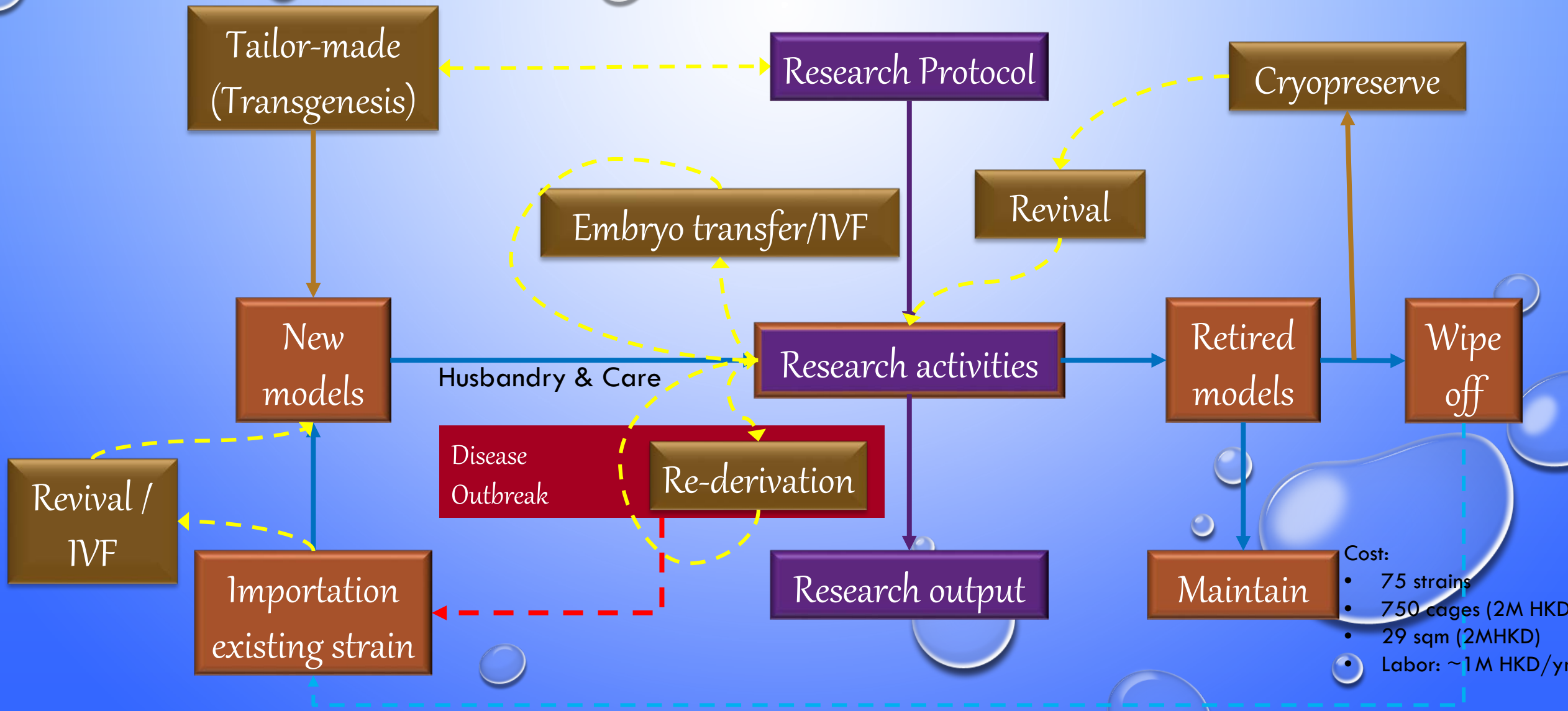
Mouse Transgenic Technologies

Siva, Wai Hung TSANG (PhD), Scientific Officer

Animal and Plant Care Facility, HKUST

Advancement of Animal Care & Use Programs by "Transgenic" Services

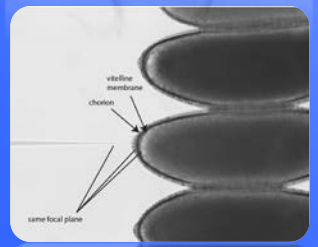


Definition of a Transgenic Animal (1981)

- An animal carrying a DNA molecule that was intentionally introduced by human intervention.
- It is expected that the exogenous DNA is able to be transmitted to the next generations.

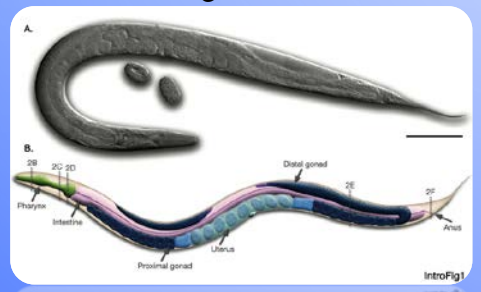
Drosophila melanogaster

Wikipedia

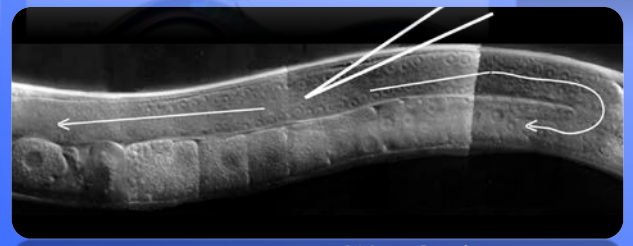


Gompel lab

Caenorhabditis elegans



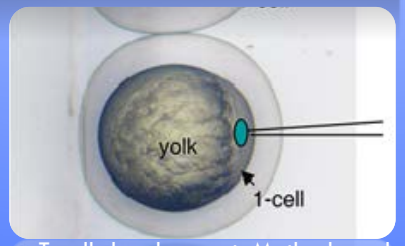
WormAtlas



WormBook

Danio rerio

FishBase



T-cell development: Methods and protocols

Mus musculus

JAX

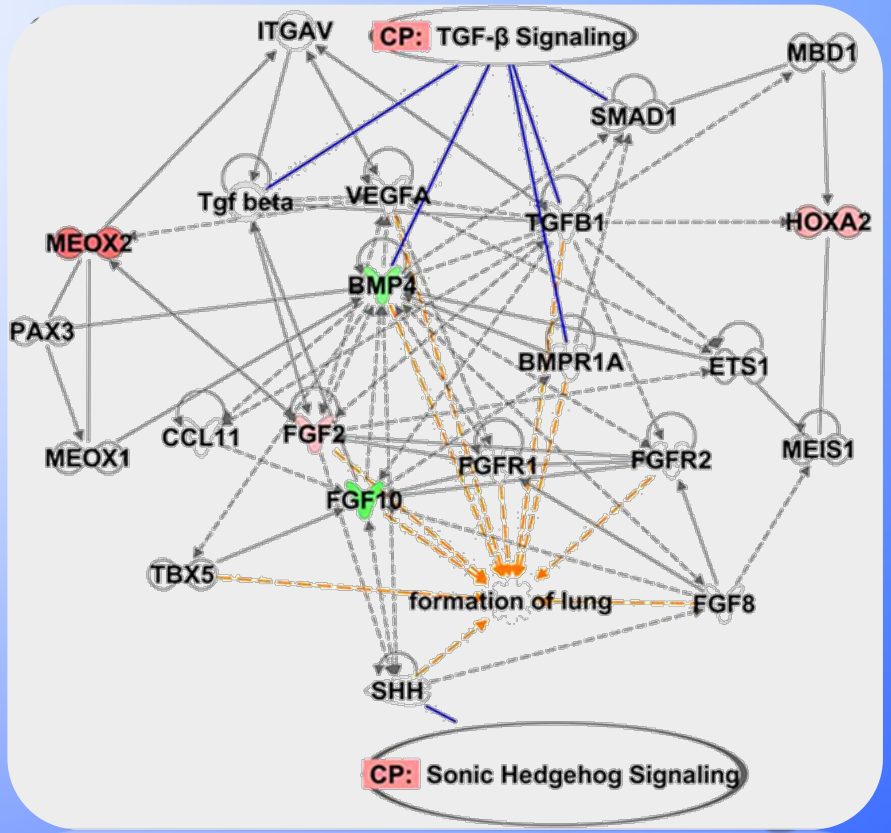
HKUST

Livestock

OCPA

Needs of studying gene function (i.e. genetics)

- Advancement of knowledge in different fields (e.g. developmental biology, biochemistry, immunology, oncology, evolution, microbiology etc.)
- Understand congenetial diseases
- Foundation and platform (disease model animals) for drug and treatment development
- …



