ANAT2341 – Lab 4

The mouse as model for human disease

Why mice?

Gene and protein expression analysis methods

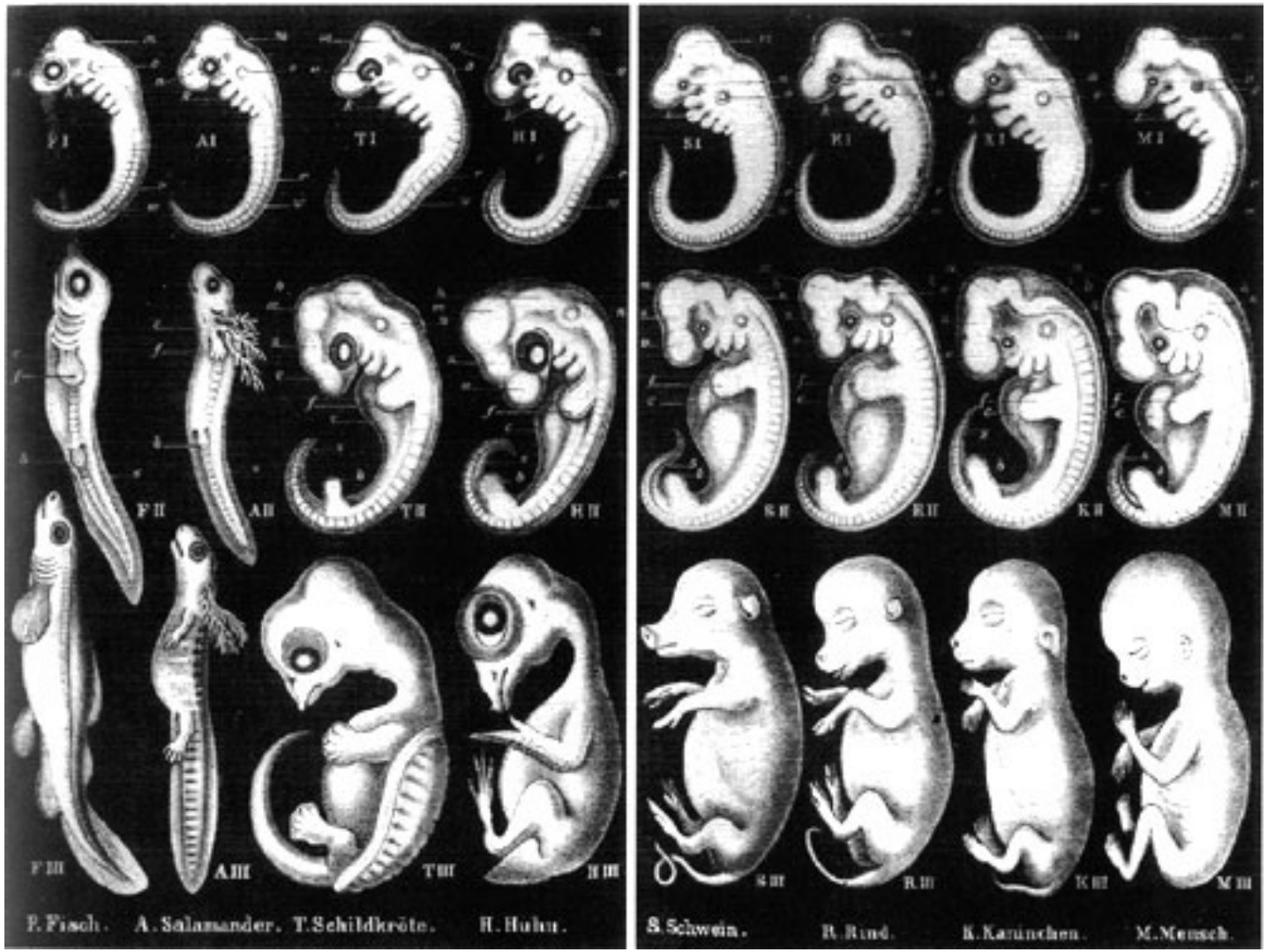
Methods to study gene function in mice *in vivo*

