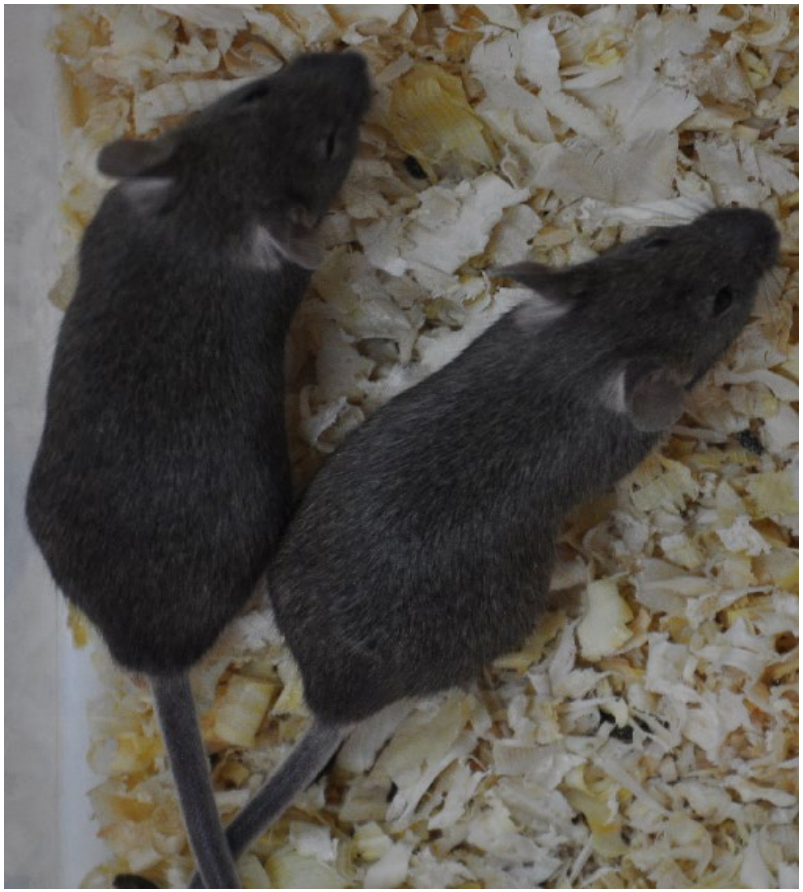
Generation of transgenic, knock-in and knock-out animal models for human disease

George A. Garinis
www.garinislab.gr

Why we use mouse models ?

- Powerful system for studying mammalian genetics
- Mirror human phenotypes and pathologies
- Over 95% of the mouse genome is similar to our own
- “Cost-effective”, efficient tool for the development of drug therapies



Inbred strains

"Inbred": ~20 back-Xs (F0 x F1 or F1 x F1 matings)

Advantages:

- Well characterized & genetically uniform
- Enable reproducible studies

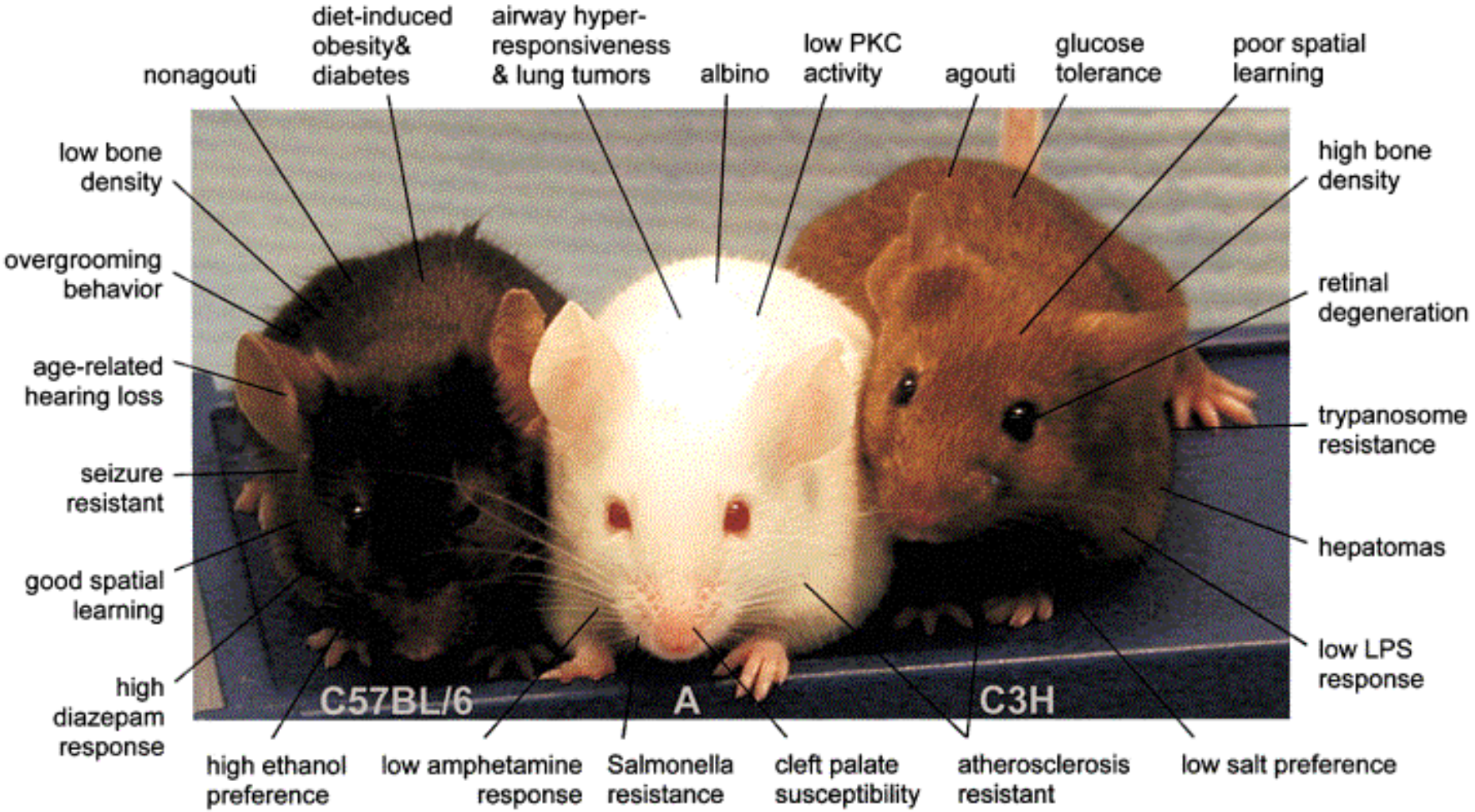
Disadvantages:

-Heterogeneity is sometimes necessary!

-Some strains have strain-specific traits

- *C57BL/6 rarely develop spontaneous cancer*
- *C57BL/6 males are highly susceptible to diet-induced obesity & atherosclerosis*
- *A/J mice are relatively resistant*
- *A/J mice have a high incidence of lung tumors and mammary cancer.*

Inbred Strain Characteristics

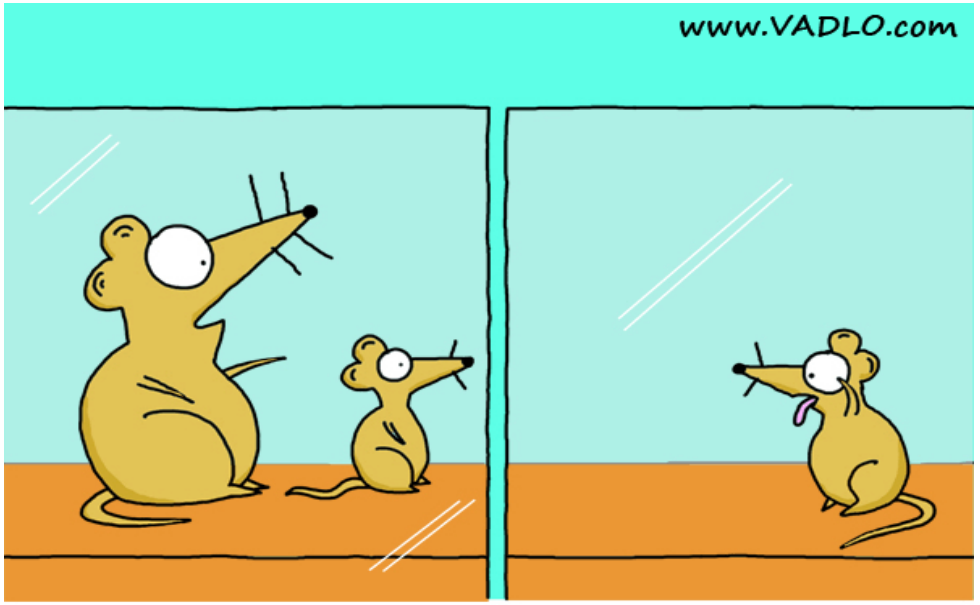


Genetically engineered mouse models

"…a mouse that has had its genome altered through the use of genetic engineering techniques"

- Explore mechanisms with a greater translational potential: preclinical mouse models
- Mouse models commercially available: Jackson Laboratory, Charles River, Taconic,
- Company custom designed

- Areas:
    1. Oncology
    2. Cardiovascular disease
    3. Neurodegenerative
    4. Metabolic disorders
    5. Musculoskeletal
    6. Immunology

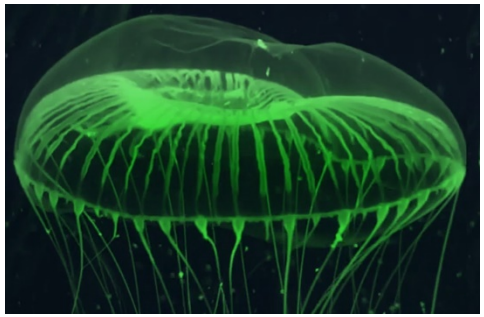


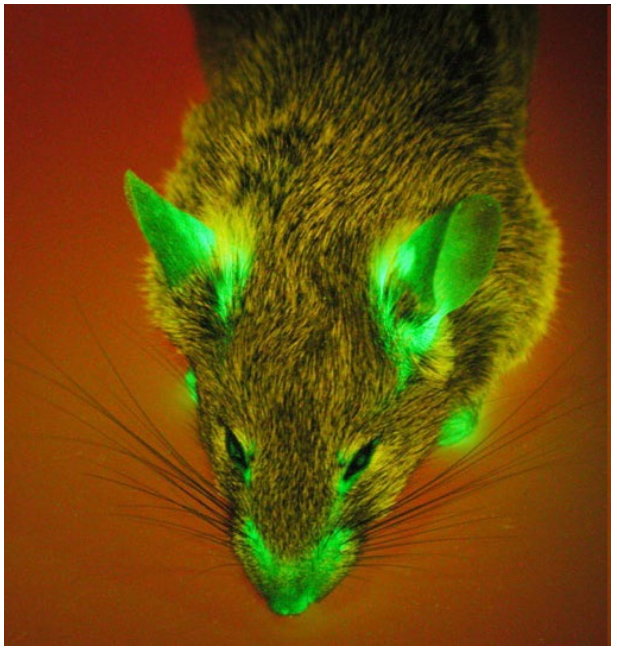
"Don't play with him, he is **Wild Type**."

