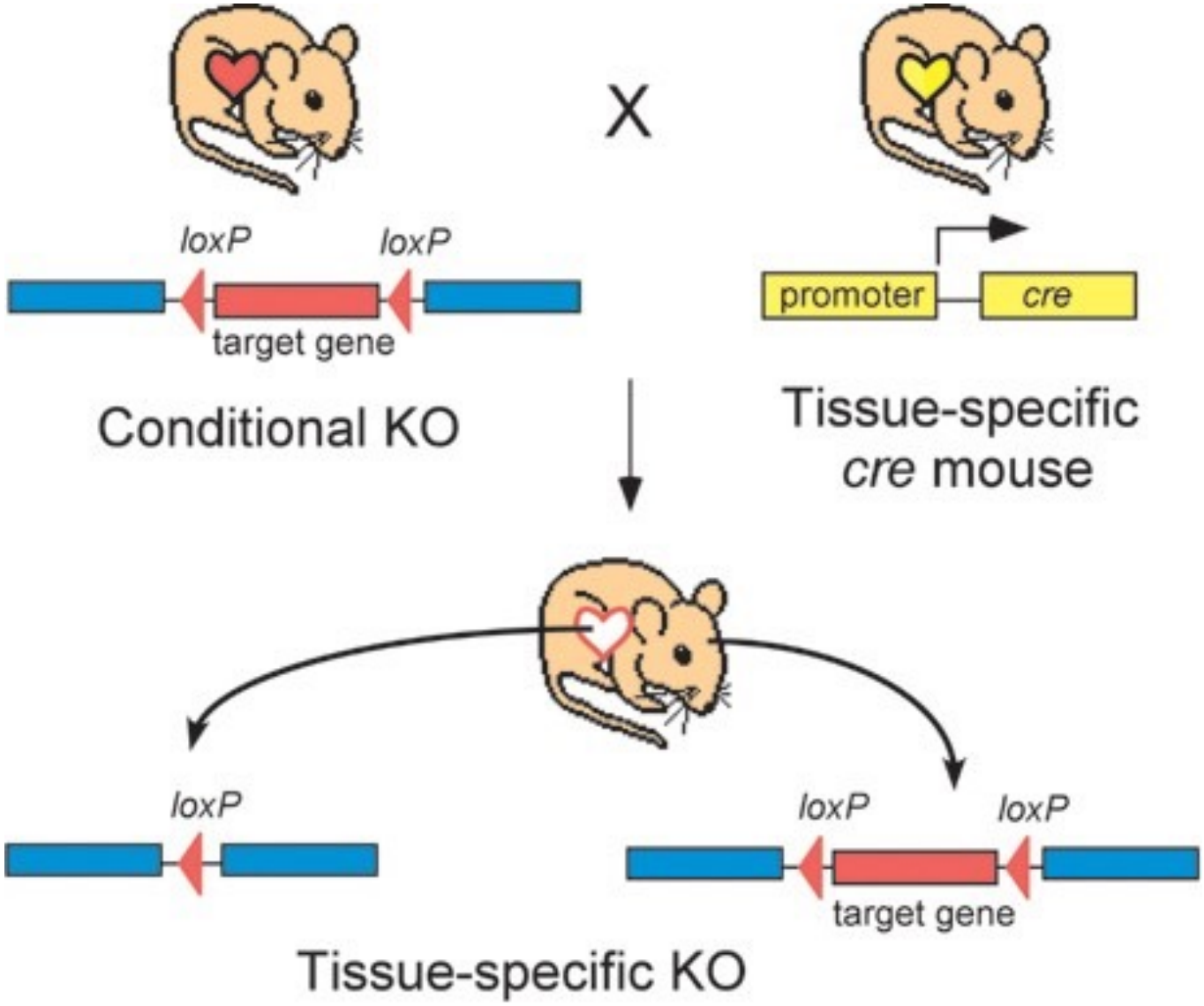
Cre-Lox strategies

Tissue specific KO



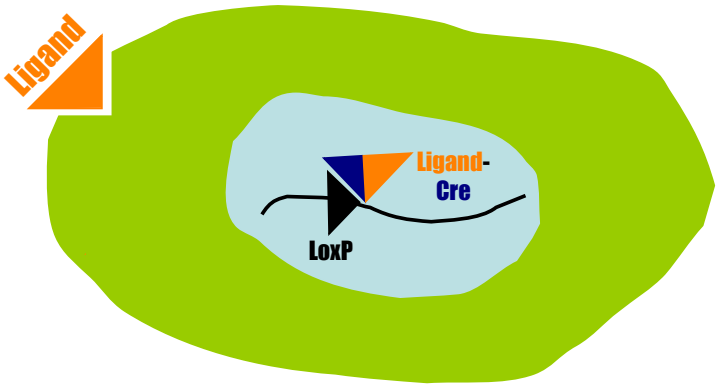
Cre-Lox strategies

Tissue specific KO



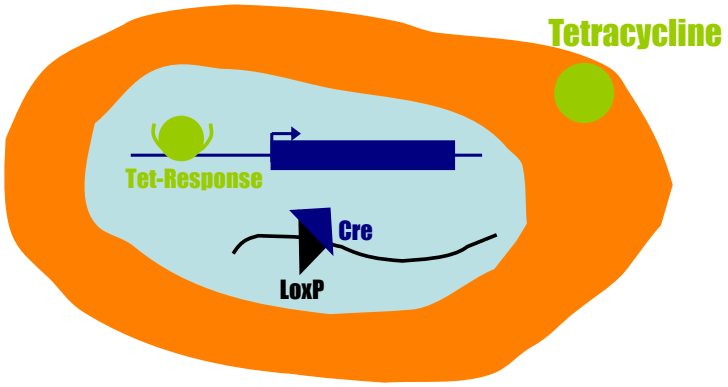
Inducible Cre-Lox systems

Induction of traslocation



Cre entries the nucleus in a ligand depedent manner

Induction of expression



Cre is expressed in presence of tetracyclin

Transgenic mice

Applications

- **Model system for basic research**
  - Gene regulation, cancer, development, etc.
  - Human genetic diseases

- **Model system for protein production**
  - (Initial testing in mice, followed by cows and others)