"Forward" Genetics

Classical genetics

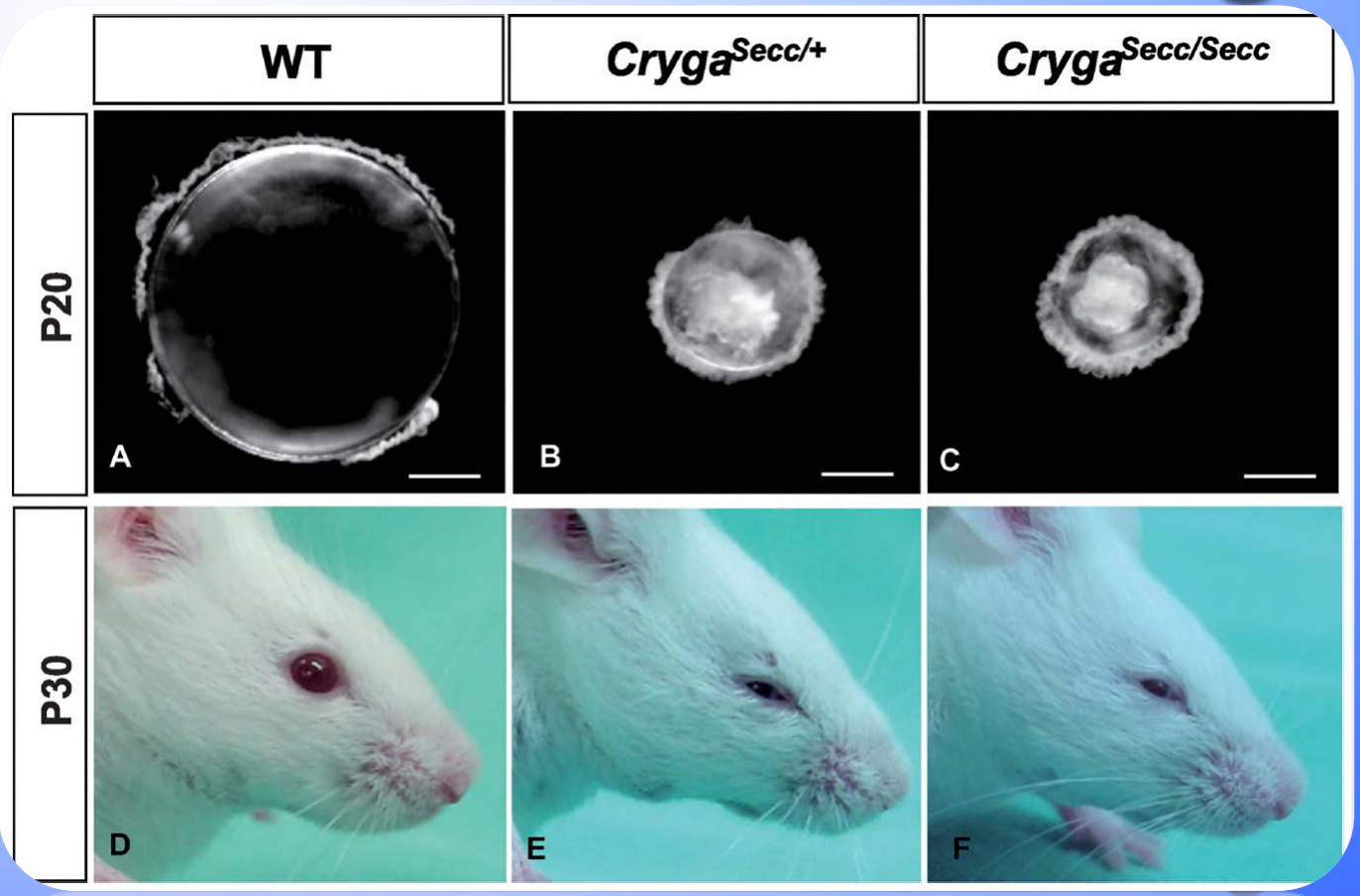
- Studying a gene by finding the basis of a trait (natural/mutagen-induced mutation)
- **Phenotype → Gene**
- a mutant must be present to work with

Phenotype:

- ***Secc (small eye, closed eyelid and cataract)***

Gene:

- Mutation in *γA-Crystallin* →$Cryga^{Secc}$



PLoS One. 2016 Aug 11;11(8):e0160691.

Reverse Genetics

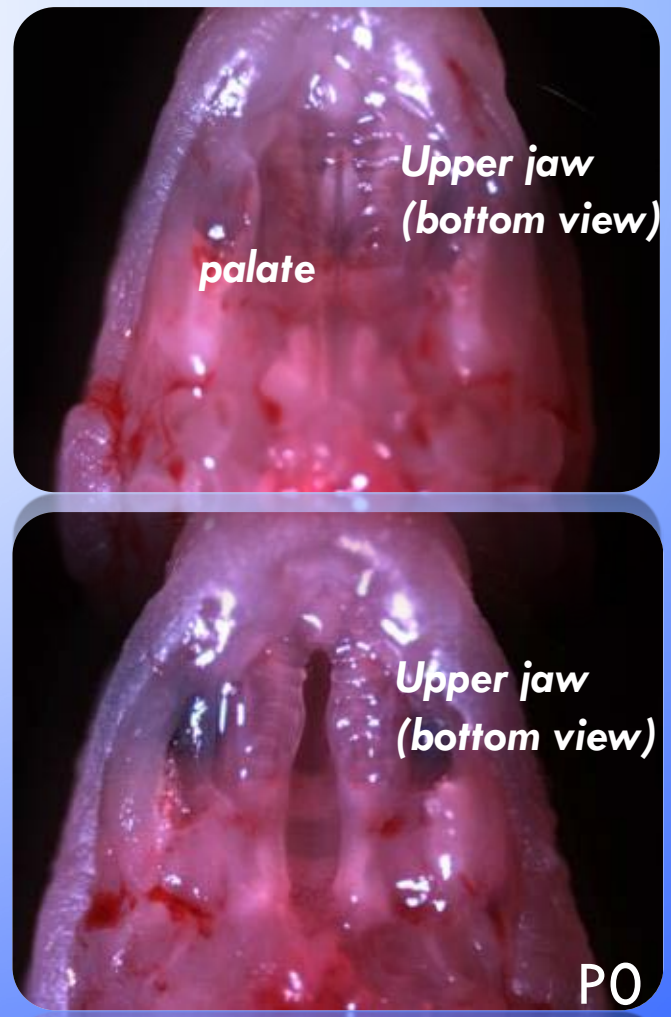
- by engineered gene sequences and analyzing the consequence.
- **Phenotype ← Gene**
- **Any gene of interest can be studied**

Gene:

- ***X***

Phenotype:

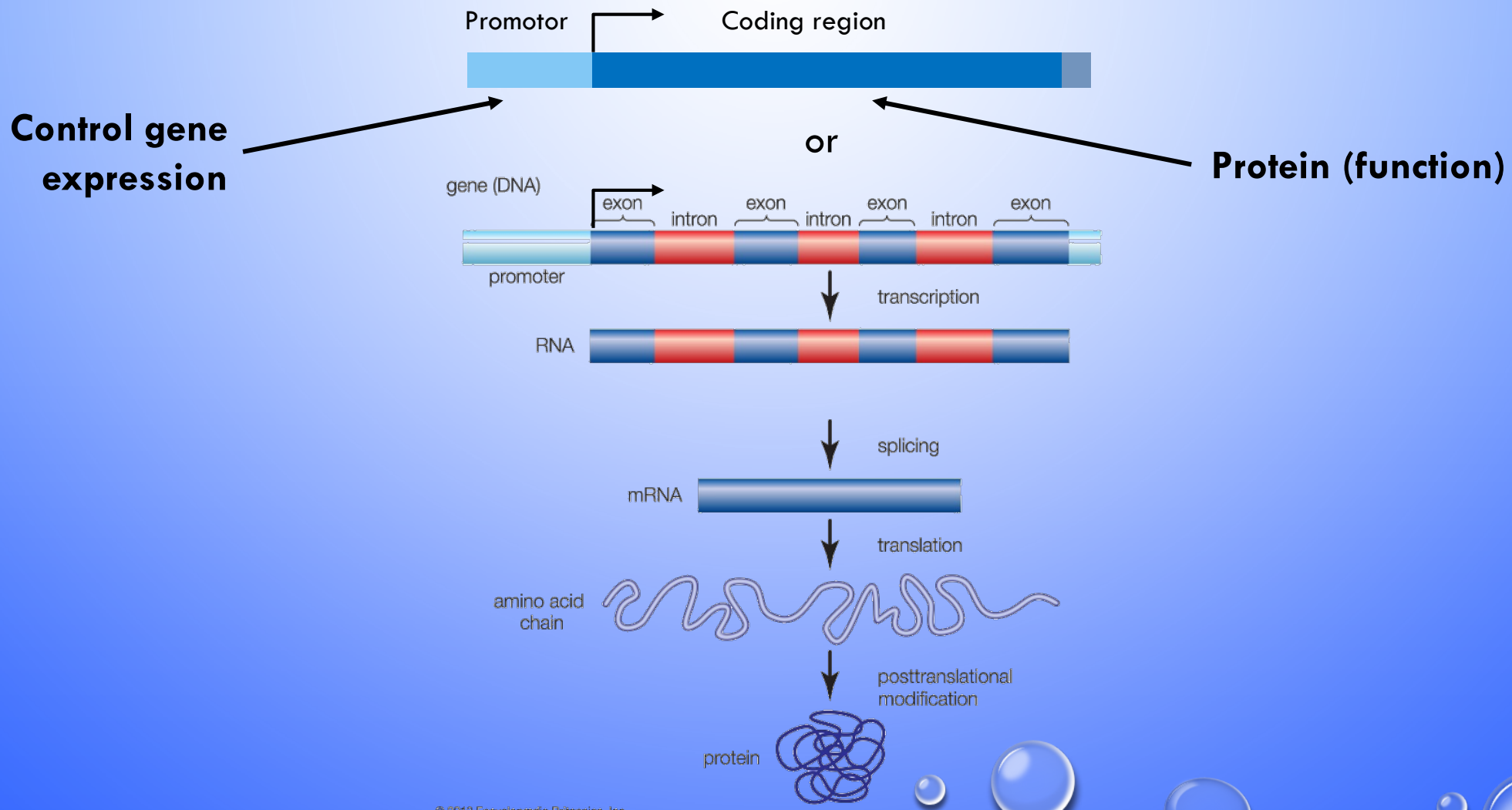
- ***Cleft palate***



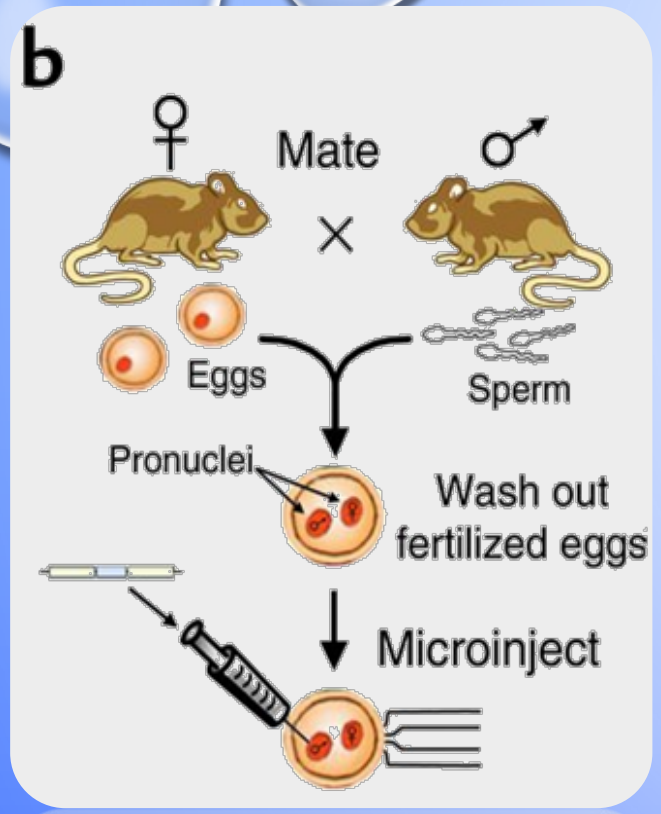
Gene X KO
(tissue specific)