Transgenic mice

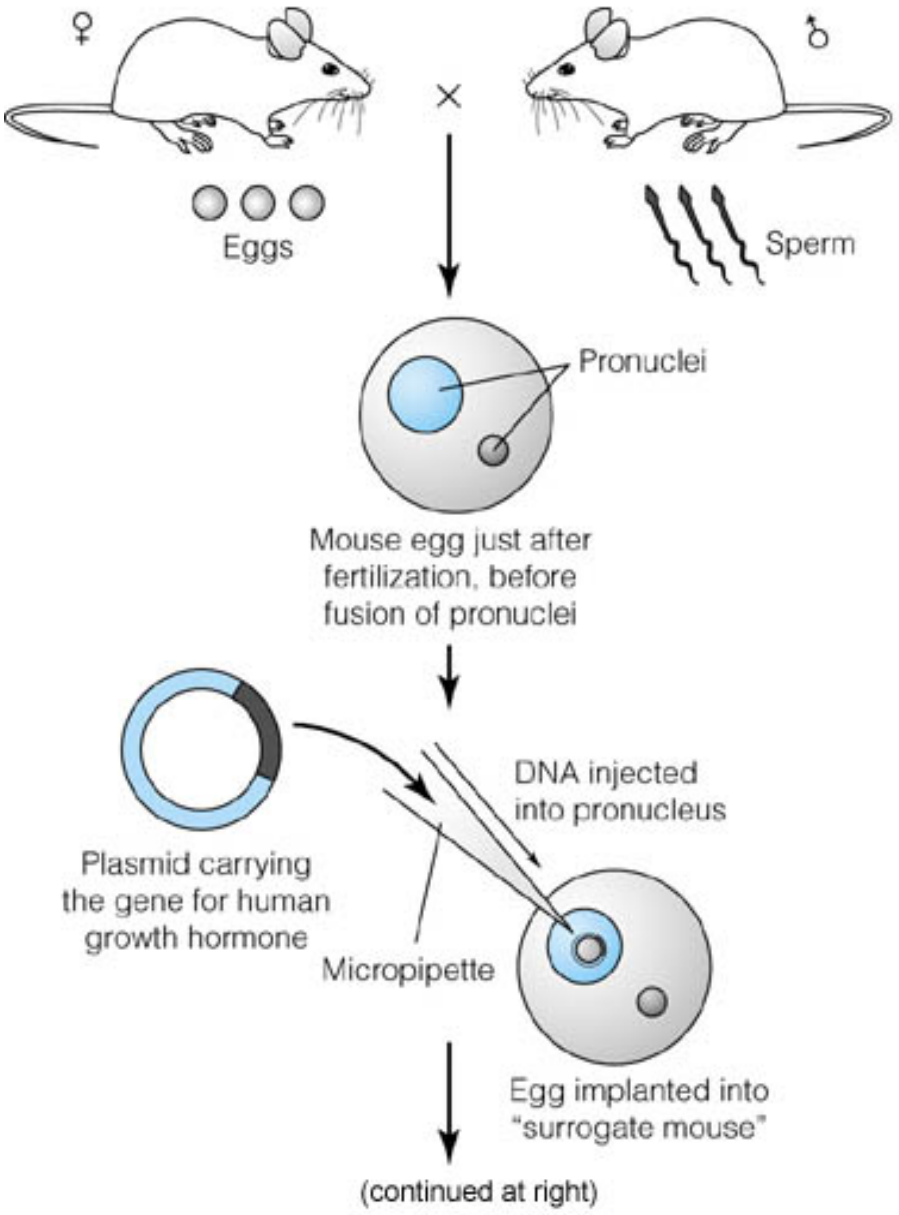
"A transgenic mouse caries a piece of DNA from a different species"

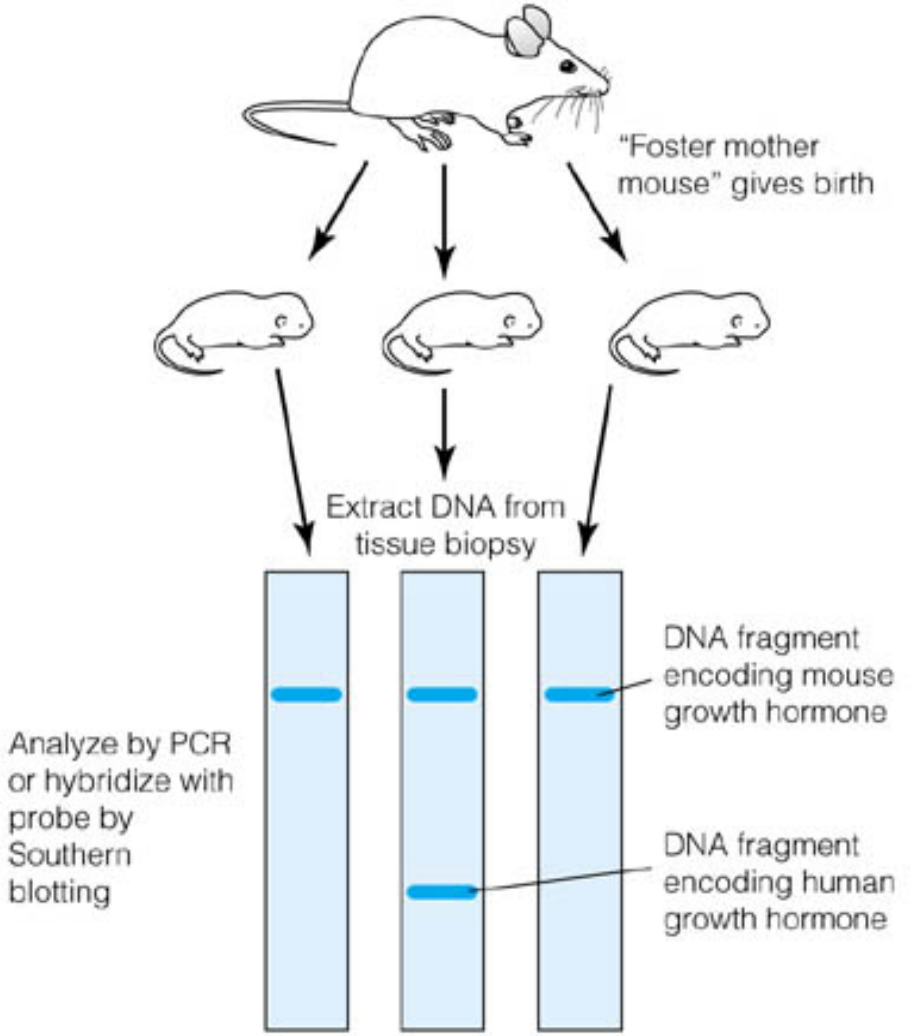
a GFP gene was isolated from the jellyfish Aequorea victoria





Generation of transgenic mice





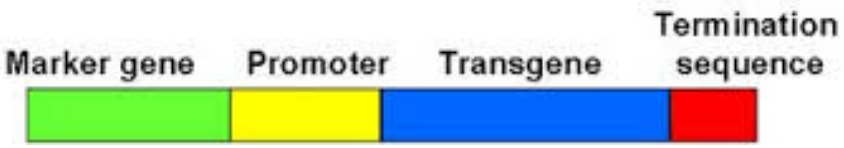
Transgenic animals

Advantages:

- Produce a “gain-of-function” model → proteins are over-expressed
- Transgenes are inherited dominantly → only 1 copy is required for observable expression
- Shorter time for founders

Disadvantages:

- Random integration
- Could disrupt an existing gene
- Could be expressed in some or all cell types
- Multiple copies
- Longer times to validate