Transgenic mice

Methodologies

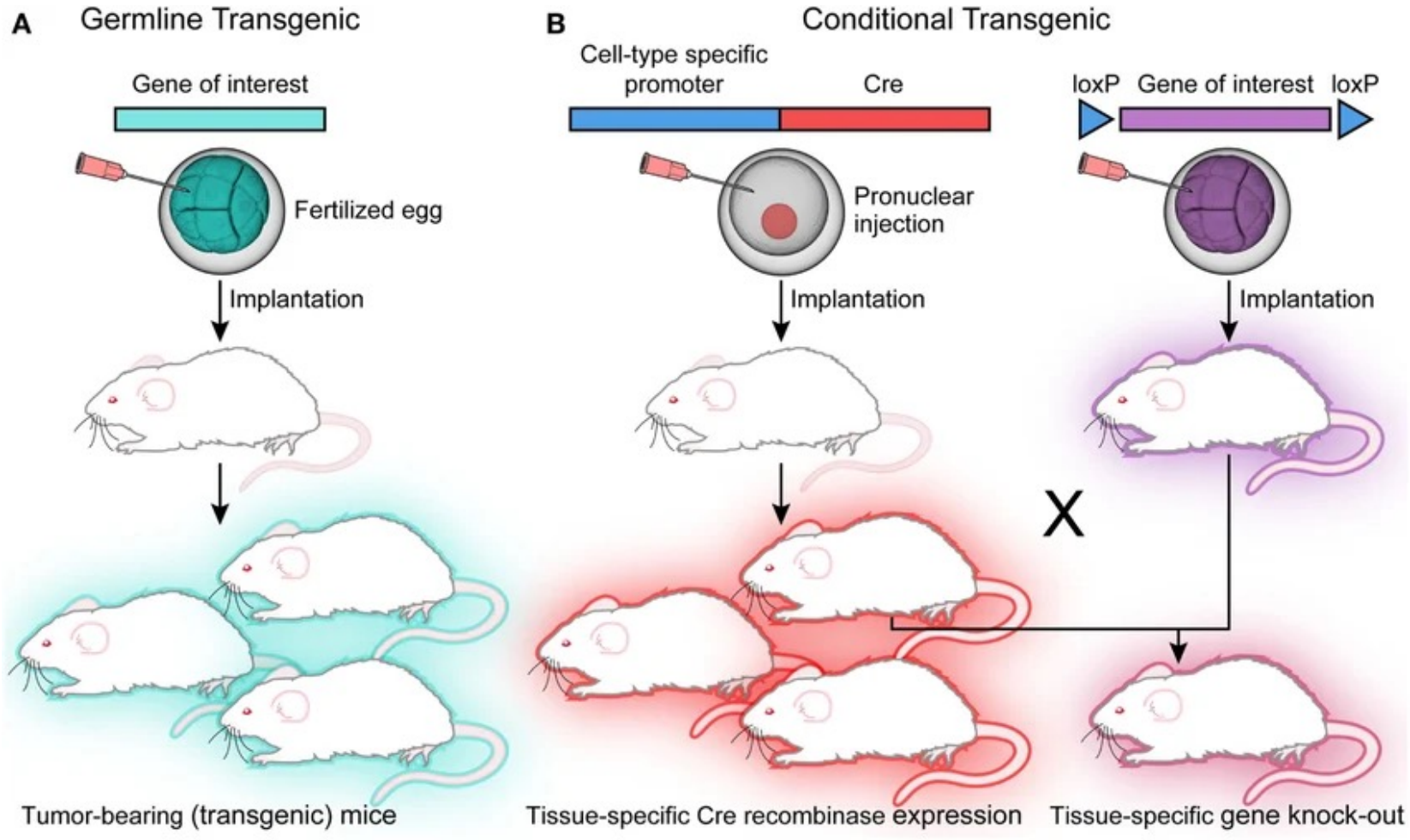
1. Retro/Lentiviral vectors

2. DNA microinjection

3. Engineered Embryonic Stem (ES) cells

Transgenic mice

(A) Injection of the vector construct into a fertilized egg will generate transgenic progeny that expresses the gene of interest in every cell. (B) Conditional transgenics can be generated for tissue-specific expression under the control of a Cre recombinase. These can be crossed with mice carrying the floxed gene of interest, resulting in progeny that carries the tissue-specific knockout.



Mouse model expressing oncogenic fucion protein