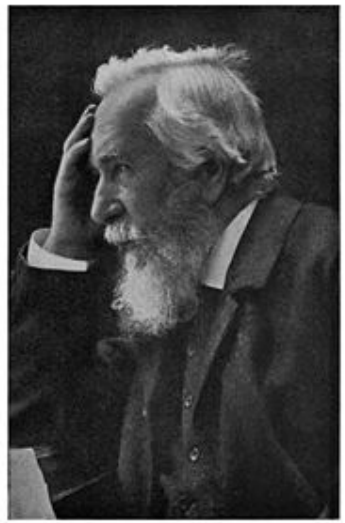
Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

Why is the mouse used as an experimental animal in developmental biology?

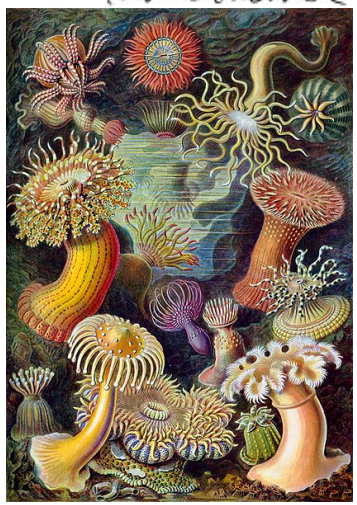
- Small, cheap to house and feed, breed quickly.
- Embryology resembles that of humans.
- Genetically similar to humans.
- Fully sequenced genome.
- Amenable to genetic manipulation.

Mouse and human embryology are very similar

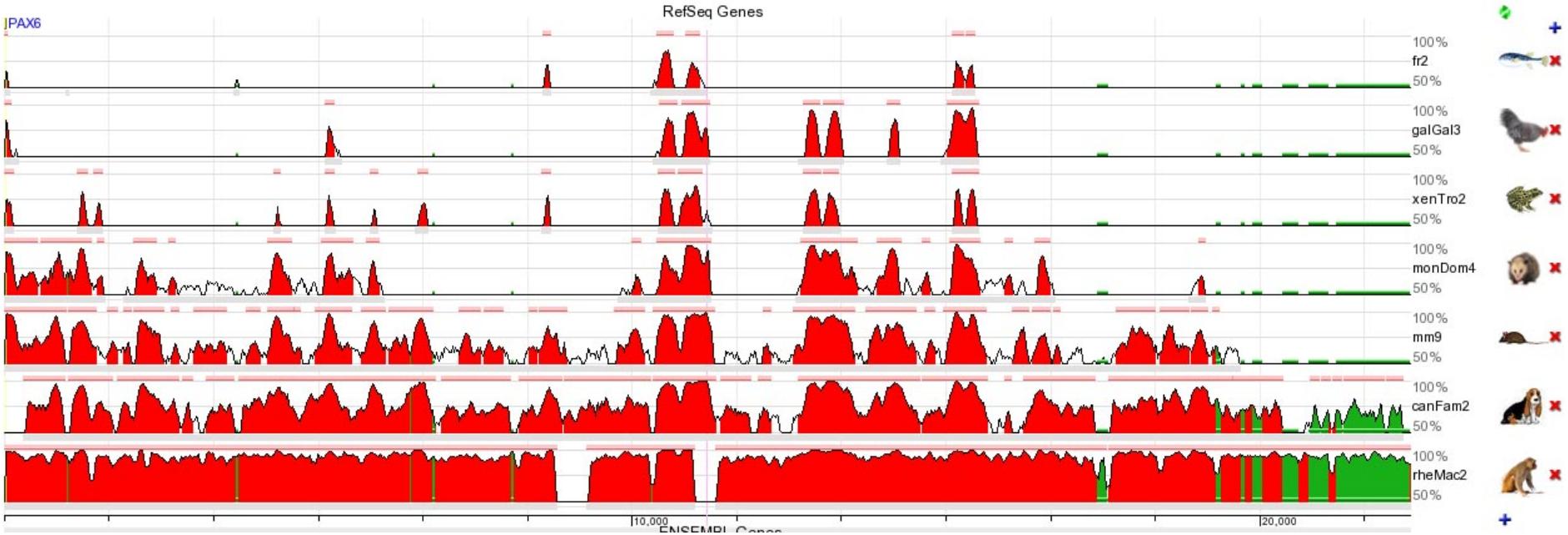




Ernst Haeckel



Mice are genetically similar to humans



Sequence homology not as good as monkeys, but much better than chick, fish, frogs