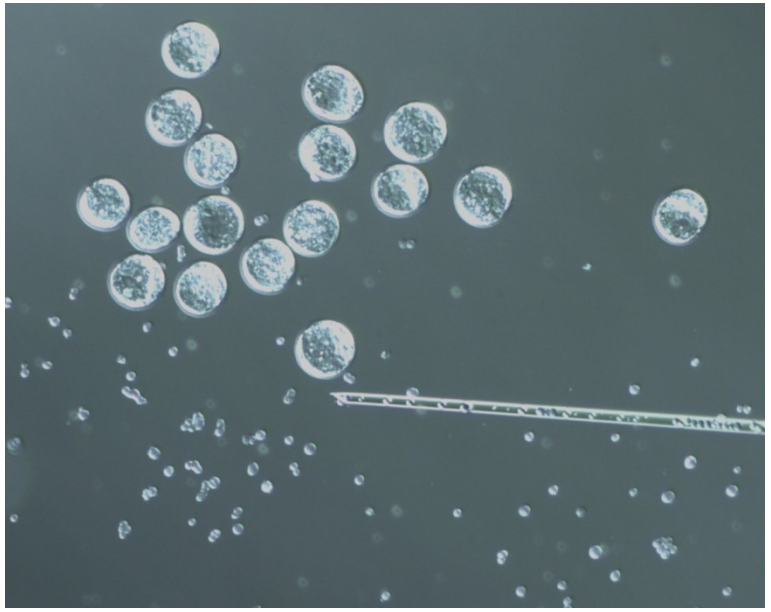


- Enhancers
- LCRs
- Mutations
- Fluorescent proteins

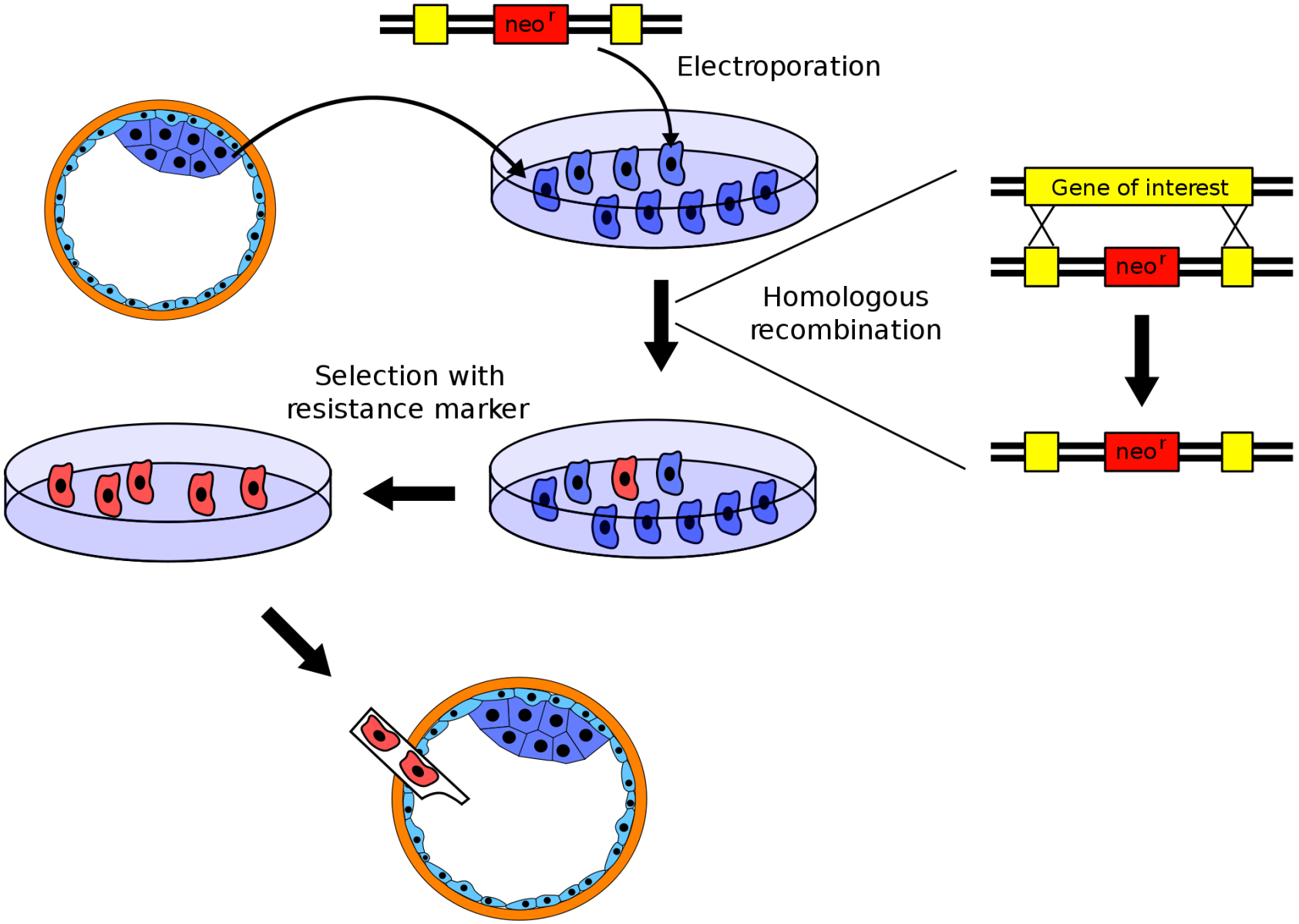
Nobel Prize in Physiology or Medicine 2007

Jointly to Mario R. Capecchi, Sir 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".

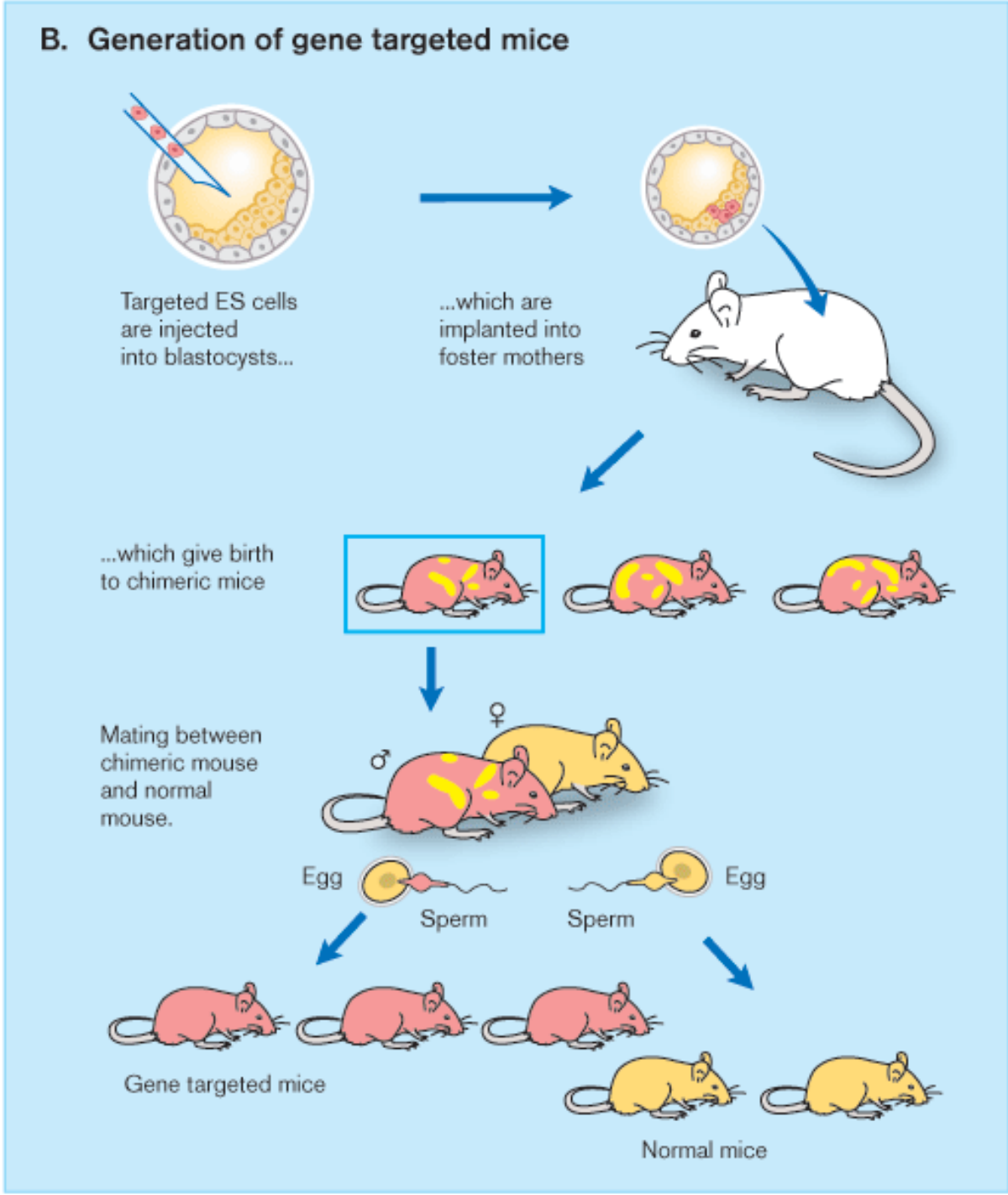


Gene targeting of embryonic stem cells

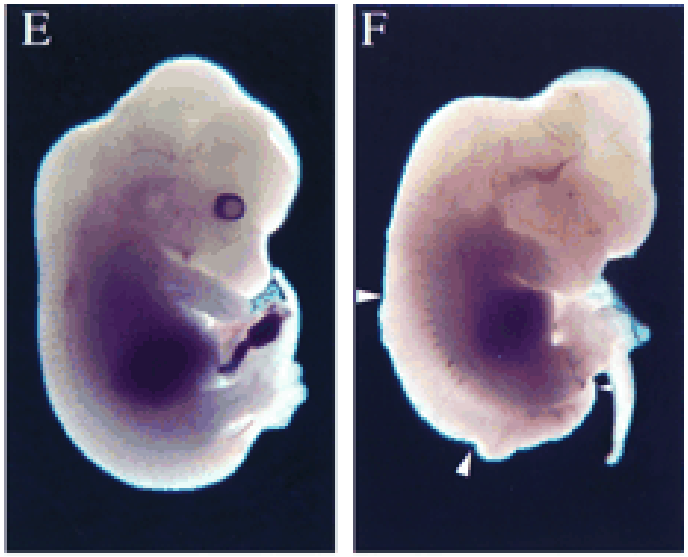


Transfer of targeted ES cells to blastocysts

Germline transmission



Sometimes, knockouts are embryonic lethal!



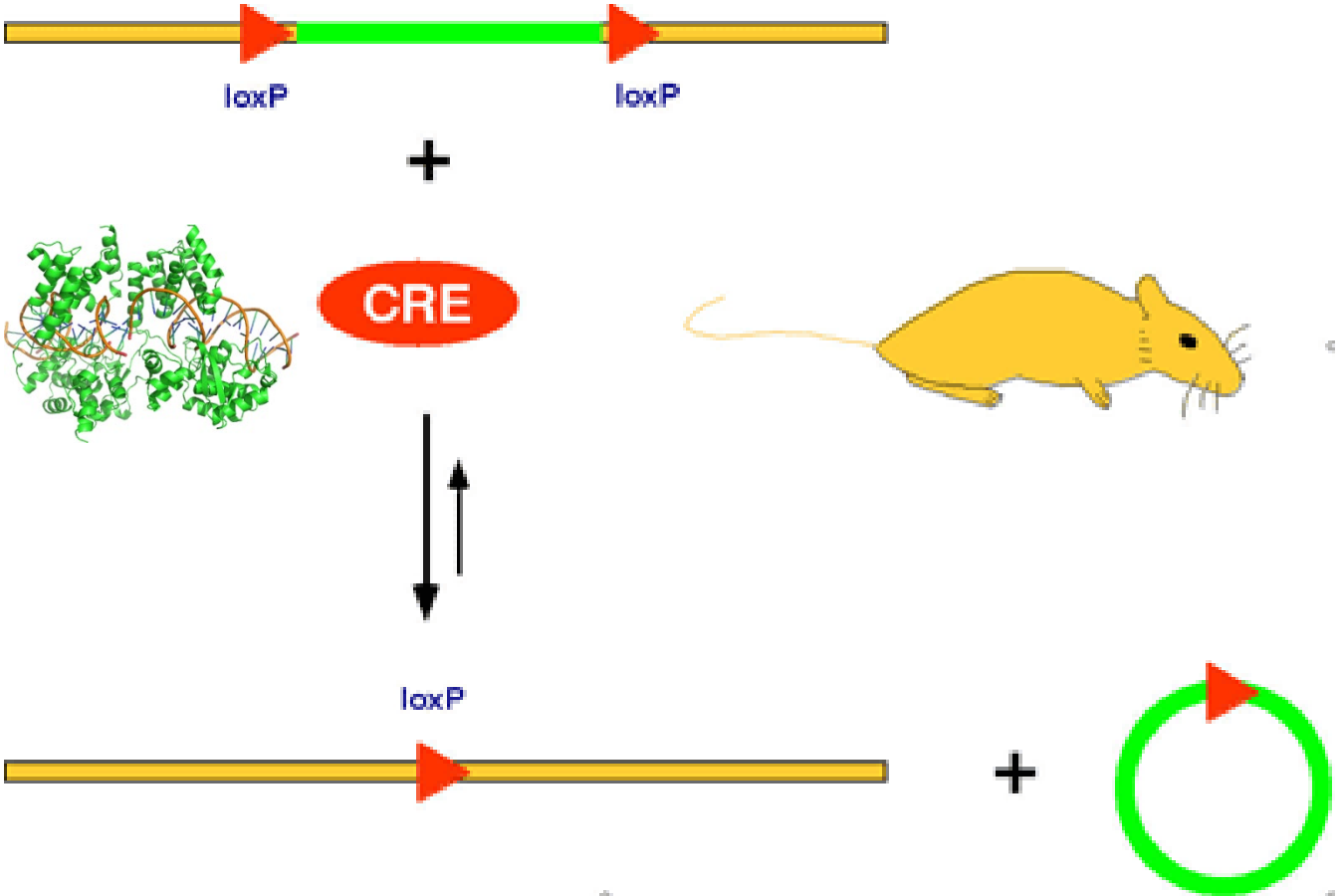
Perhaps, it is better to knockout a gene on a single cell/tissue type.