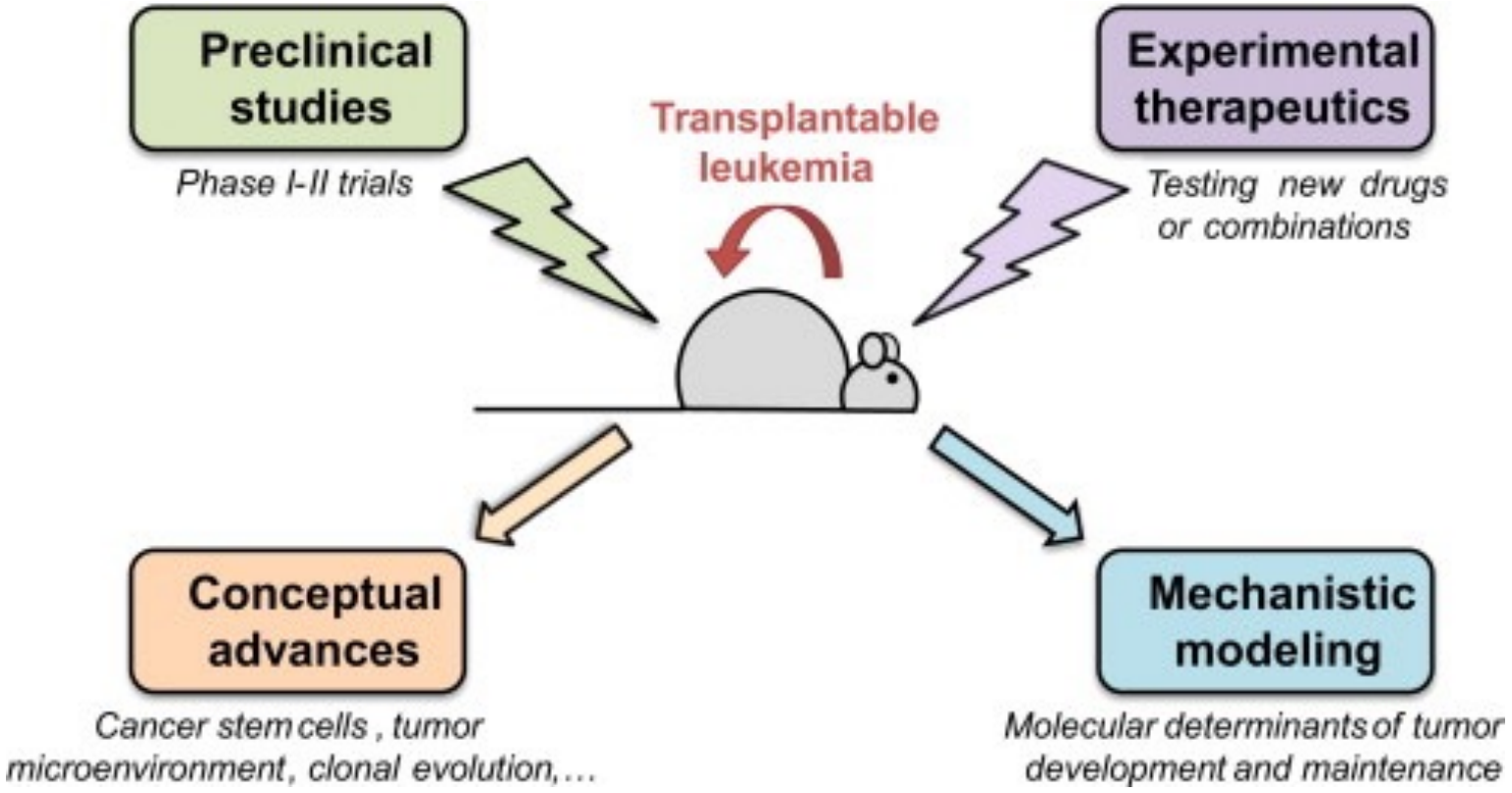
Disease	Fusion protein	Model
AML	BCR–ABL1	NSG xenograft with MSC scaffold
	Mixed-lineage leukemia–AF9	NSG xenograft with MSC scaffold
	AML1–ETO	Irradiated C57BL/6J with intravenous autologous transfected BM cells
CML	BCR–ABL1	BCR-ABL retrovirus co-expressing GFP in a triple gene system
ALL	ETV6–RUNX1	Inter-cross ETV6–RUNX1 and Pax5 heterogeneic mice
	E2A–PBX1	Conditional transgenic E2A–PBX1 under the control of Mb1 or Mx1 promoter-Cre
Peripheral T-cell lymphoma	ITK–SYK	ITK-SYK cloned into ROSA26 targeting vector, crossed to CD4-Cre