Mouse genome is fully sequenced

Genome sequence of the mouse was complete in 2002
C57Bl
There are about 23.000 mouse genes
99% of mouse genes have human orthologues

Understand human biology and disease

Gene hunting:
Identification of mutated genes in diseases
'*In silico*' gene identification

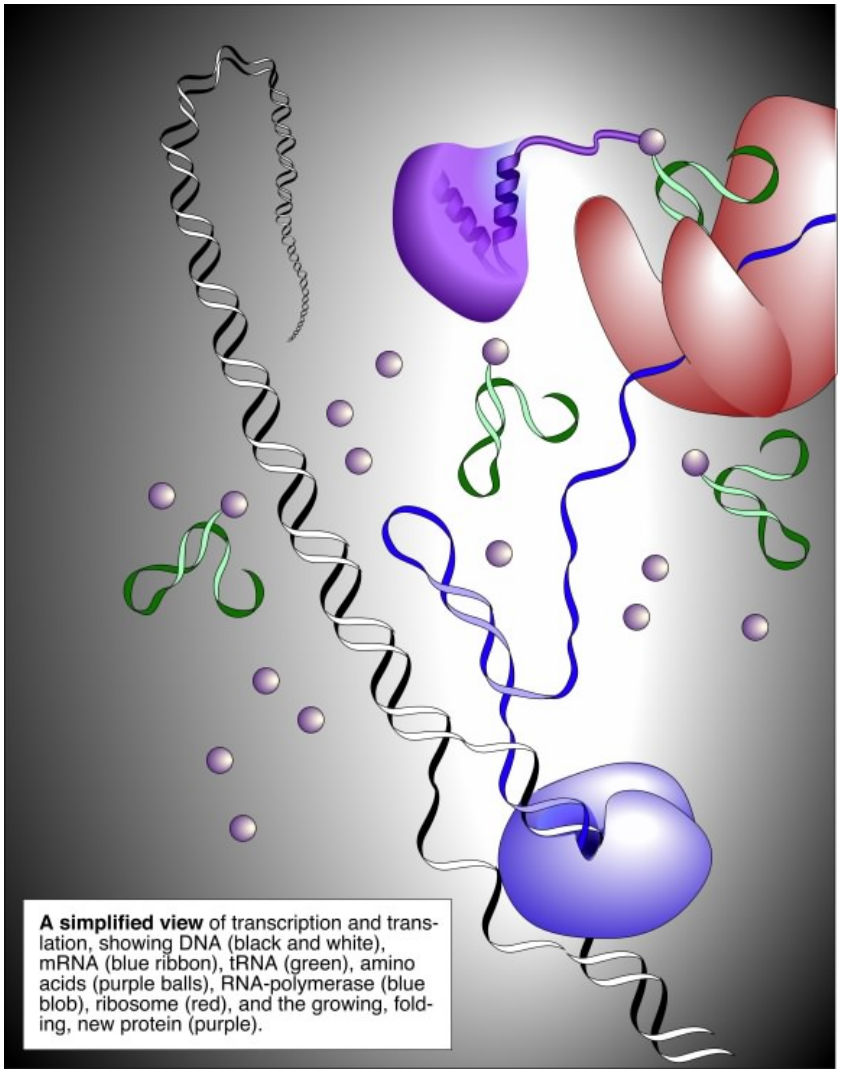
Mouse amenable to genetic manipulation

Mouse gene loss and gain-of-function studies

What can we do with a mouse to address developmental biology questions?

1. Gene and protein expression analyses
2. Gene function analyses

Gene and protein expression analysis methods



A simplified view of transcription and translation, showing DNA (black and white), mRNA (blue ribbon), tRNA (green), amino acids (purple balls), RNA-polymerase (blue blob), ribosome (red), and the growing, folding, new protein (purple).