A tissue-specific knockout mouse: a mouse model in which a gene of interest is inactivated in specific cell types or in a certain tissue

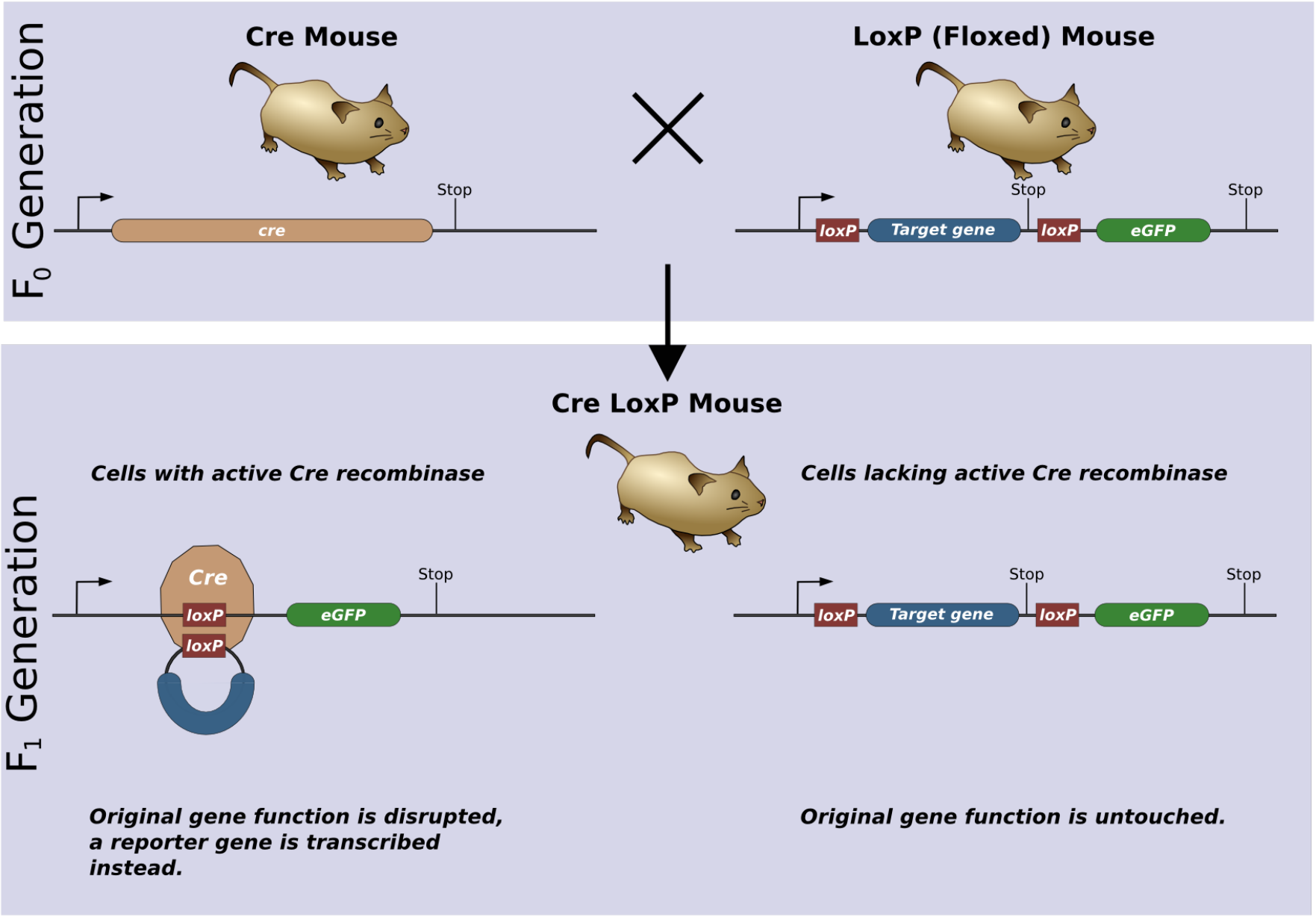
Cell type-specific knockouts: Cre-LoxP

Cre: 38kDa recombinase from bacteriophage P1
Cre recombines DNA between specific 34-bp sequences, called loxP.

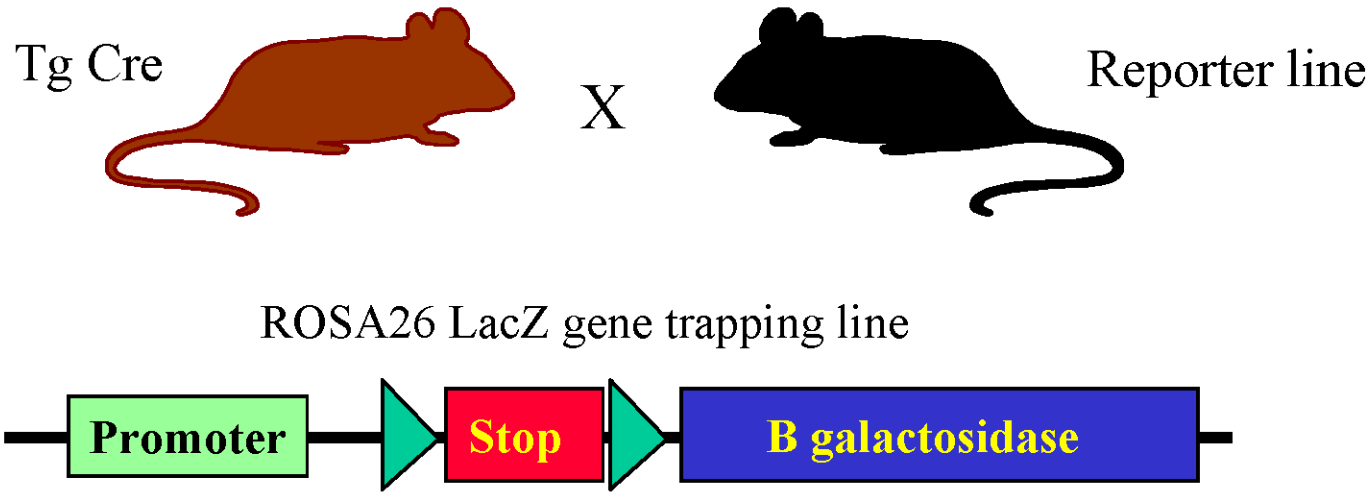
LoxP consists of a central 8-bp asymmetric sequence flanked by two identical 13bp inverted repeats.



The Cre-LoxP system



How to test for the specificity of Cre expression?