NSG, NOD/SCID IL2Rγ−/− mouse; MSC, mesenchymal stem cell; BM, bone marrow.

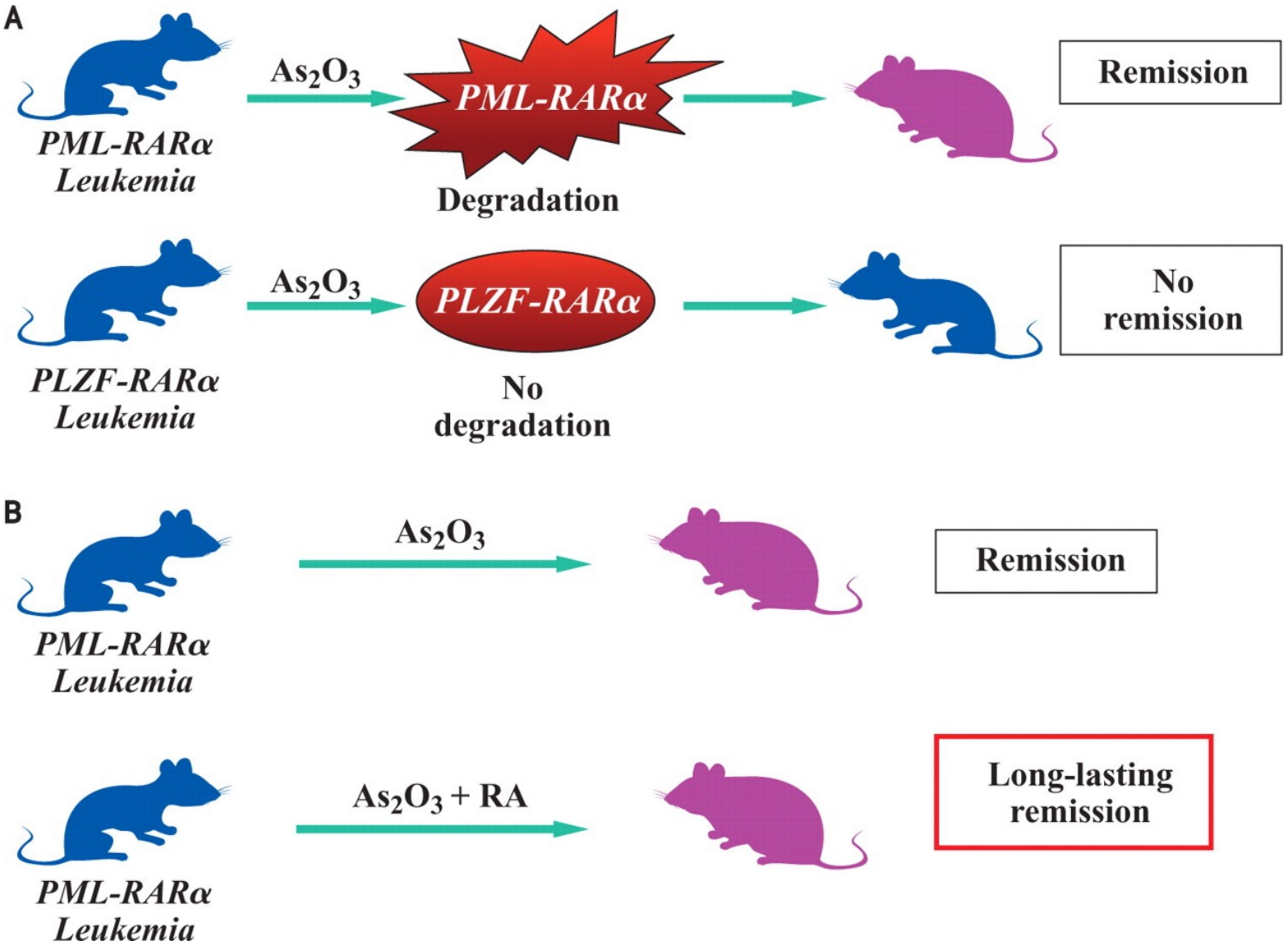
mmunodeficient mouse—the **NOD scid gamma (NSG)** mouse