Gene expression:

DNA

Transcription ↓

mRNA

Translation ↓

Proteins

↓

Function

Gene and protein expression analysis methods

Detection of **protein** expression:

-Immunodetection

-Proteomics

Detection of **RNA** expression:

- RTPCR

- Quantitative RT PCR

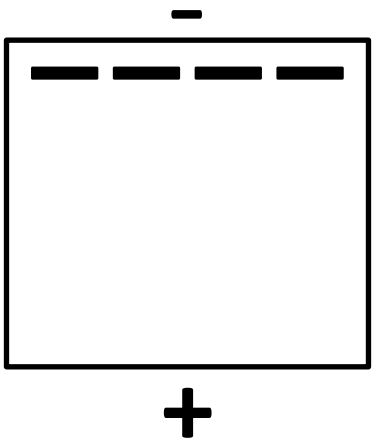
-In situ hybridization

-Microarrays

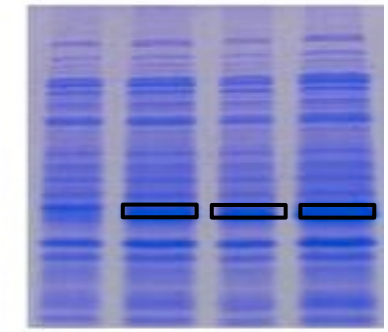
Protein expression analyses