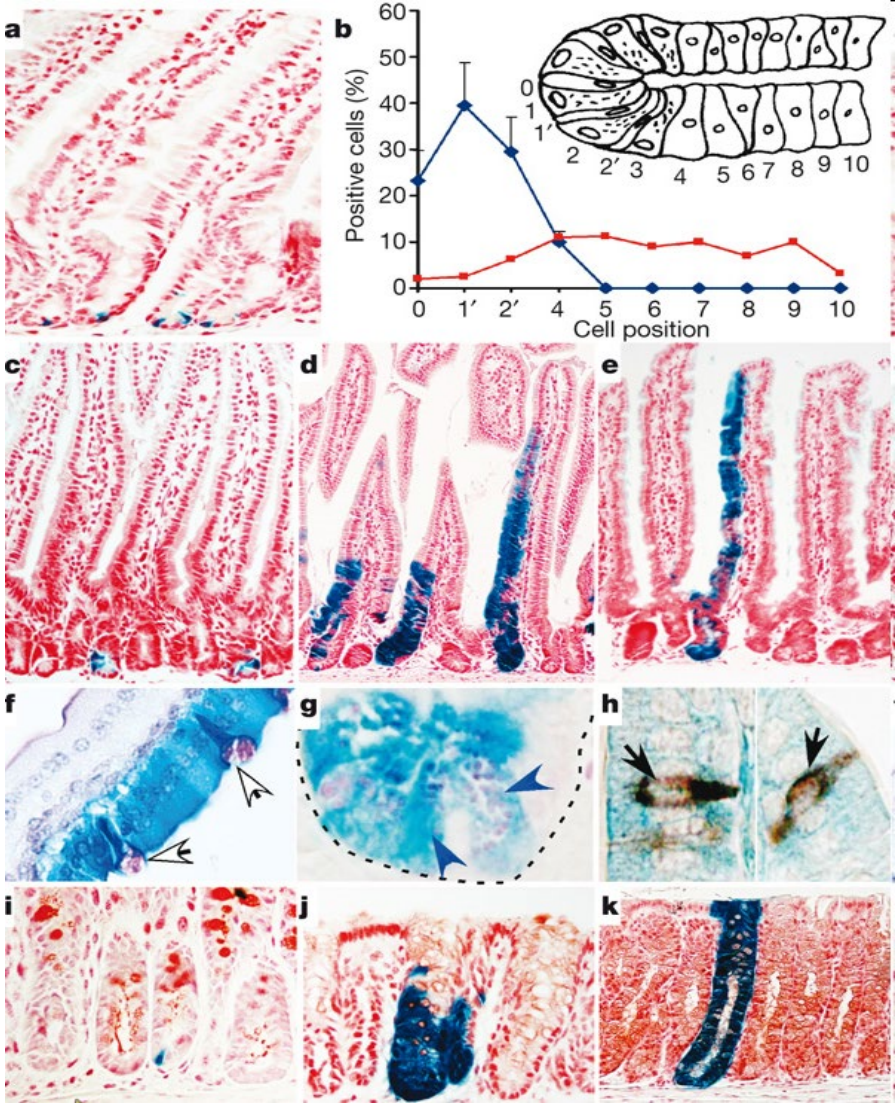


Tissue-specific testing for Cre recombinase expression

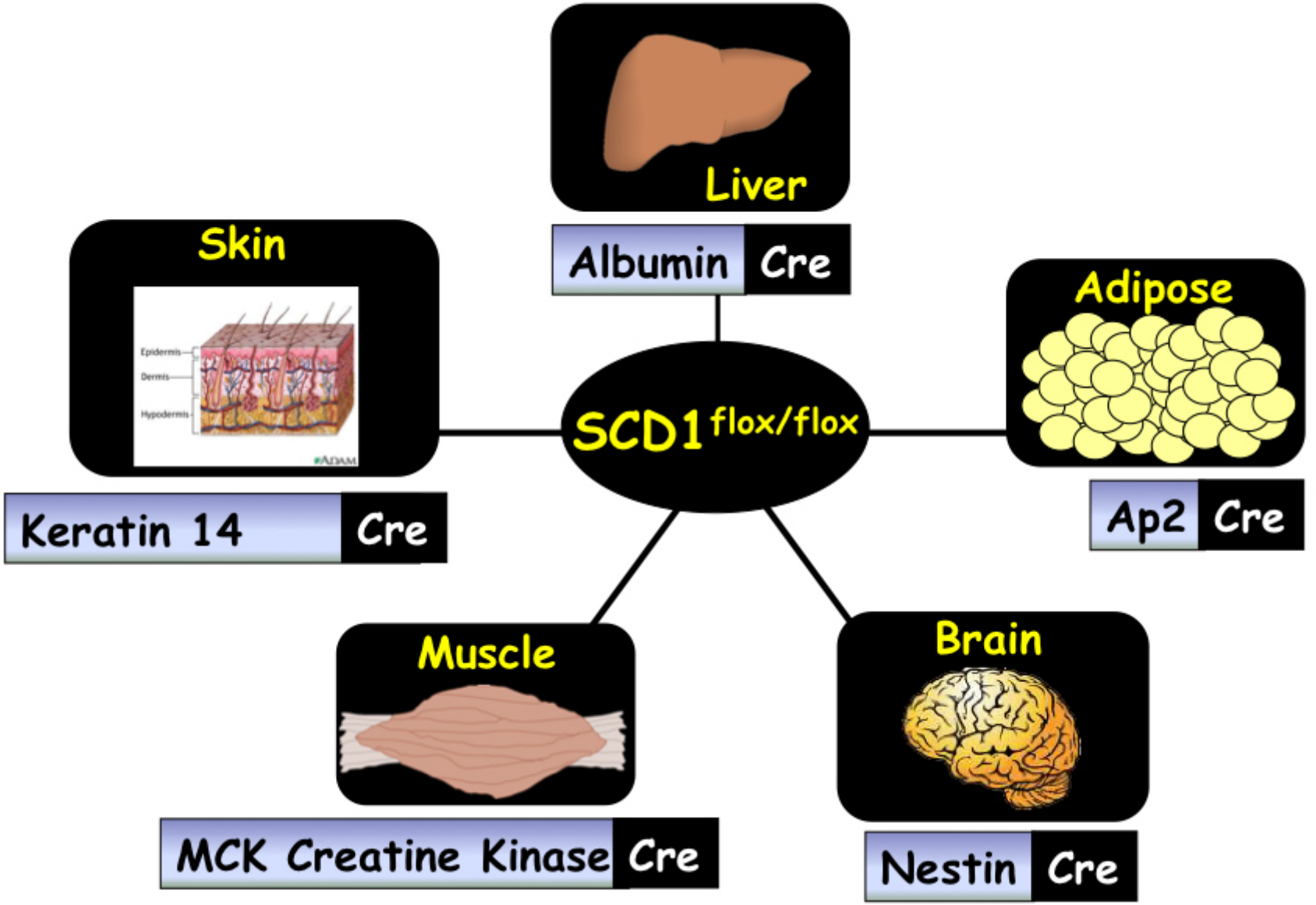
Rosa26 x P (gene of interest)-Cre

X-gal

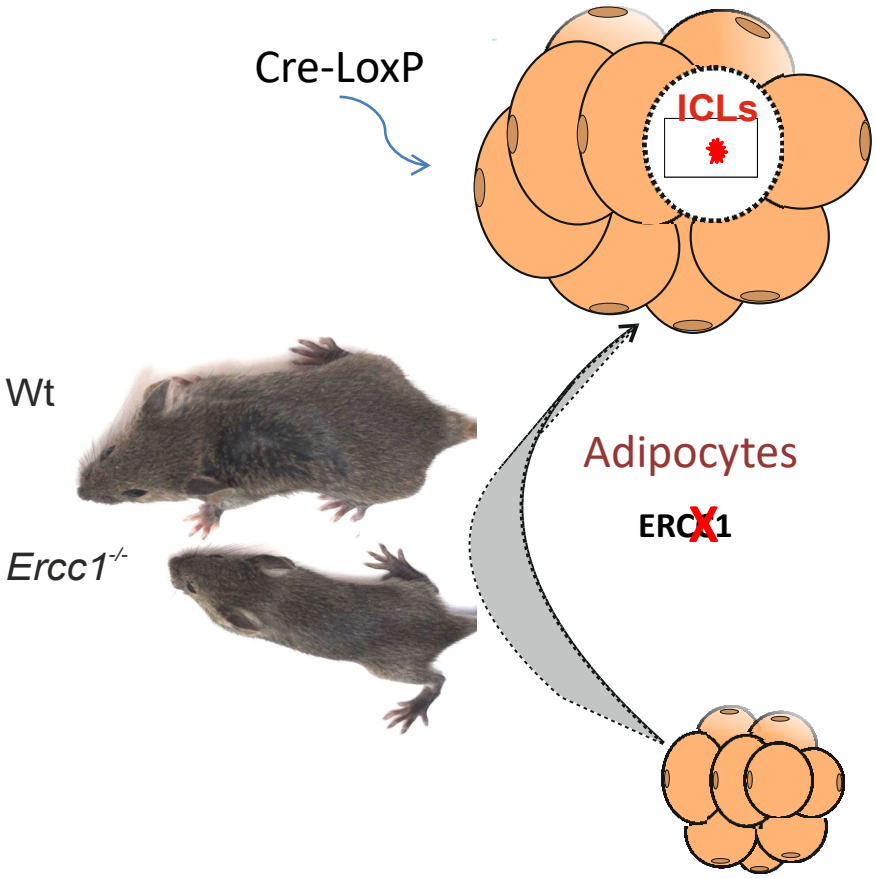
B-galactosidase activity



Several Cre lines to target distinct types of tissues



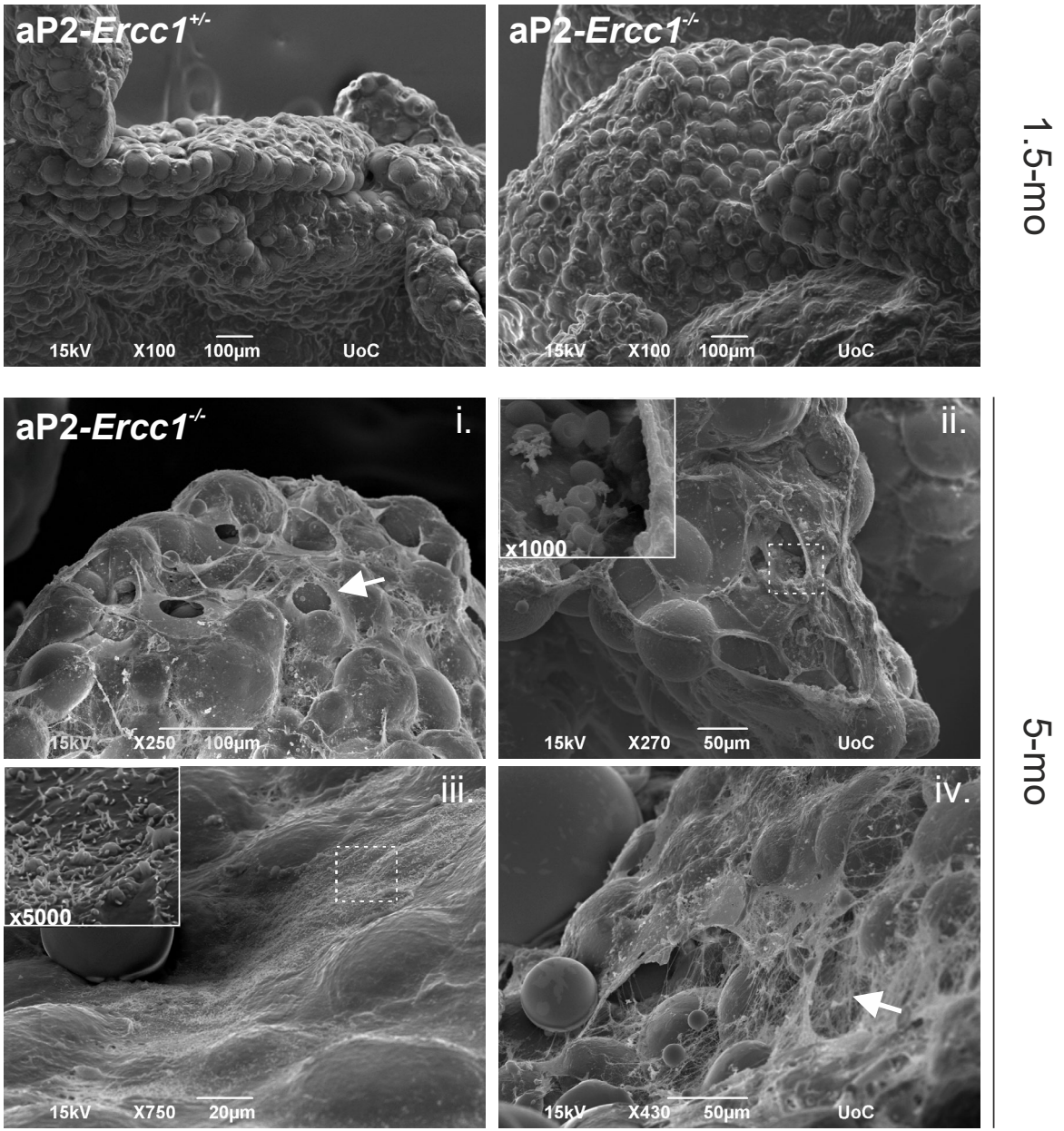
Targeting a DNA repair gene in the white adipose tissue