Use of Leukemia Mouse model

These mouse models are particularly suitable for evaluating the effects of new drugs, but also for identifying which existing drugs might have the maximum efficacy alone or in combination. Leukemic blasts are easily and rapidly transplantable, allowing for the creation of large cohorts of mice with identical diseases that can be used for preclinical purposes.



Pre-clinical trials using APL mouse models



Clinically relevant mouse trial

Trial Design	Cancer Type	Model Type	Engineered Drivers	Drugs/ Treatment	Significance	Relevant Publications
Preclinical	Hematopoietic (APL)	GEM	PML-RARα fusion PLZF-RARα fusion	Retinoic acid	Demonstrated the efficacy of retinoic acid plus As_2O_3 in specific APL subtypes, validated in clinic	(Ablain and de Thé, 2014; Pandolfi, 2001)
Preclinical	Pancreas (Neuro-endocrine)	GEM	RIP1-Tag2	Sunitinib	Demonstrated the efficacy of Sunitinib plus Imatinib, validated in clinic. FDA approved for pancreatic cancer treatment in 2011.	(Pietras and Hanahan, 2005; Raymond et al., 2011)
Preclinical	Medulla-blastoma	GEM	$Ptc1^{+/-}$ $P53^{-/-}$	GDC-0449 (SMO inhibitor)	Demonstrated the efficacy of an Shh pathway small molecule inhibitor, validated in clinic	(Romer et al., 2004; Rudin et al., 2009)
Preclinical	Pancreas (Neuro-endocrine)	GEM	RIP1-Tag2	Erlotinib Rapamycin	Demonstrated efficacy of combining drugs targeting EGFR and mTOR	(Chiu et al., 2010)
Co-clinical	Pancreas (PDA)	GEM	LSL-$Kras^{G12D}$ LSL-$Trp53^{R172H}$ Pdx-1-Cre	Gemcitabine Nab-Paclitaxel	Provided mechanistic insight into clinical cooperation between Gemcitabine and Nab-Paclitaxel	(Frese et al., 2012; Goldstein et al., 2015)
Co-clinical	Pancreas (PDA)	GEM	LSL-$Kras^{G12D}$ LSL-$Trp53^{R172H}$ Pdx-1-Cre	CD40 monoclonal antibody Gemcitabine	Demonstrated that targeting stroma was effective in treatment of metastatic PDA	(Beatty et al., 2013)
Co-clinical	Lung (NSCLC)	GEM	$KRAS^{G12D}$ $p53^{fl/fl}$ $Lkb1^{fl/fl}$	Selumetinib Docetaxel	Validation of improved response of adding Selumetinib to Docetaxel treatment	(Chen et al., 2012; Jänne et al., 2013)
Co-clinical	Lung (NSCLC)	GEM	EML4-ALK fusion	Crizotinib Docetaxel Pemetrexed	GEM model predicted clinical outcome of drug combinations	(Chen et al., 2014; Lunardi and Pandolfi, 2015)
Co-clinical	Various Sarcomas	PDX	N/A	Various chemotherapies	PDX testing predicted clinical outcome of drug combinations	(Stebbing et al., 2014)
Postclinical	Ovarian (SEOC)	GDA; PDX	RB/p53-deficient BRCA1/2-deficient	Olaparib Cisplatin	Validation of treatment efficacy in BRCA mutant tumors in both GDA *and* PDX models	(Kortmann et al., 2011; Szabova et al., 2014)
Postclinical	Pancreas (Neuro-endocrine)	GDA	RIP1-Tag2	Anti-VEGFR1 and anti-VEGFR2 antibodies	Identification of mechanisms of resistance to anti-angiogenic therapies	(Casanovas et al., 2005)
Biomarker	Lung (NSCLC)	GEM; Carcinogen-induced	Various Models	N/A	Used in-depth quantitative MS-based proteomics to profile plasma proteins	(Hanash and Taguchi, 2011)
Biomarker	Pancreas (PDA)	GEM	$Kras^{G12D}$ $Ink4a/Arf^{fl/fl}$ Pdx-1-Cre	N/A	Used in-depth proteomic analyses to identify candidate markers applicable to human cancer	(Faca et al., 2008)