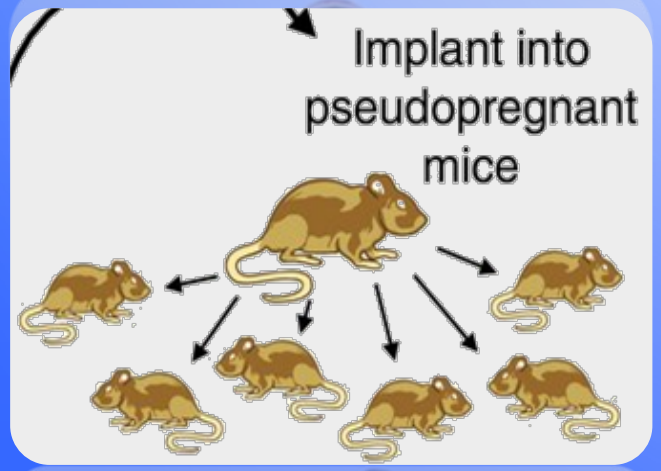
Tsang and Chow, HKUST

A Typical Gene Structure



Generation of Transgenic Mice by Pronuclear DNA Microinjection



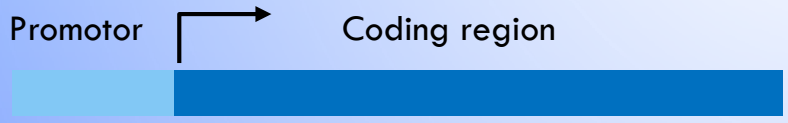


- Five groups simultaneously reported their success in 1980s
- Addition of a transgene. Ideal for gain-of-function study
- Stably integrated into the genome with an acceptable frequency (in a100 of injected and survived zygotes, one can have a few transgenic founders).
- Random integration

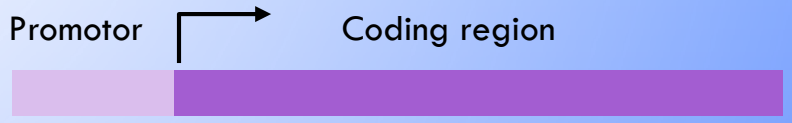
Transgenes for "Gain-of-Function Study"

- Transgene: materials from existing genes

Gene *Eye*



Gene *Finger*



Transgene:

(From gene *Eye*) (From gene *Finger*)

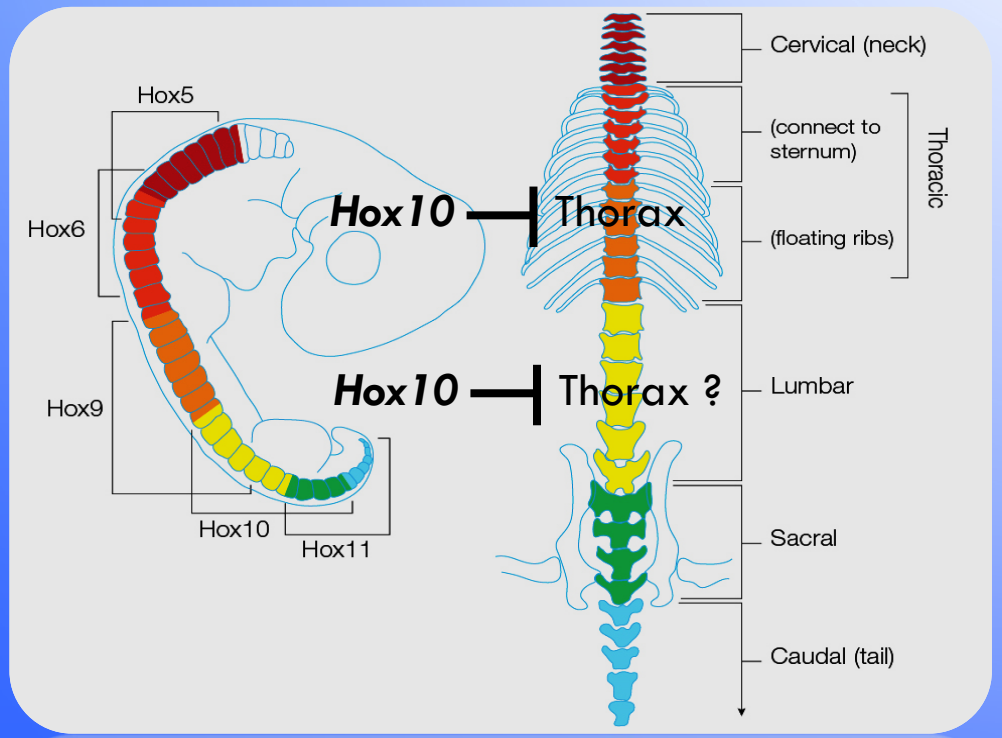
?

(hypothetical image)

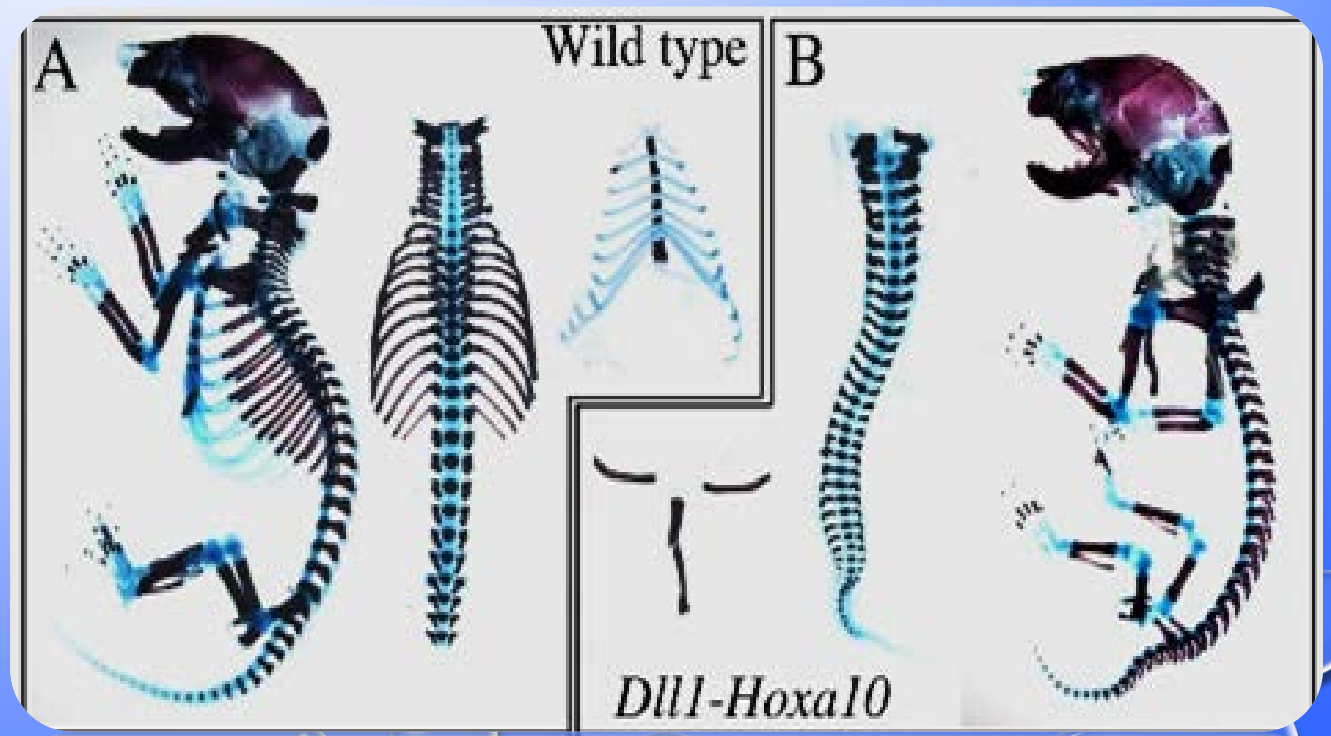
Transgene name abbreviation: ***Eye*** **-** ***Finger***

Gain-of-Function Study using Transgenic Mice

- Expression of *Hox10* in all axial level (Dll1-Hoxa10): a visual example
  - Applying to the lumber ? *Hox10* determines lumbar identity (partly by suppressing the formation of ribs)