Fat-specific *Ercc1*$^{f/-}$ mice

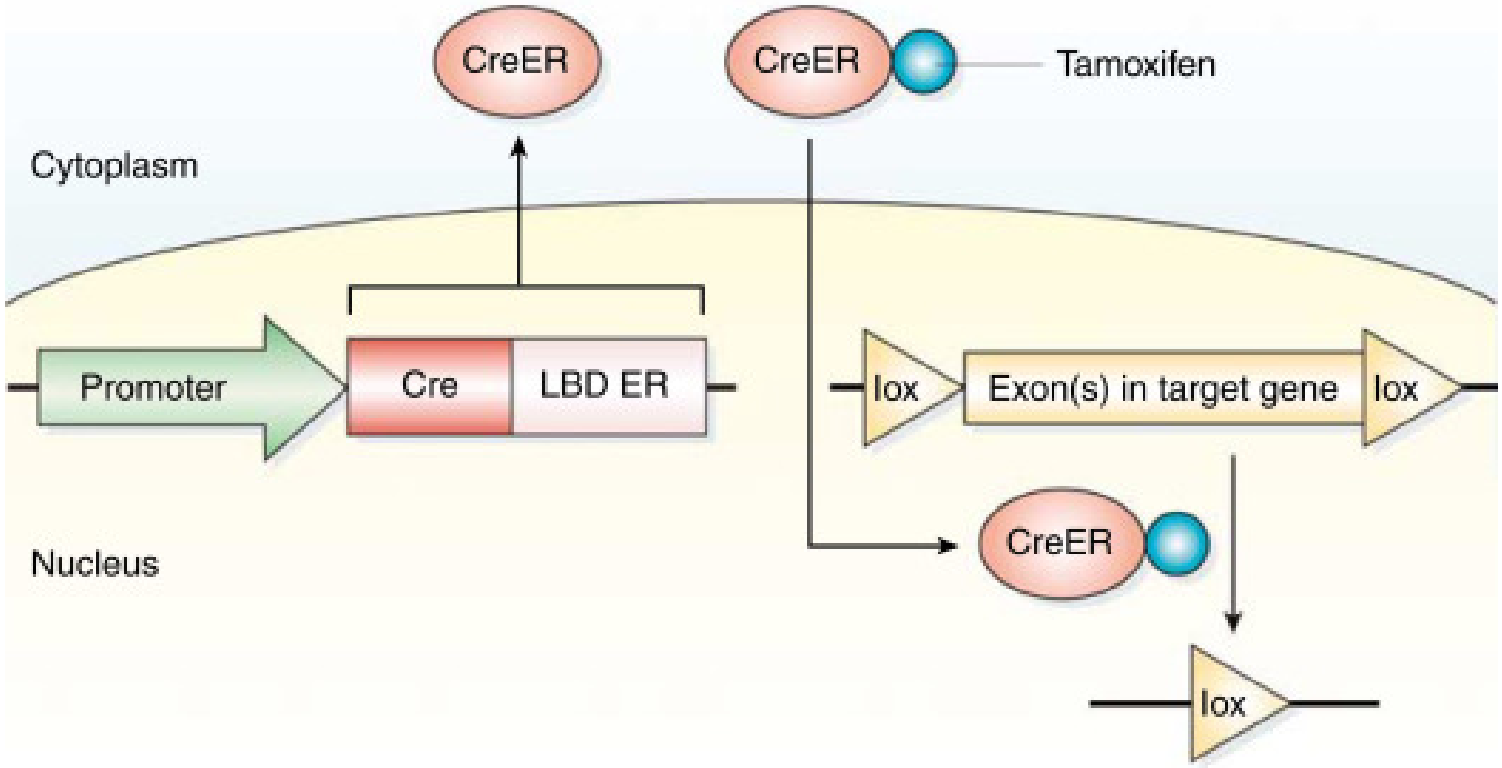
aP2-Ercc1$^{-/-}$ mice show signs of lipodystrophy

B.



Tamoxifen systems

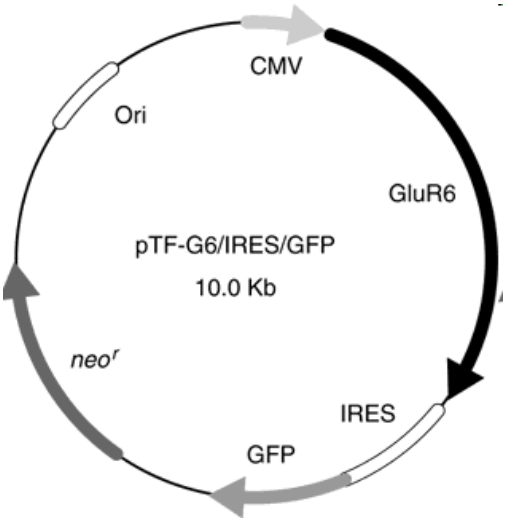
Site-specific recombination + inducible system for the temporal control of gene expression

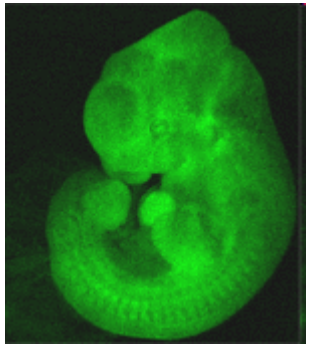


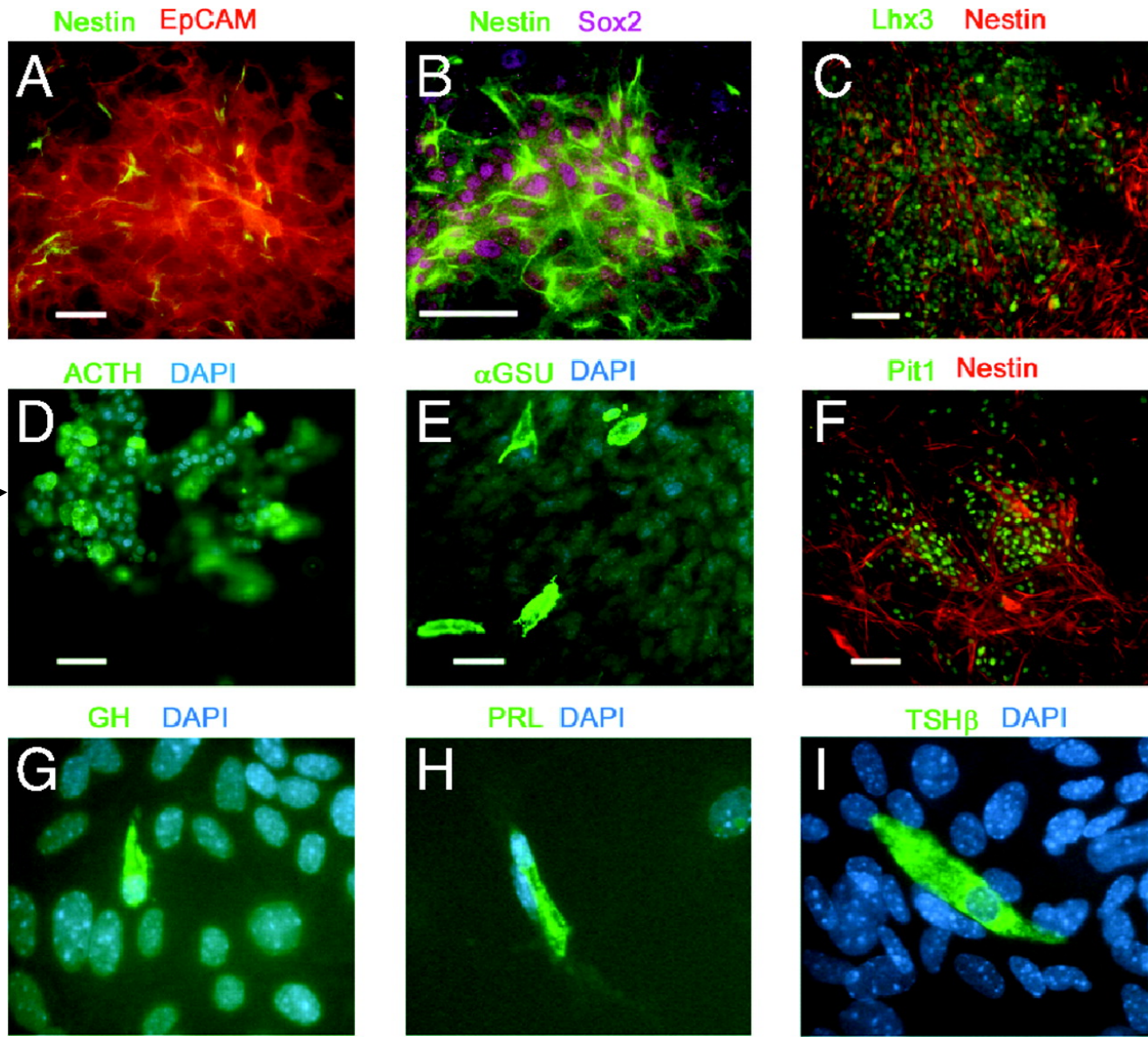
CreER: a Cre recombinase fused to the ligand binding domain (LBD) of the estr. receptor (ER).

When tamoxifen binds to CreER protein, CreER translocates into the nucleus, and then mediates site-specific recombination

In vivo tagging of gene expression



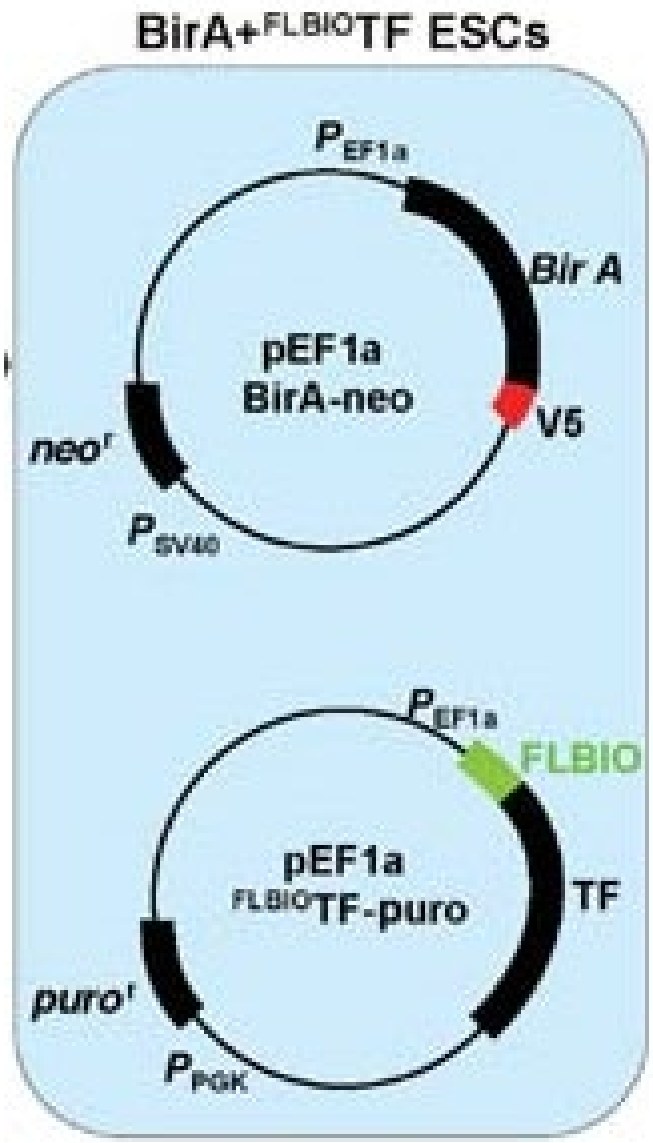




In vivo targeting of proteins

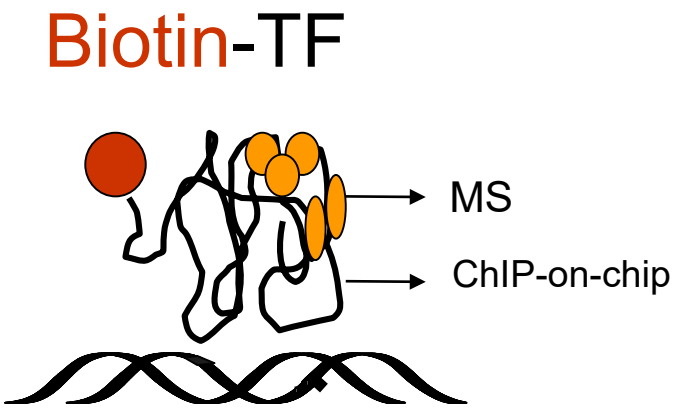
Knockin animals

In vivo biotinylation targeting of proteins:



In cells:

In vivo biotinylation tagging of transcription factors



Tagging Proteins *in vivo*

Peptide

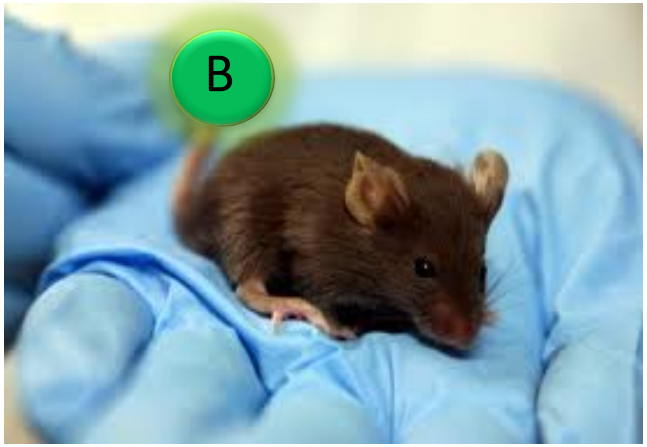
B FL NER factor

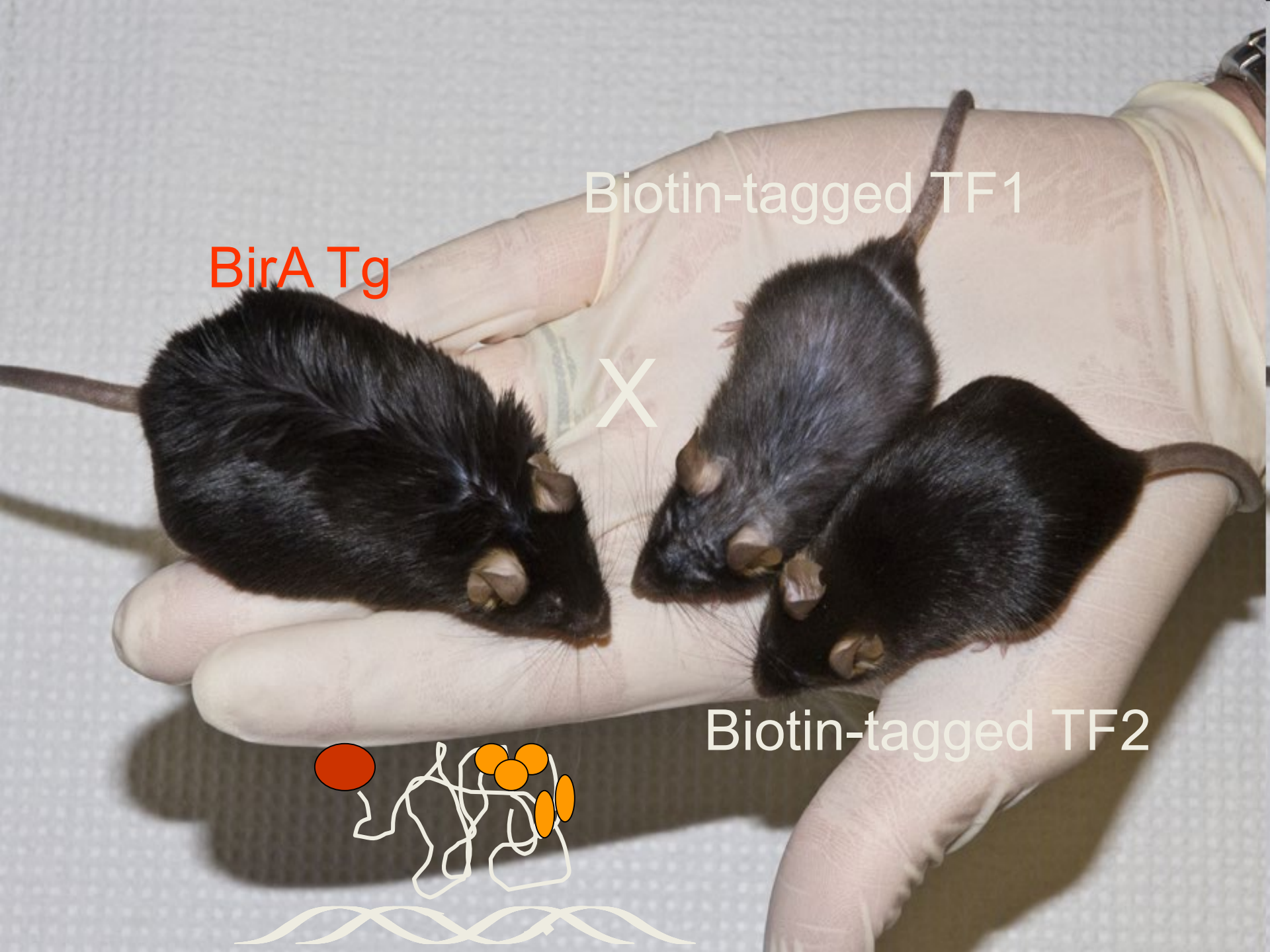
Bacterial ligase

Knock-in

Transgenic

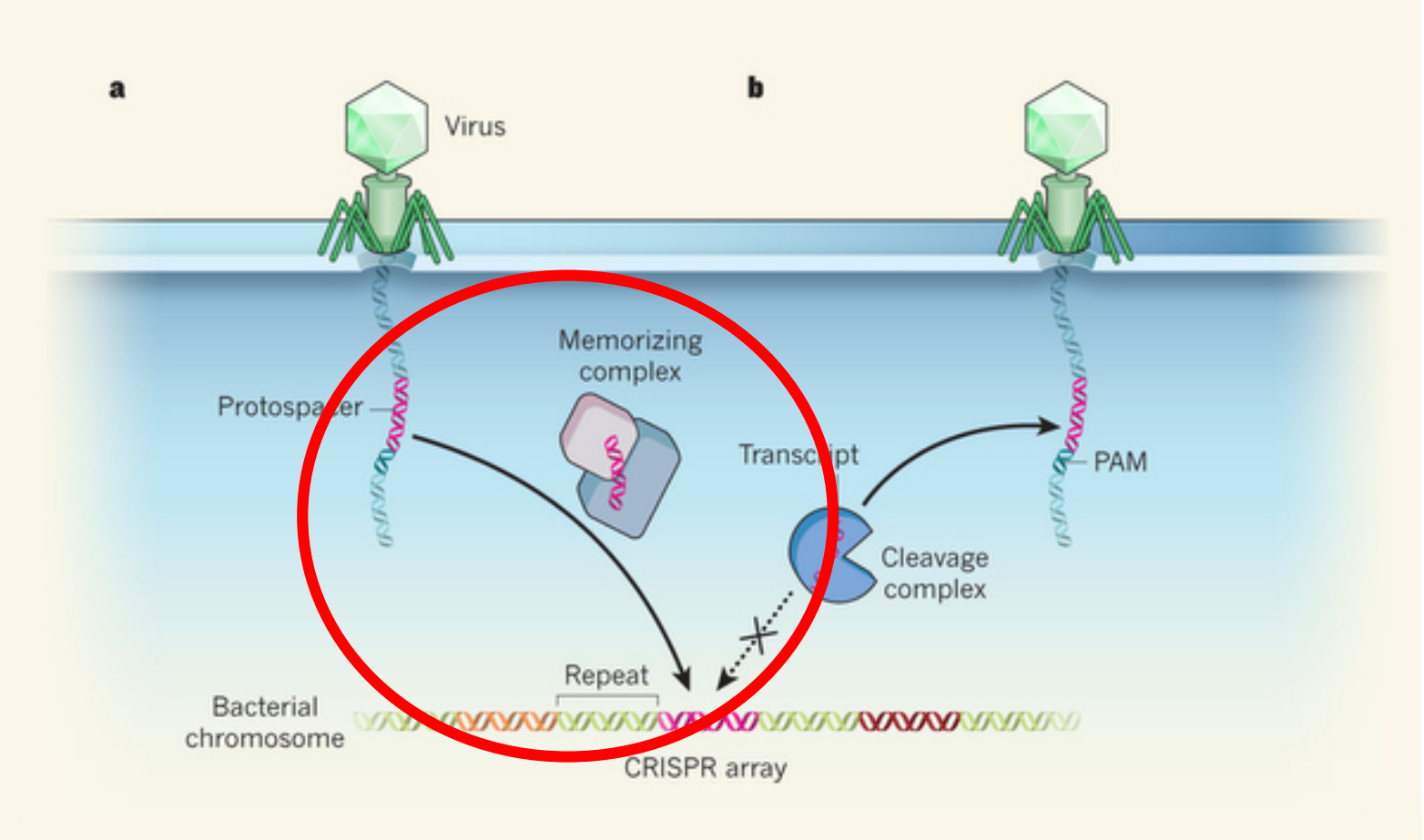
BioNER mice




Biotin-tagged TF1
BirA Tg
X
Biotin-tagged TF2

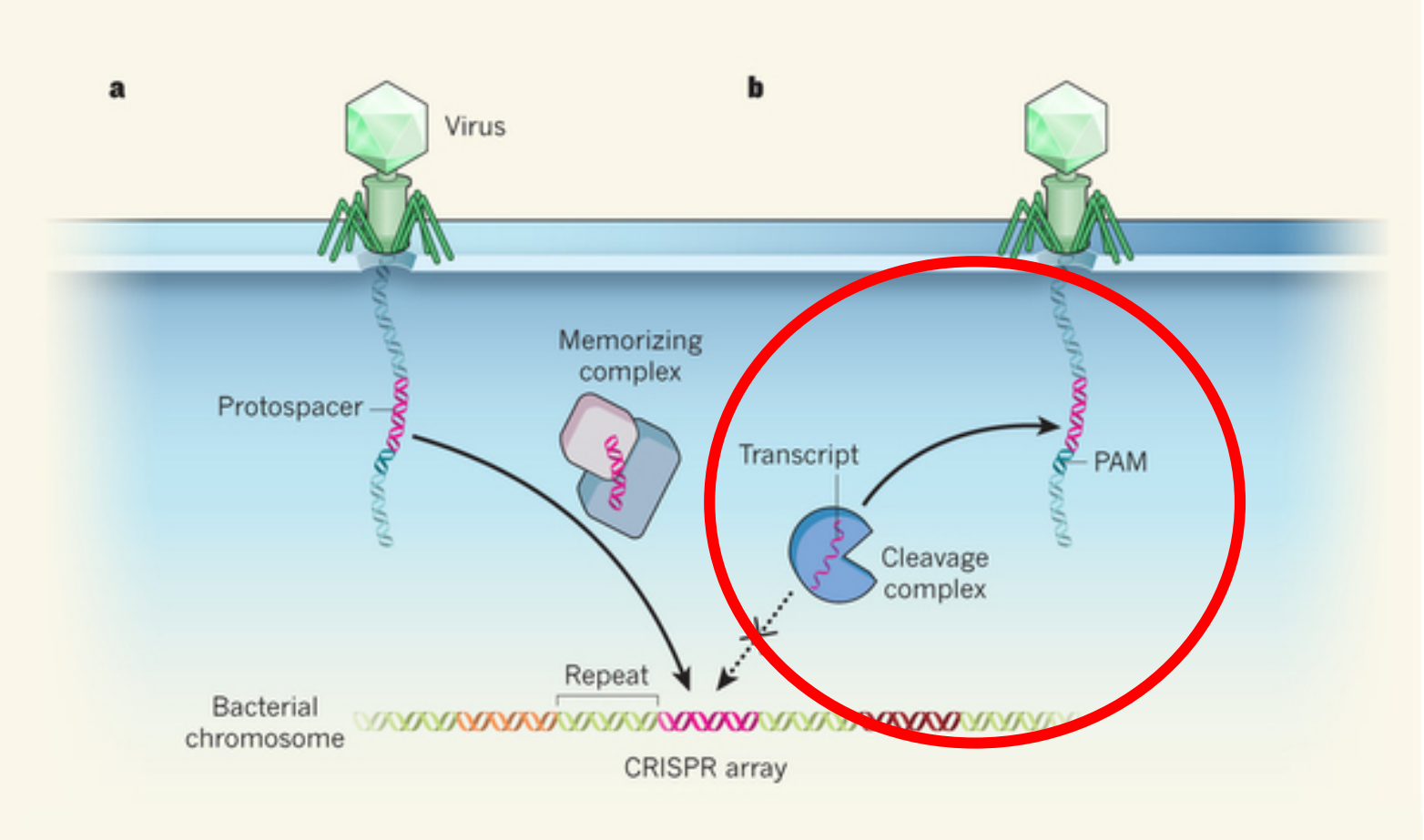
The CRISPR-CAS9 system

The CRISPR-CAS9 system

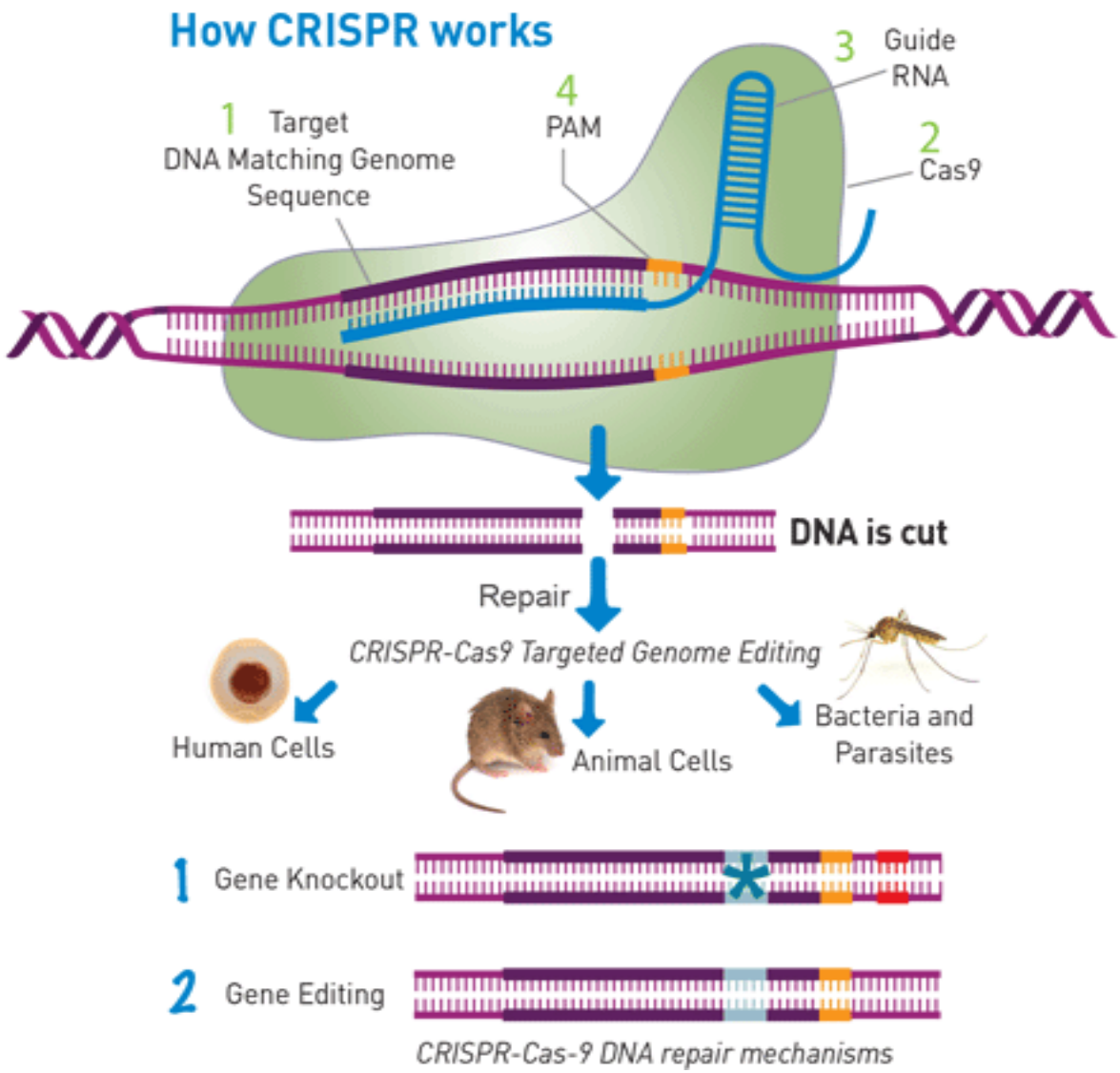


When bacteria become infected from viruses, some memory protein complexes of the CRISPR–Cas immune system select viral sequences (protospacers) to incorporate them within their own chromosome. Such sequences are incorporated in tandem in terms of repeats and are called «clustered regularly interspaced short palindromic repeats (CRISPRs)».

The CRISPR-CAS9 system