Human-Animal Chimeras

The main objective of chimera research is to produce human cellular traits in model animals.

- Human cell line or tumor transplant
- Primary cell transplant
- Organ transplant
- hES (human Embryonic Stem cells) transplant

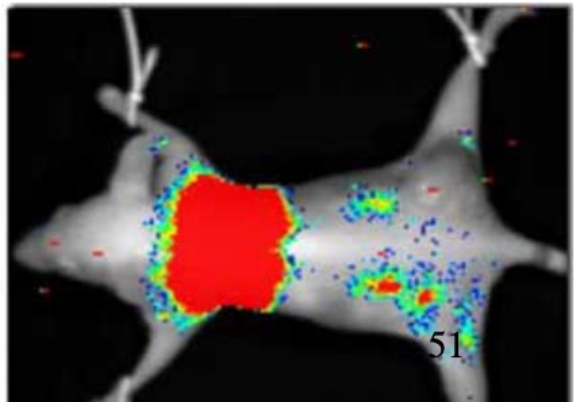
Xenograft models

Are produced by grafting human cells into an immune-deficient animal

1. The **immune-deficient athymic nude mouse** is the most utilized for xenograft. ***Nude*** is a recessive mutation that results in loss of T cells. They still have B cells and NK cells.

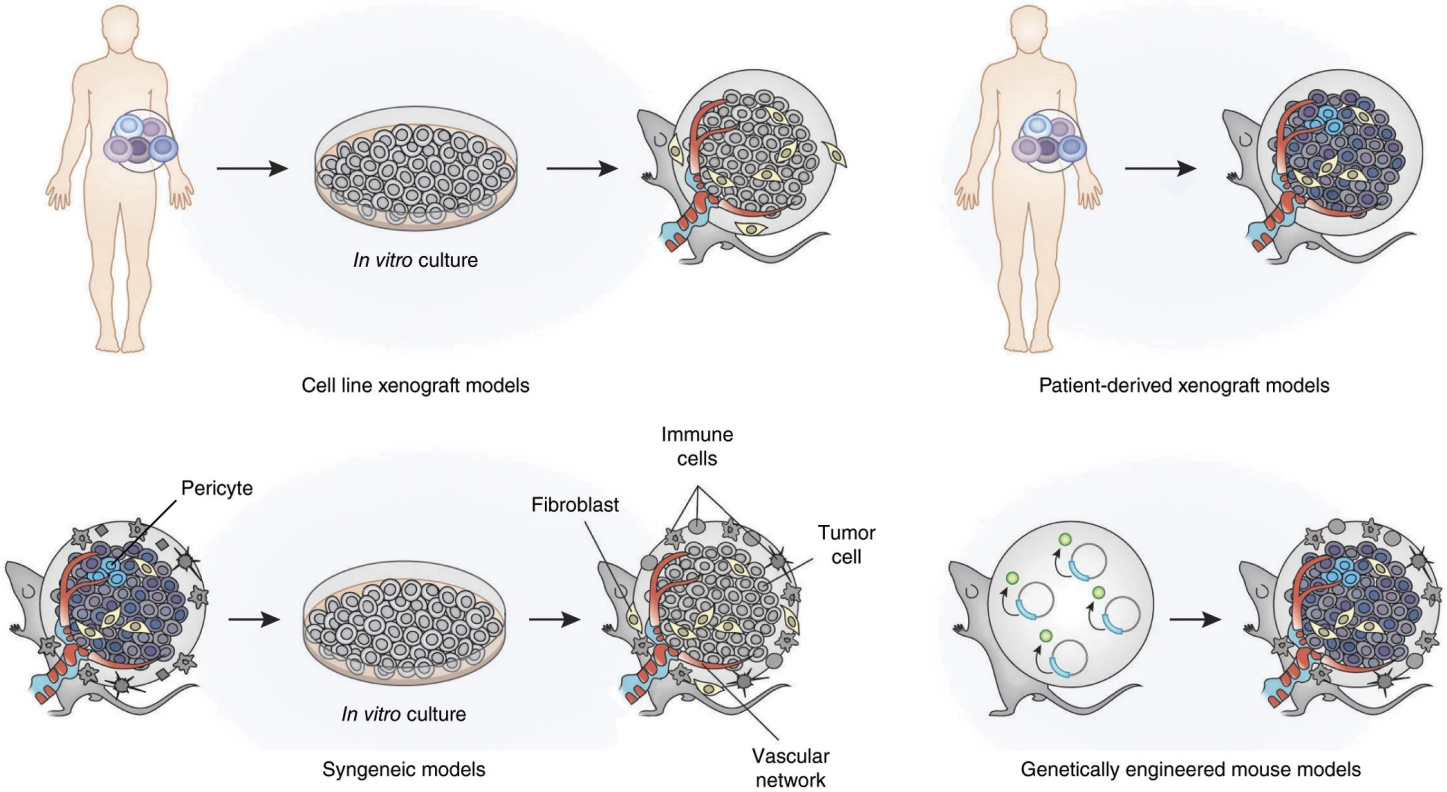
2. Mice with the **sever combined immunodeficinecy (SCID)** crossed with **nonobese diabetic (NOD)**, NOD-SCID mouse, show geater human engraftment.

Useful to understand cancer mechanism and to test therapies against tumors



Preclinical Cancer Models

In **cell line xenograft models**, established human tumor cell lines are transplanted into an immunodeficient murine (mouse) host. In **patient-derived xenograft (PDX) models**, human tumor explants are implanted into immunodeficient mice. In **syngeneic mouse models**, allografts of established murine cell lines are transplanted into immunocompetent recipients of a compatible strain. Finally, in **genetically engineered mouse (GEM) models** of cancer, genomic modification allows for the spontaneous or induced development of tumors directly within the desired target tissue of an immunocompetent animal.