Immunodetection

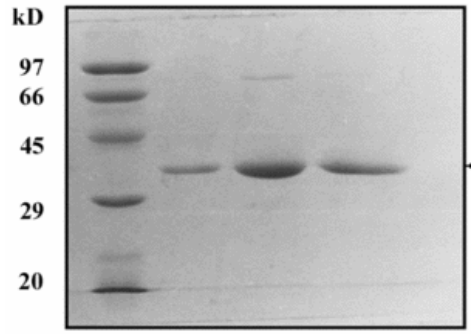
PAGE and Western Blotting



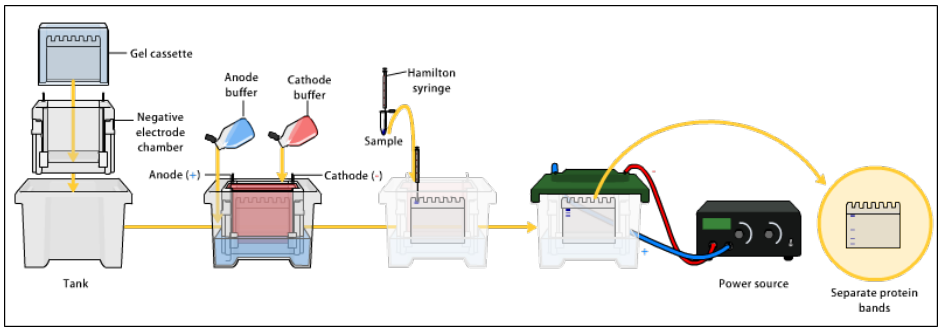
Electrophoresis



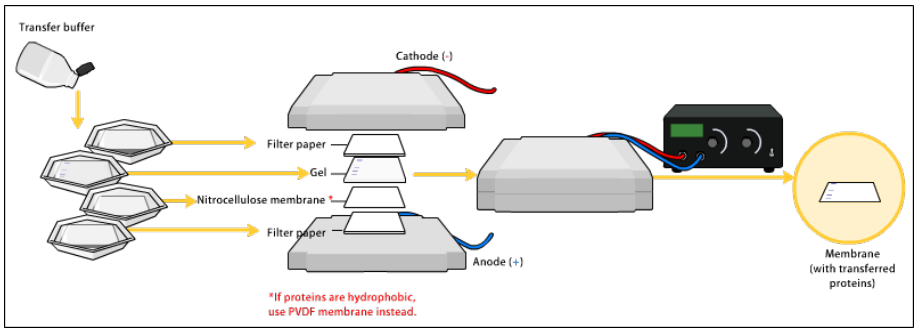
Electroblotting
immunodetection



Electrophoresis



Electroblotting



Protein expression analyses

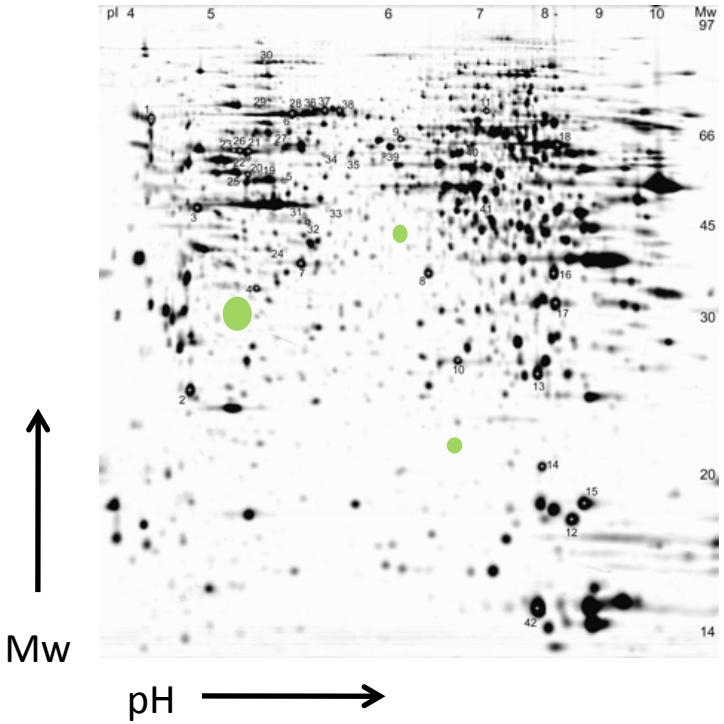
Immunodetection

Proteomics

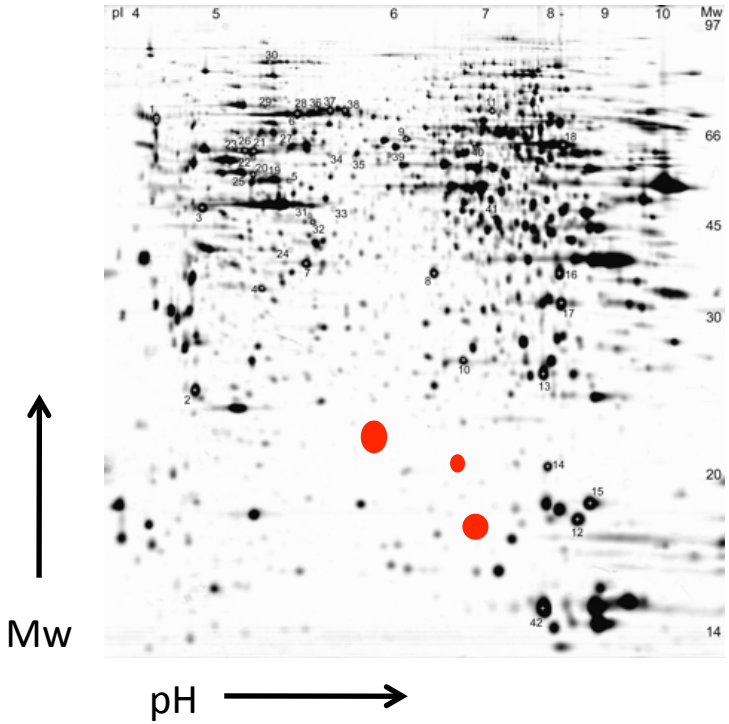
Large scale study of protein expression