Learn Genetics @ Utah - University of Utah



Dll1 mesoderm Development 2012 139: 2703-2710

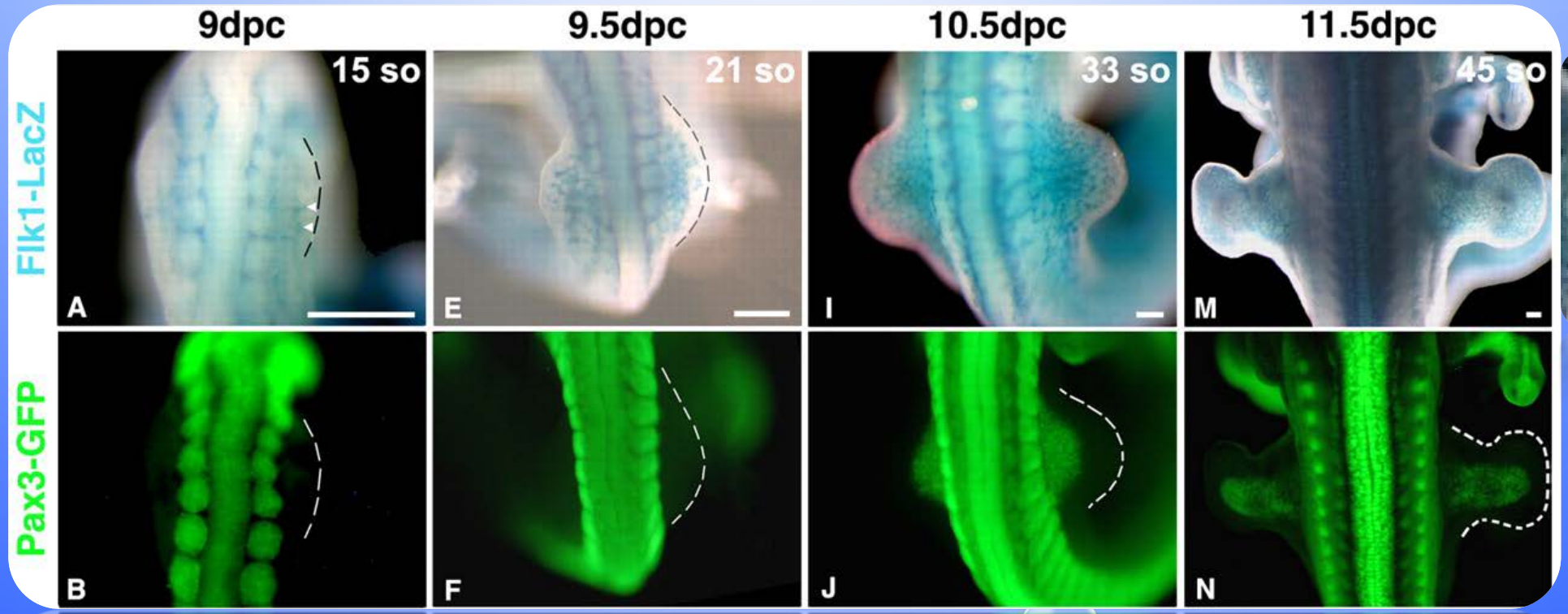
Monitoring Biological Processes in Transgenic Mice

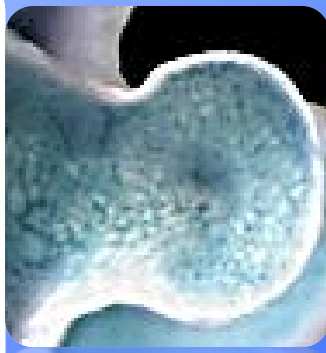
- Expression of reporter gene
- e.g. Limb development

Other popular reporter genes: RFP, YFP

Flk1-LacZ (blood vessel cells)

Pax3-GFP (muscle progenitor cells)



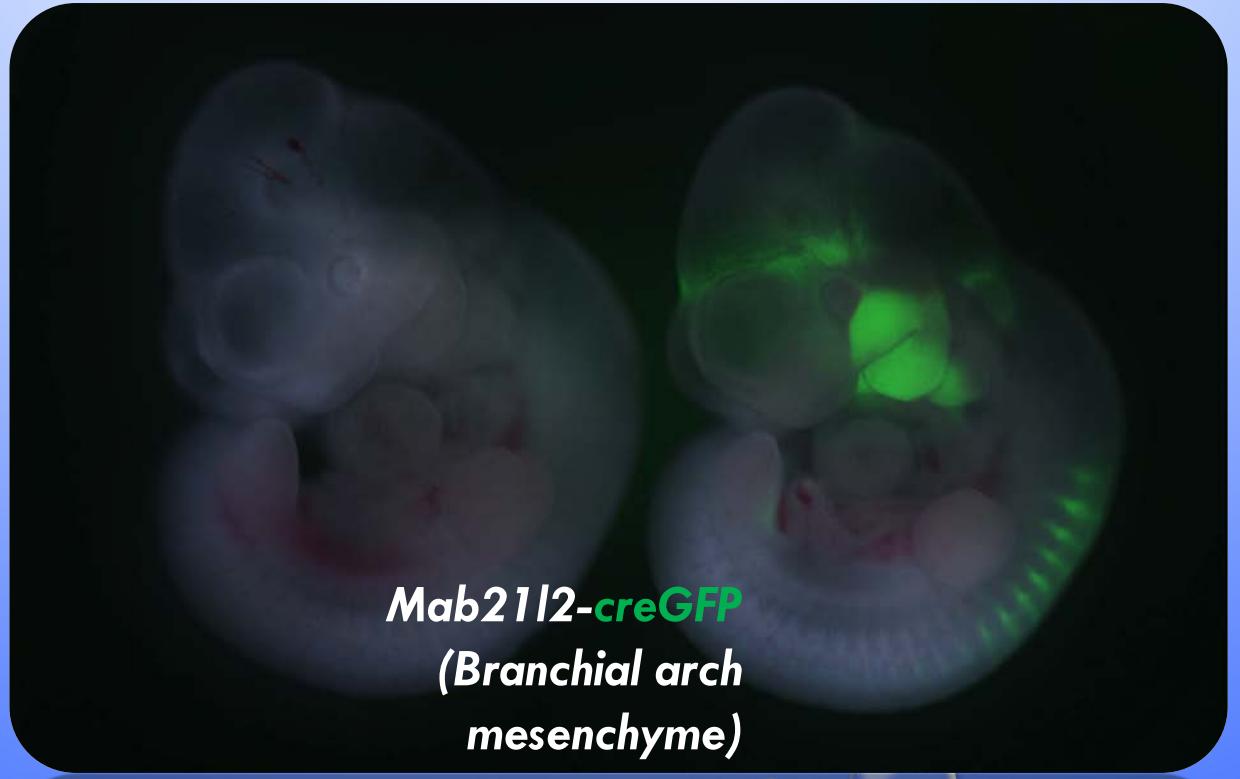


Development 2012 139: 277-287

Dual Function of a Transgene

e.g. creGFP

- Cre recombinase: gene editing
- GFP: marking the tissue



Tsang and Chow, HKUST

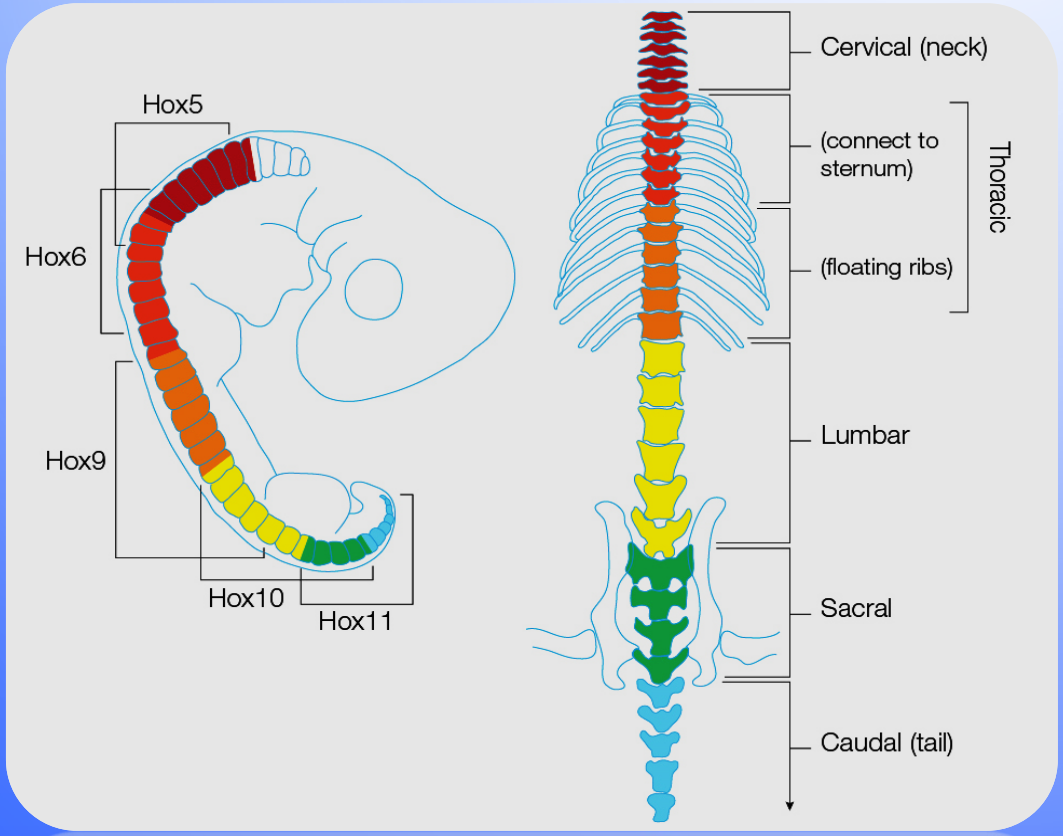
Other use of Transgenic Mice

- Marking protein subcellular localization
  - Protein::GFP fusion protein (nucleus, membrane, clustered etc)
- Genetic cell ablation
  - Diphtheria toxin
- Expressing genetic engineering tool proteins
  - Recombinase, transposase
- Modeling diseases
  - Transgene genes with human mutations