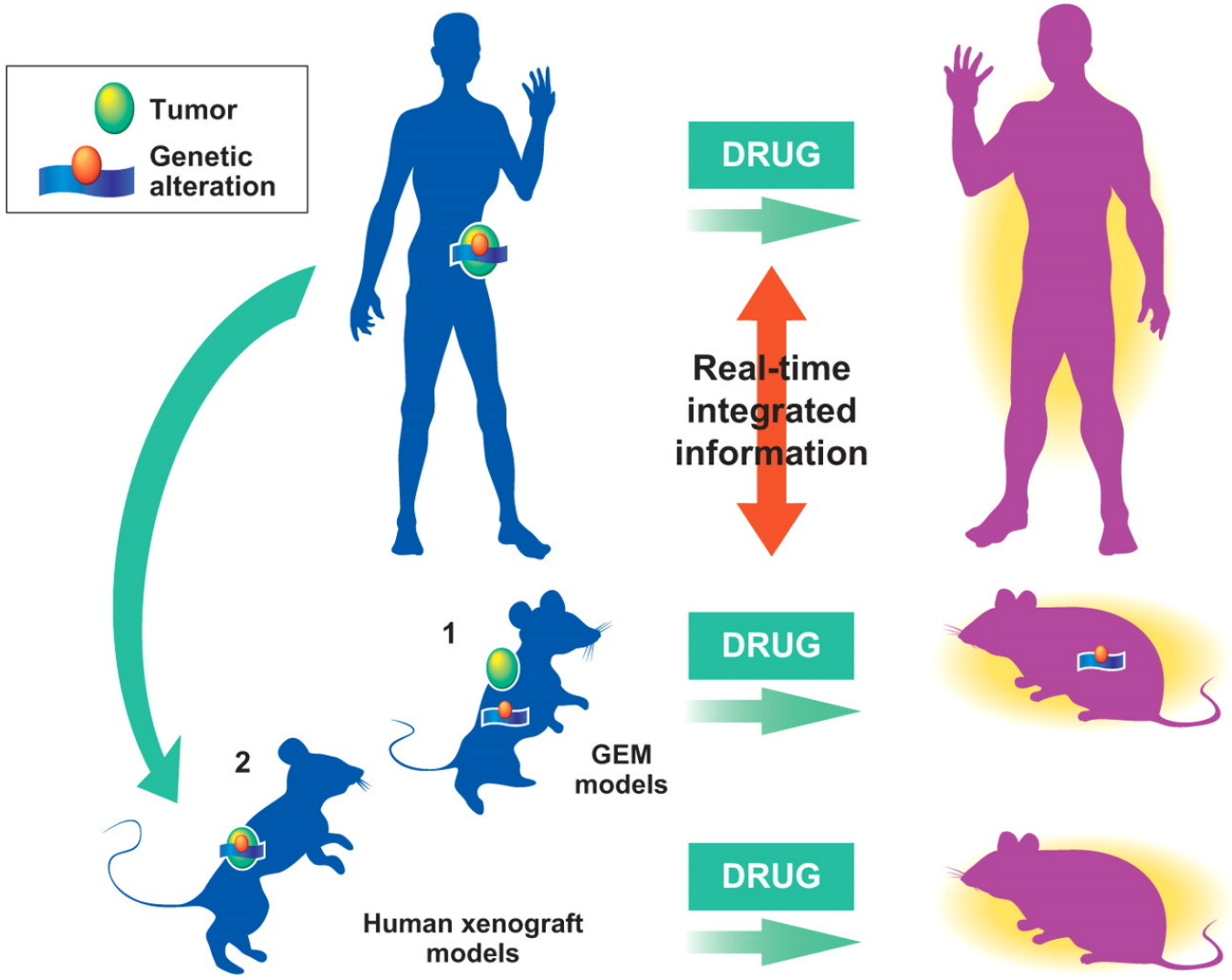


Preclinical oncology models

	Principal components	Benefits	Caveats
Cell line xenograft models	Established human tumor cell lines transplanted into immune-deficient host	i) Numerous established and well-annotated cell lines ii) Representation from various human tumor types iii) Features of the tumor microenvironment, including vascular and stromal cells incorporated within the tumor iv) Tumors are easily and precisely measured	i) Immune-deficient ii) Subcutaneous location may not foster important tissue-specific stromal infiltrate iii) Cross-species disconnect, stromal components are mouse whereas tumor cells are human iv) Limited or no genetic heterogeneity is present within tumor
Patient-derived xenograft models	Human tumor explants grown in immune-deficient host	i) Genetic diversity and heterogeneity within tumors ii) Representation from various human tumor types iii) Features of the tumor microenvironment, including vascular and stromal cells incorporated within the tumor iv) Tumors are easily and precisely measured	i) Immune-deficient ii) Subcutaneous location may not foster important tissue specific stromal infiltrate iii) Surgical implantation required iv) Cross-species disconnect, stromal components are mouse whereas tumor cells are human v) Genetic and phenotypic drift with passage