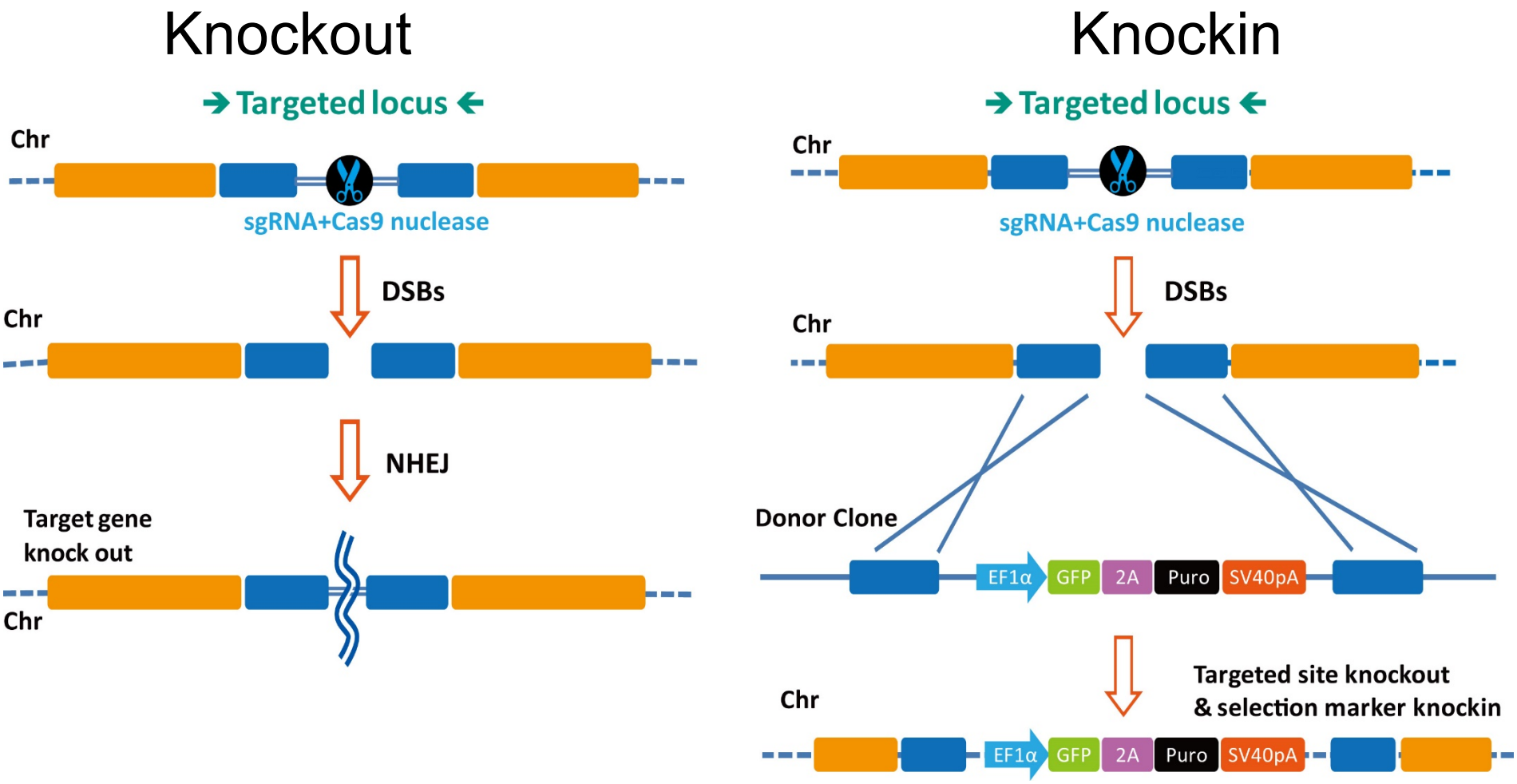


The next time the bacteria will become infected from their own viral DNA, the transcripts from spacers will guide an endonuclease to the viral DNA to cut it.



1. Target DNA: This is the region of the genome to be modified.
2. Cas9: This bacterial enzyme unzips and cuts the target DNA.
3. Guide RNA: A short fragment of RNA binds to Cas9 and contains a recognition sequence that matches the target.
4. Protospacer Adjacent Motif sequence. It is part of the target sequence DNA and is one of the factors that is required to define the cutting site.

CRISPR-CAS9 and DNA repair



The CRISPR-CAS9 in gene therapy

Cryopreservation “Tank Farm”

>7,900 strains

Cryopreservation of embryos

The Jackson Laboratory BH Facility

~1200 live strains

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New JAX® Sperm Cryo Kit
….Just do it!