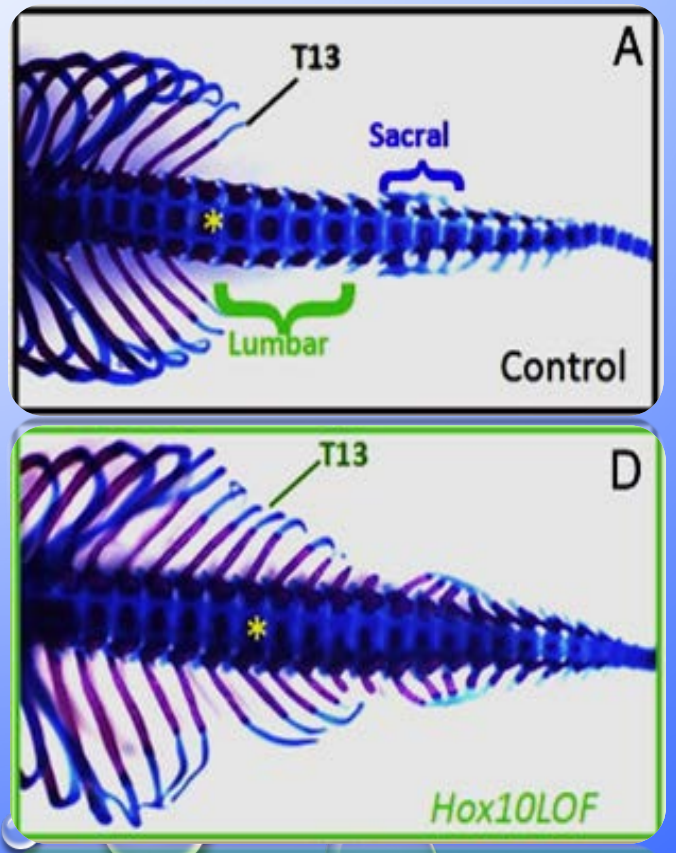
Gene targeted Mice

Loss-of-Function Study using Knockout Mice

- Example: *Hox10* – backbone identity
- Gain-of-function → *Hox10* suppresses thorax identity
- Loss-of-function → ?



Learn Genetics @ Utah - University of Utah

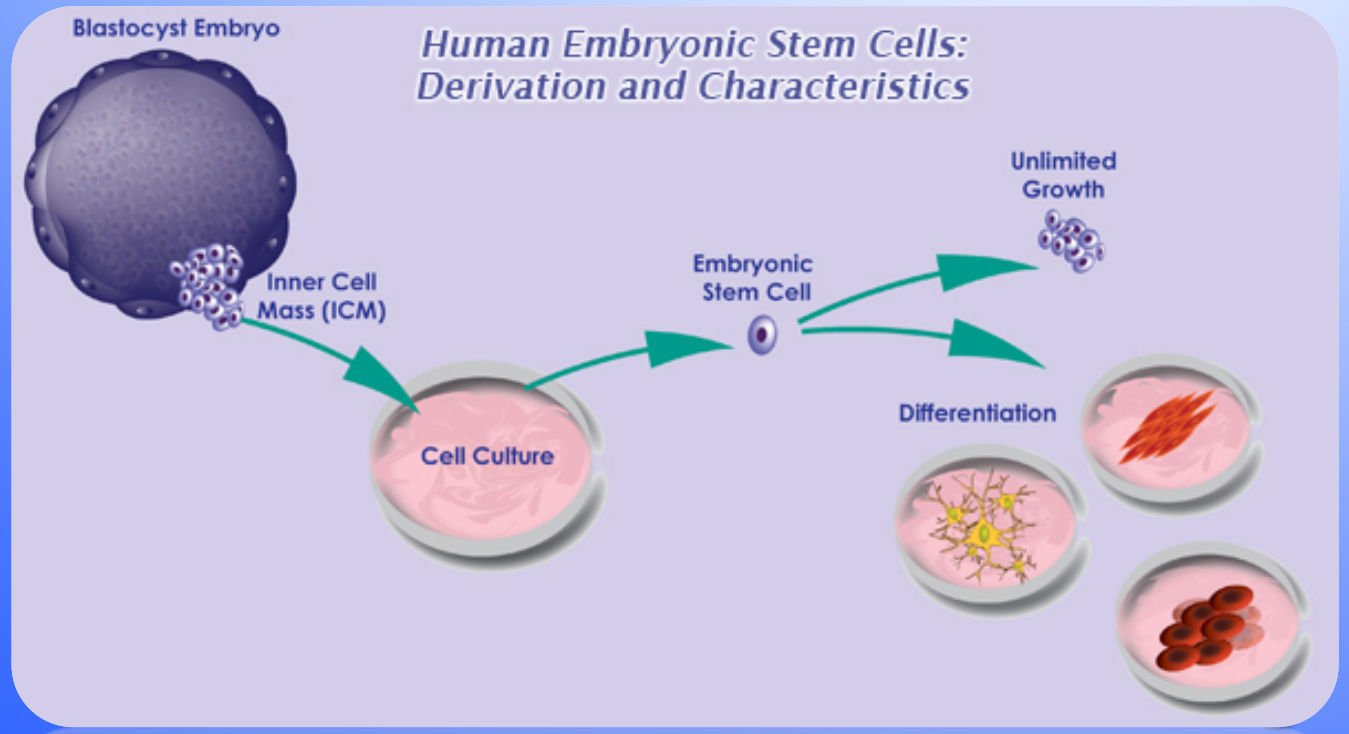


Cell 73: 279-294

Gene targeted Mice
Generated by
1. Embryonic Stem Cells (ESC) & Homologous Recombination (HR)

Embryonic stem cells (ESC)

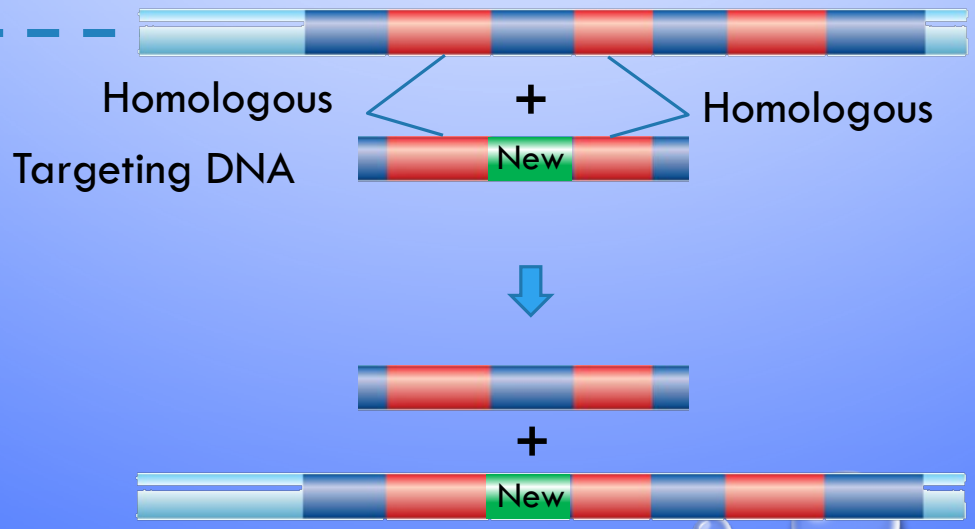
- Derived from preimplantation embryos
- ***Pluripotency***
- Ability to colonize a host embryo, including its *germline*



Homologous Recombination (HR)

HR: exchange of DNA sequences between two similar (homologous) DNA molecules (targeting DNA and genomic DNA)

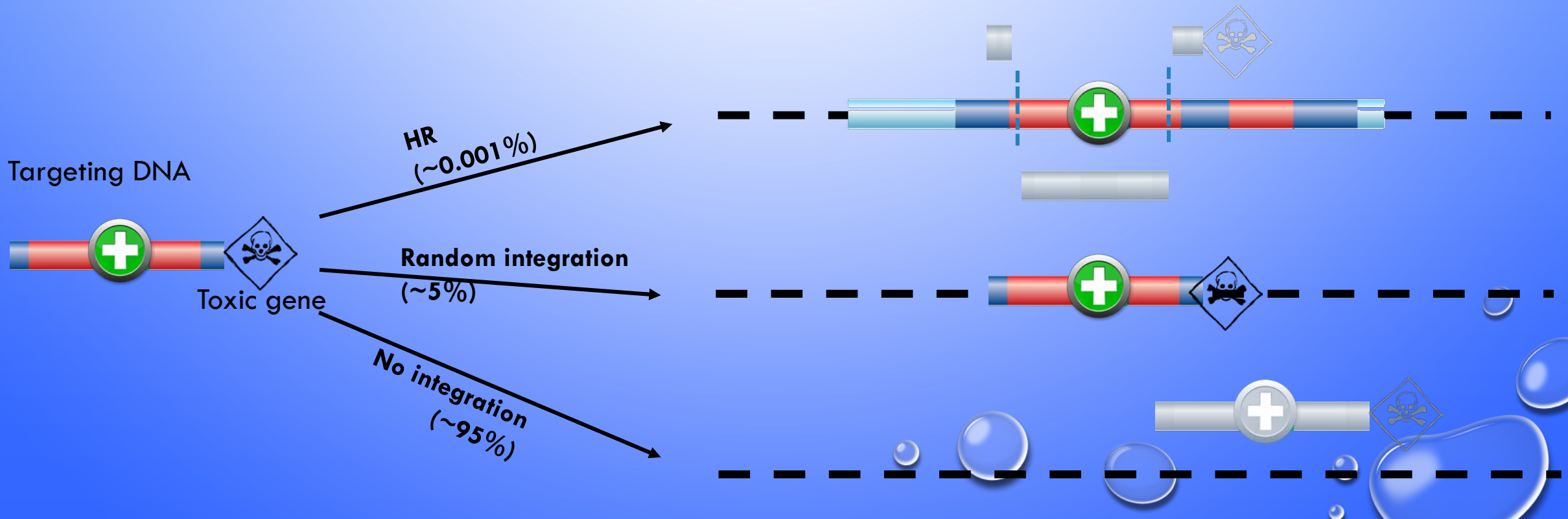
- Two homology arms: same similar sequence of the gene of interest
- Selection strategy (Frequency: 1 in 100,000 to 1,000,000)