Syngeneic models	Established mouse tumor cell lines transplanted into immune-competent host	i) Presence of an intact immune system ii) Features of the tumor microenvironment, including vascular and stromal cells incorporated within the tumor iii) All cell types within the tumor are of mouse origin iv) Tumors easily and precisely measured	i) Limited number of established cell lines, poorly annotated ii) Strong immunogenicity of some lines promotes spontaneous regression iii) Rapid growth rate of many lines limits use in longer-term studies
Genetically engineered mouse models	Genetic modification that permits spontaneous or induced tumor development	i) Tumors develop in the tissue of origin ii) Presence of an intact immune system iii) All cell types within the tumor are of mouse origin iv) Incorporates features of the tumor microenvironment, including vascular and stromal cells and immune components	ii) Limited genetic mosaicism and heterogeneity of tumors ii) Technical hurdles for monitoring tumor response when on internal organs iii) Low throughput and high investment

The Co-clinical Trial Project

The synchronization of preclinical studies with clinical trials—to be conducted in parallel on human patients and mouse models, including both **GEM models (1)** and **human xenograft models (2)**—will allow for the integration of information in real time.



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The Co-clinical Trial Project

The same drug and the exact same protocol will be used in both the mouse models and the human patients. Data will be collected and analyzed in parallel regarding disease stratification in primary tumors and acquired resistance to treatment.