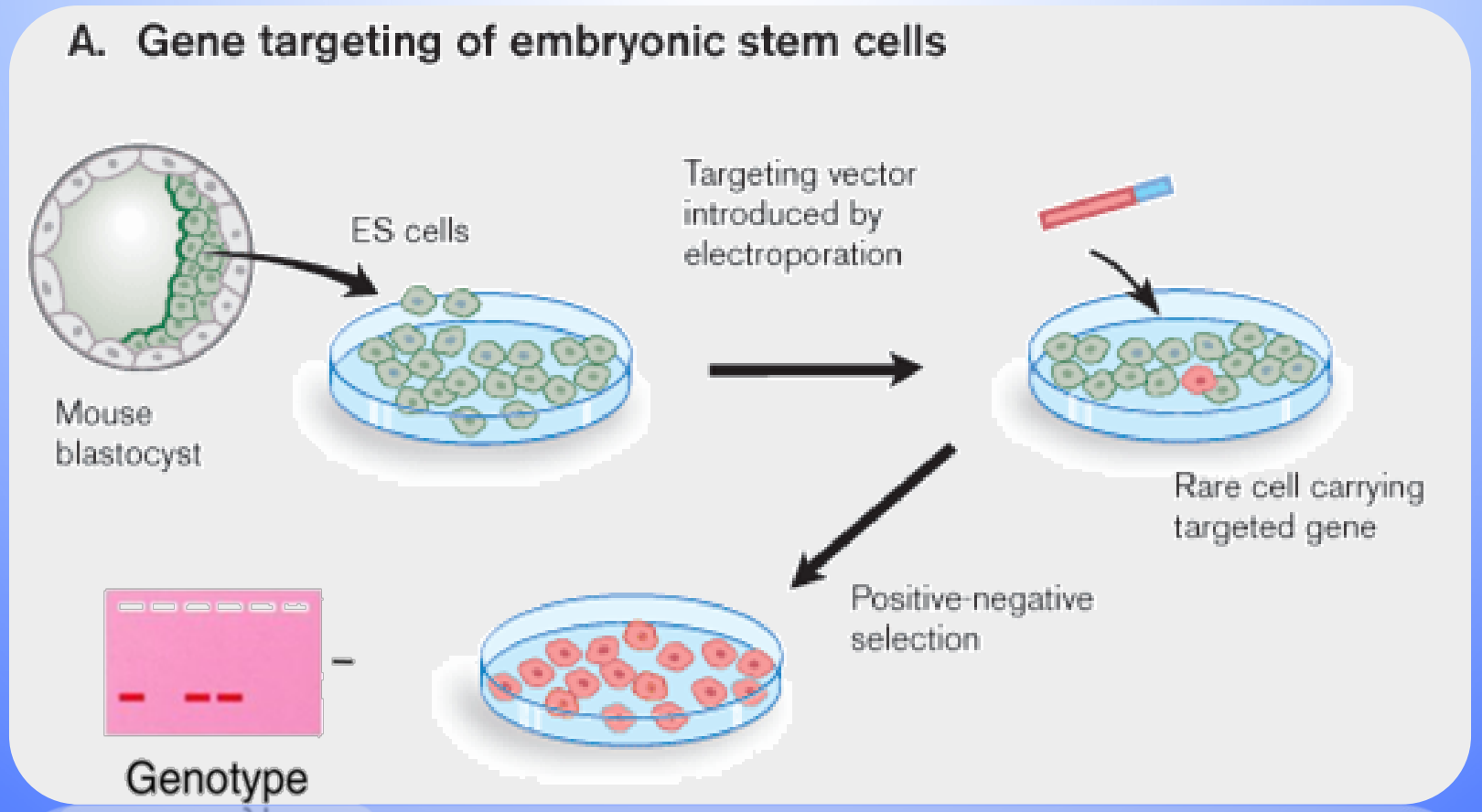
Homologous Recombination (HR)

Targeting DNA:

- a positive selection marker: resist to a toxic drug added to the culture medium
- a negative selection marker: express a toxic protein to kill a cell itself

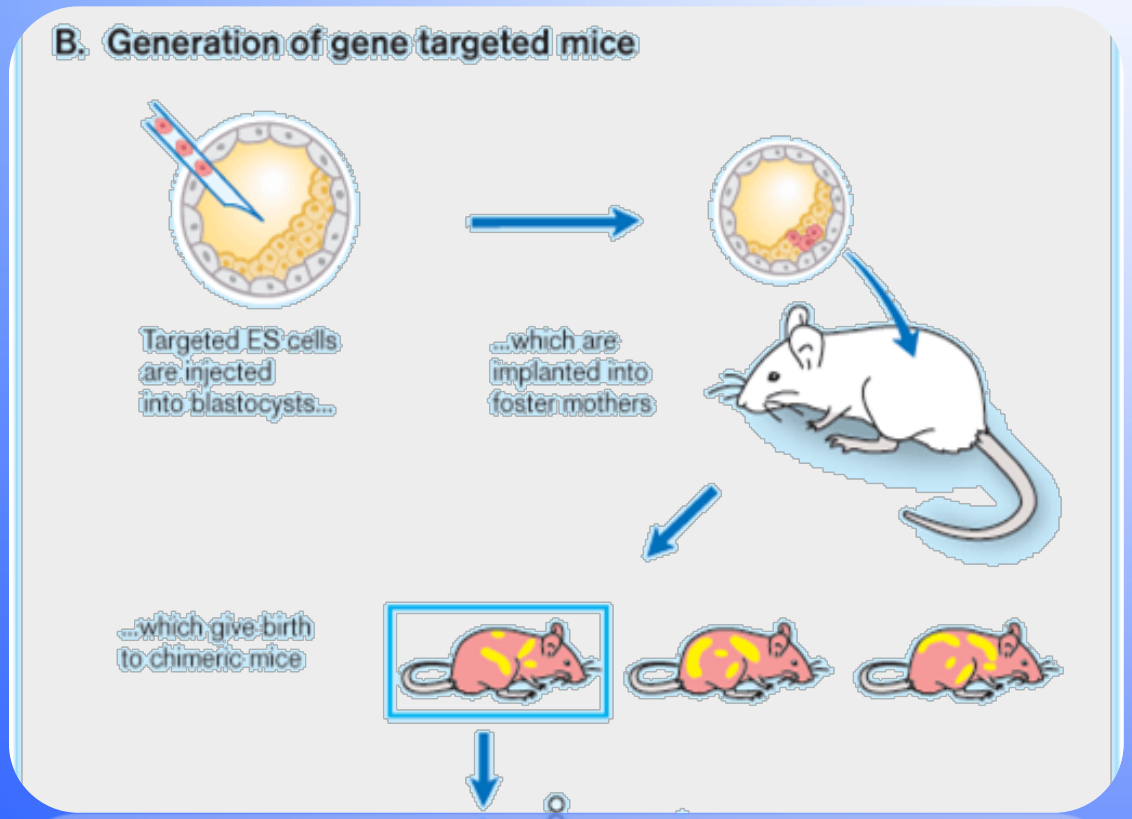


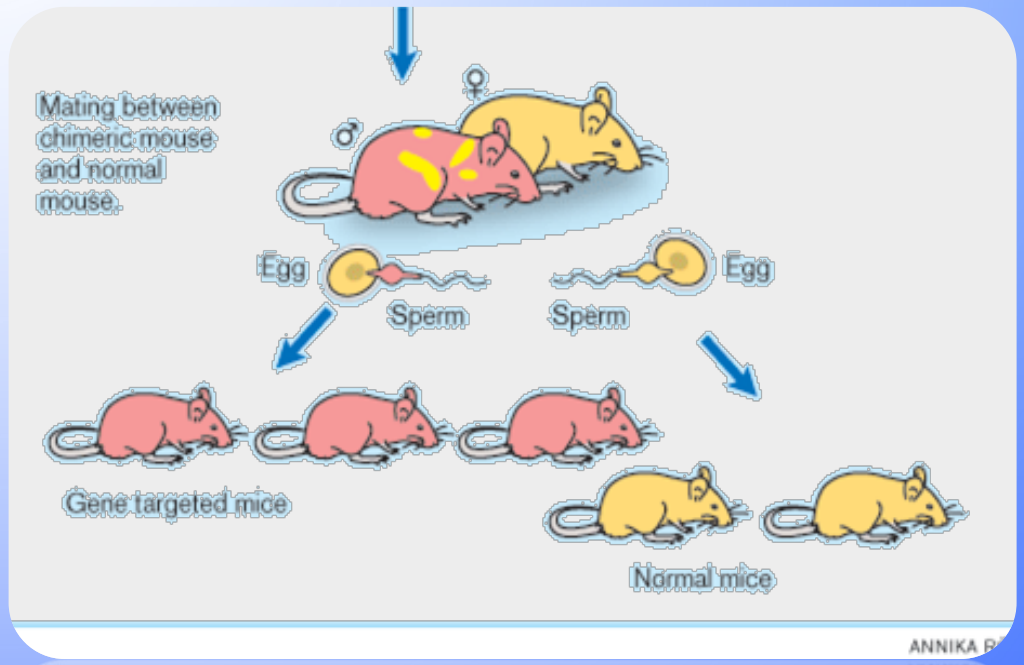
Generating gene targeted ESC



Targeted knockout Schematic. nobelprize.org (2017)

Procedures



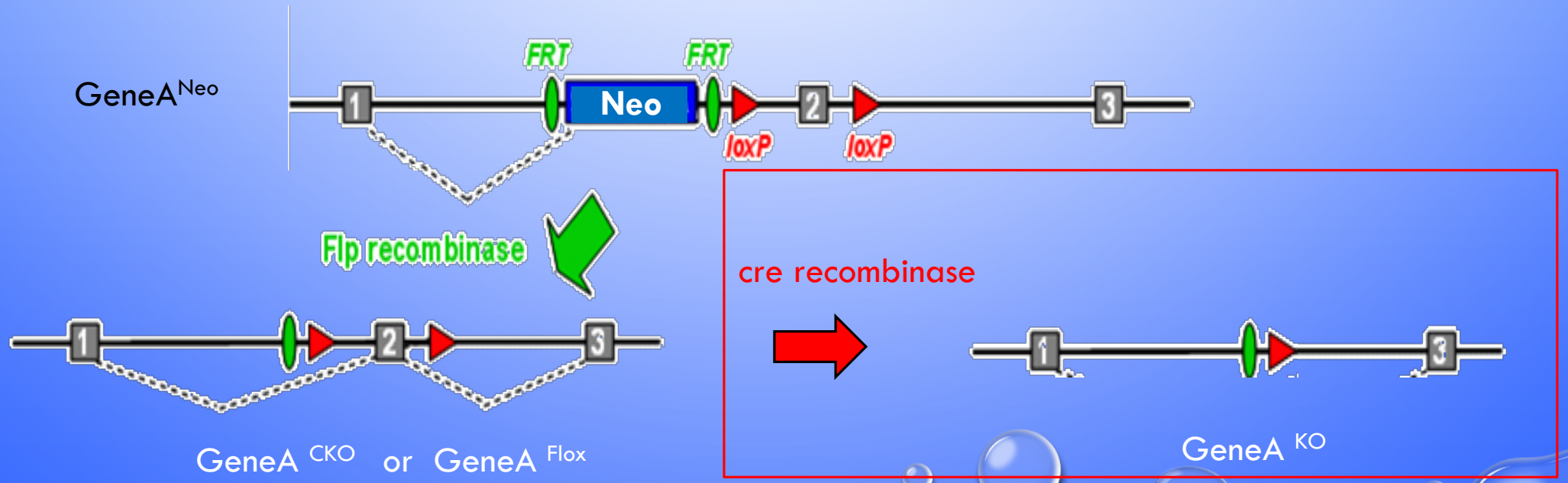


Targeted knockout Schematic. nobelprize.org (2017)

Site specific recombinase technology (SSR): advancement on gene targeting

Site specific recombinase technology (SSR)

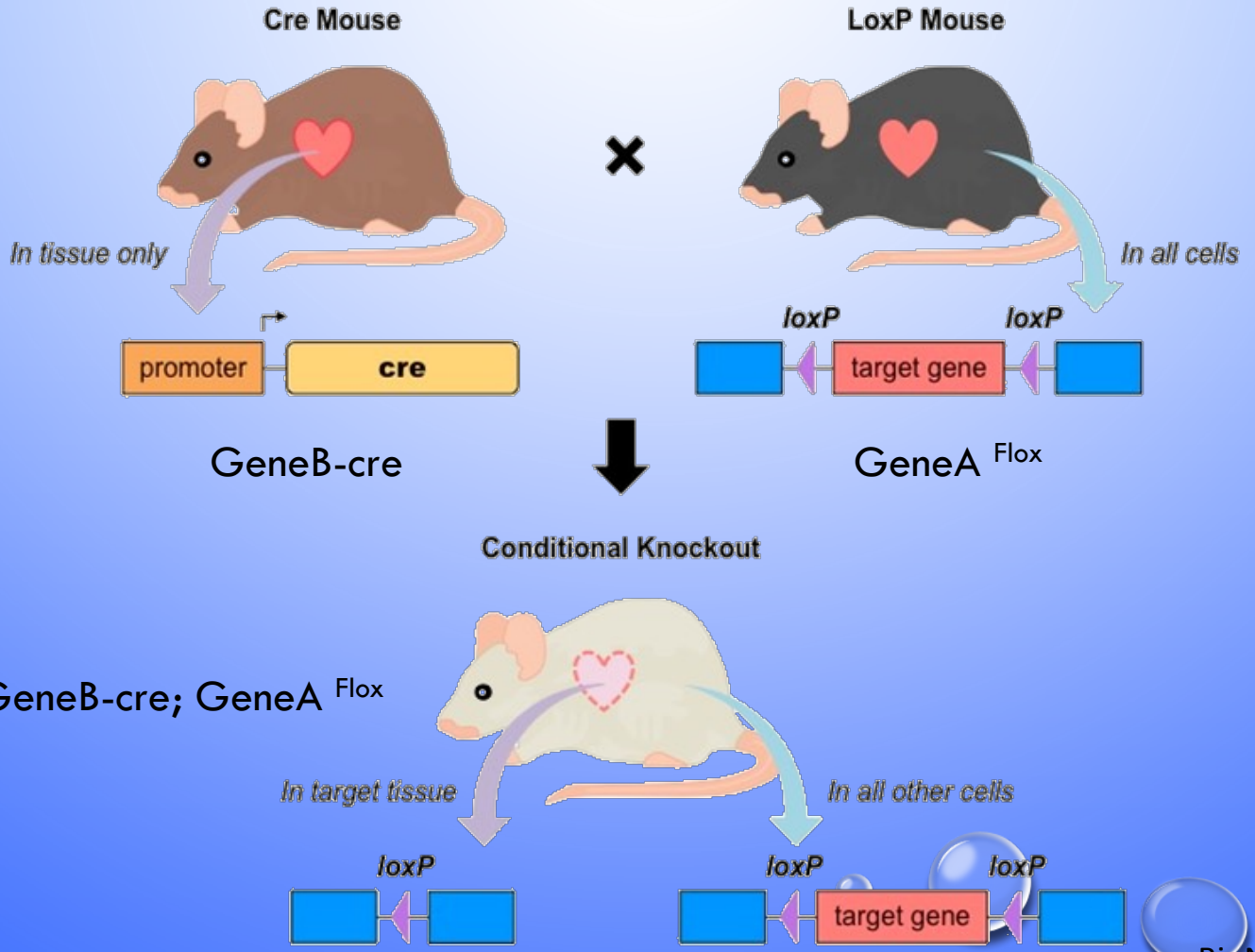
- The recombinases excise regions flanked by the respective sites.
  - Flp recombinase /FRT sites (yeast, Flp-recombinase target, 34bp)
  - Cre recombinase/ loxP sites (P1 bacteriaophage, 34bp)
  - Allowing selection marker removal
  - Allowing conditional knockout strategy (CKO)



Modified from KOMP repository

Conditional Knock-out Strategy

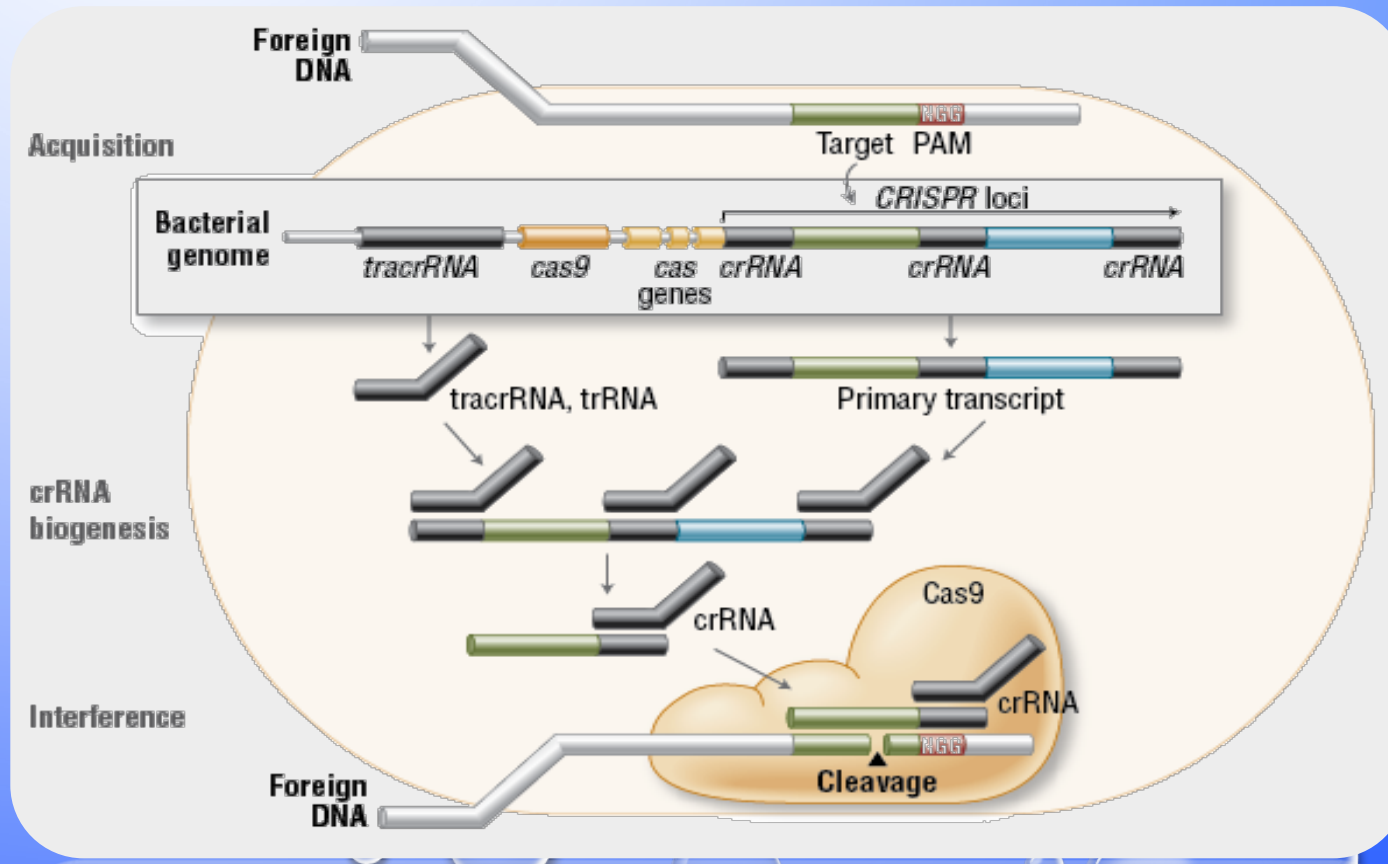
- Tissue/time-specific Knock-out



Gene targeted Mice
Generated by
2. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / CRISPR associated protein 9 (Cas9)

CRISPR/Cas9

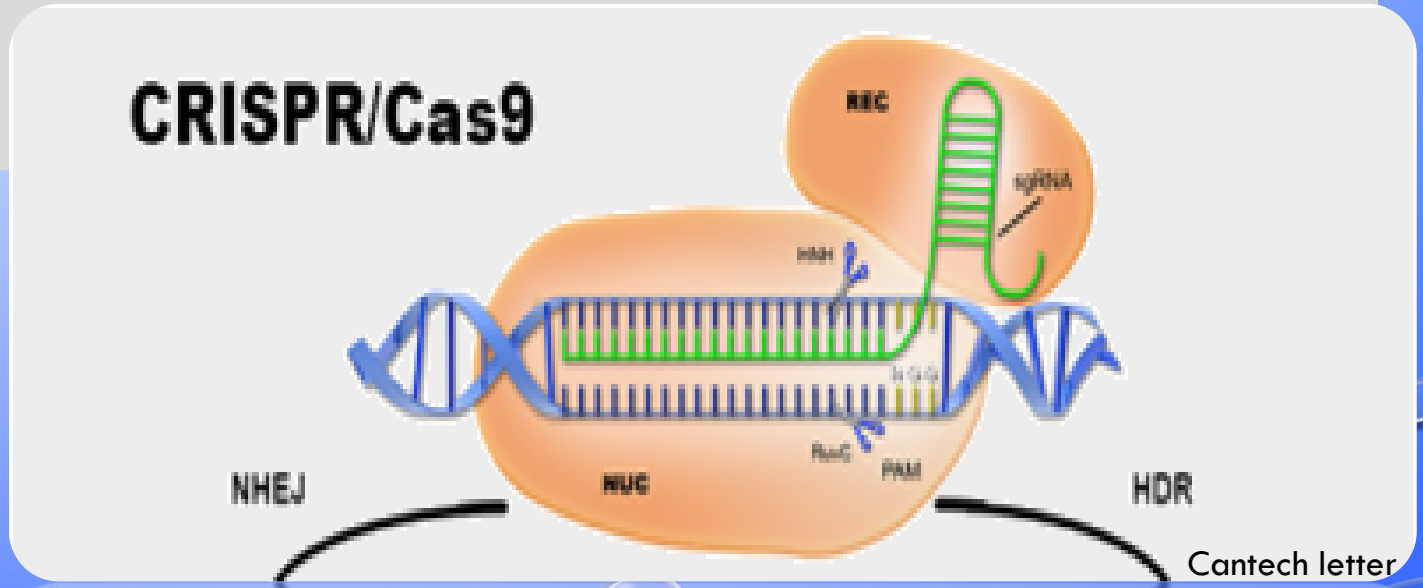
- Origin: *Streptococcus pyogenes*
- "Immune system" in response to viral infection
- Components:
  - Cas9 proteins
  - crRNA (with spacer)
  - tracrRNA



NEB

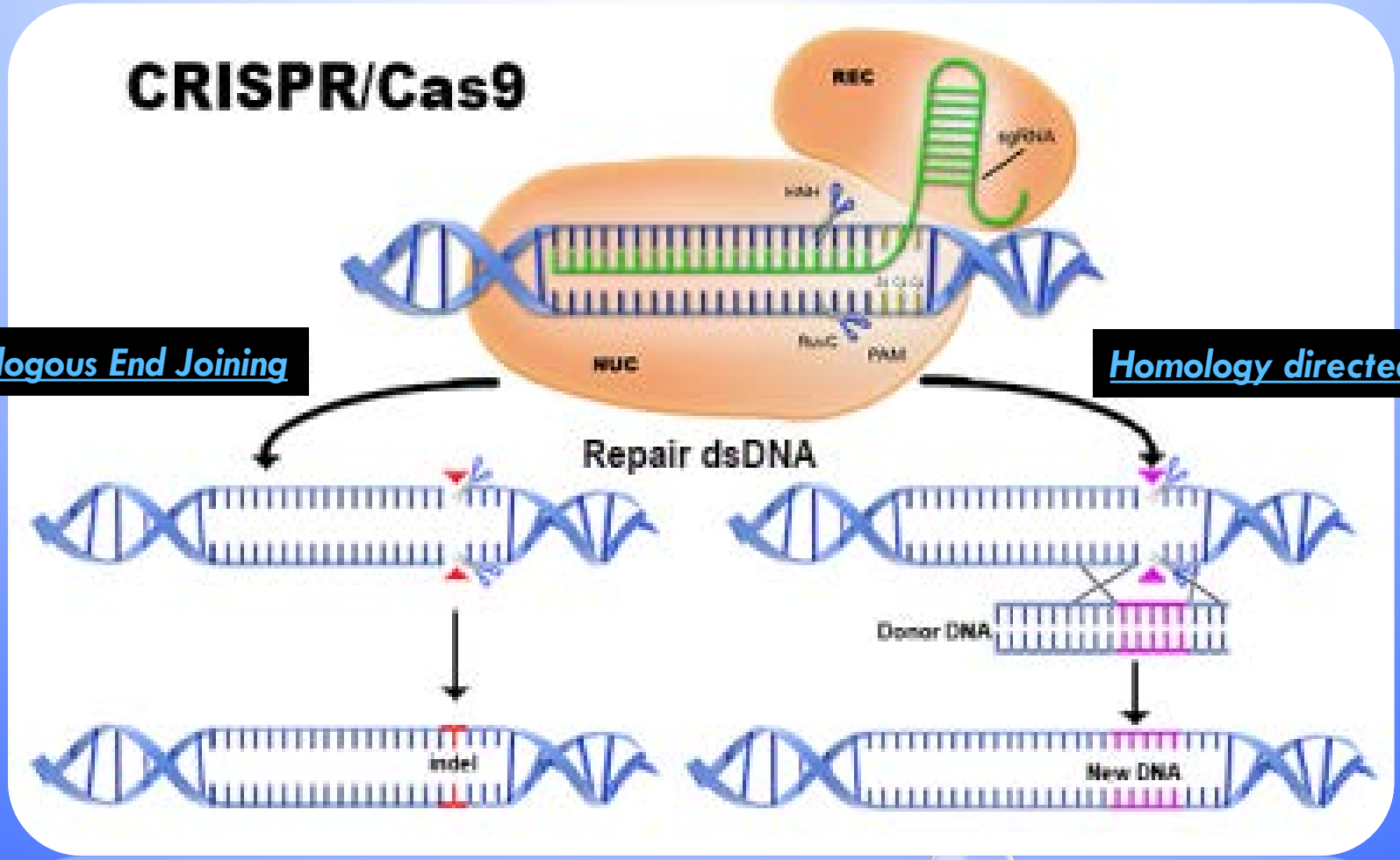
Application of CRISPR/Cas9 on Genome Editing

- Microinjection of components into the fertilized eggs
  - Cas9 mRNA
  - sgRNA (fusion of crRNA, tracrRNA sequence and a target sequence)
  - Donor DNA, if required
  - Video (time:1:50-3:15)



Cantech letter

Application of CRISPR/Cas9 on Genome Editing



Cantech letter

PAM: Protospacer adjacent motif

CRISPR/Cas9 components to be microinjected

Components	User Preparation	Procured tailor made
sgRNA	• cloning target sequence into a plasmid (for linking to cr-, tracrRNA sequence) • *in vitro* transcription	Commercial, synthetic
Cas9	mRNA, by *in vitro* transcription	mRNA/Protein (commercial)
Donor DNA	Long: DNA construction	Short: commercial, synthetic

CRISPR alternatives

Increase specificity

- eSpCas9 and SpCas9-HF: mutations minimizing complex-target binding energy → reduce non-specific binding
- SpCas9n: cutting one single strand; work by cutting two closely locating target sites by two complex → increase specificity

Increase choices of targets

- SpCas9-VQR: alternative PAM sequence (NGA G/T/A/C)
- SpCas9-EQR: alternative PAM sequence (NGAG)
- SpCas9-VRER: alternative PAM sequence (NGCG)
- SaCas9-KKH: alternative PAM sequence (NNN G/A G/A T)
- *Staphylococus aureus* SaCas9: PAM sequence (NNG G/A G/A T), gene size small enough for AAV virus gene delivery

CRISPR/CPf1 (Cas12a):

- PAM sequence (TTTN)
- no tracrRNA is involved (43nt RNA vs ~100nt RNA)

THANK YOU!