Compare proteomes in biological samples using 2D gels

Control sample



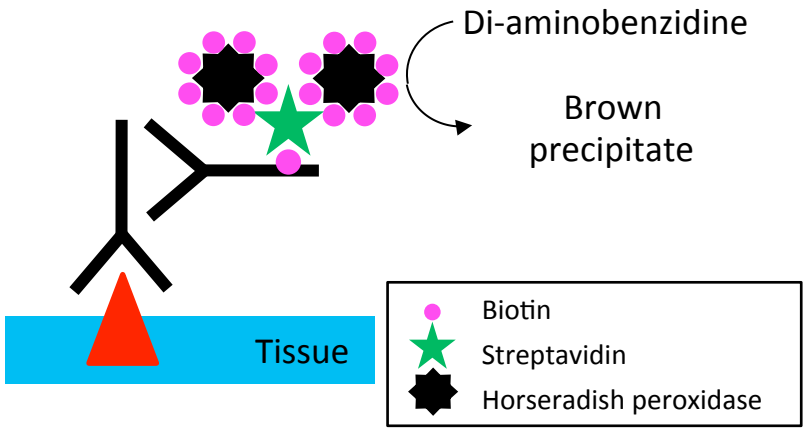
Test sample

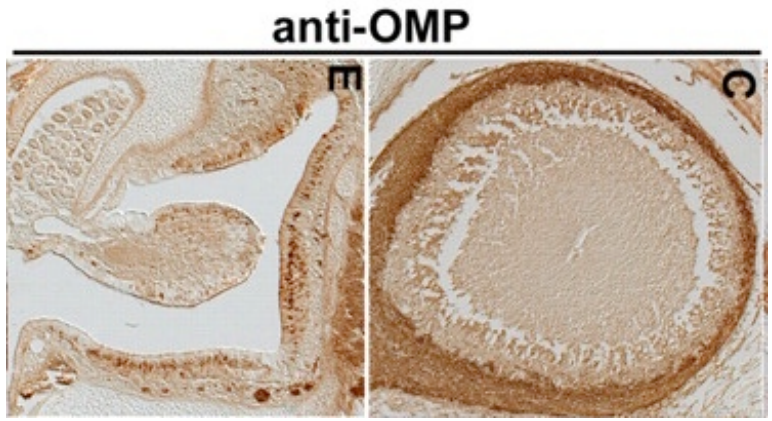


Protein expression analyses

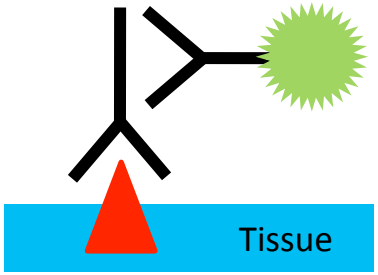
Immunodetection: IHC and IF

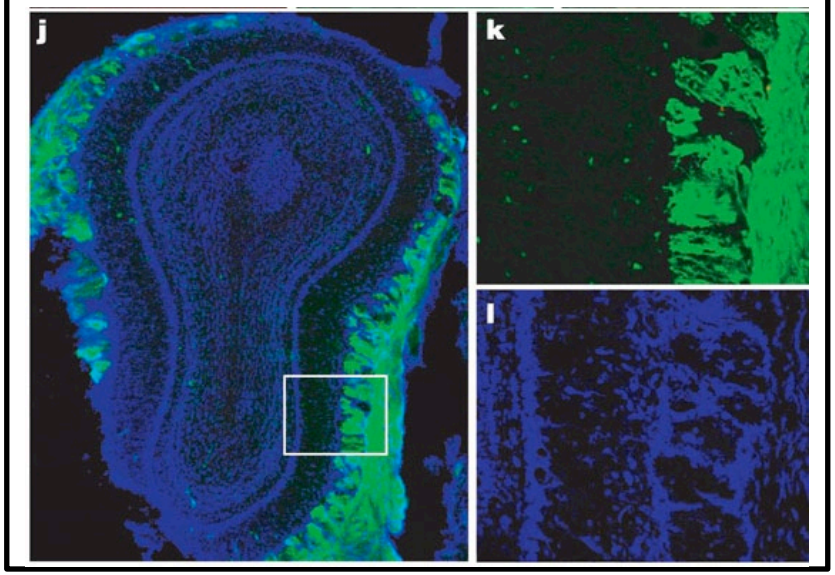
Immunohistochemistry





Immunofluorescence

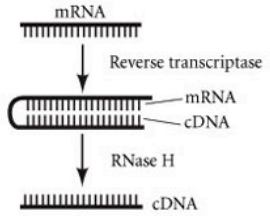




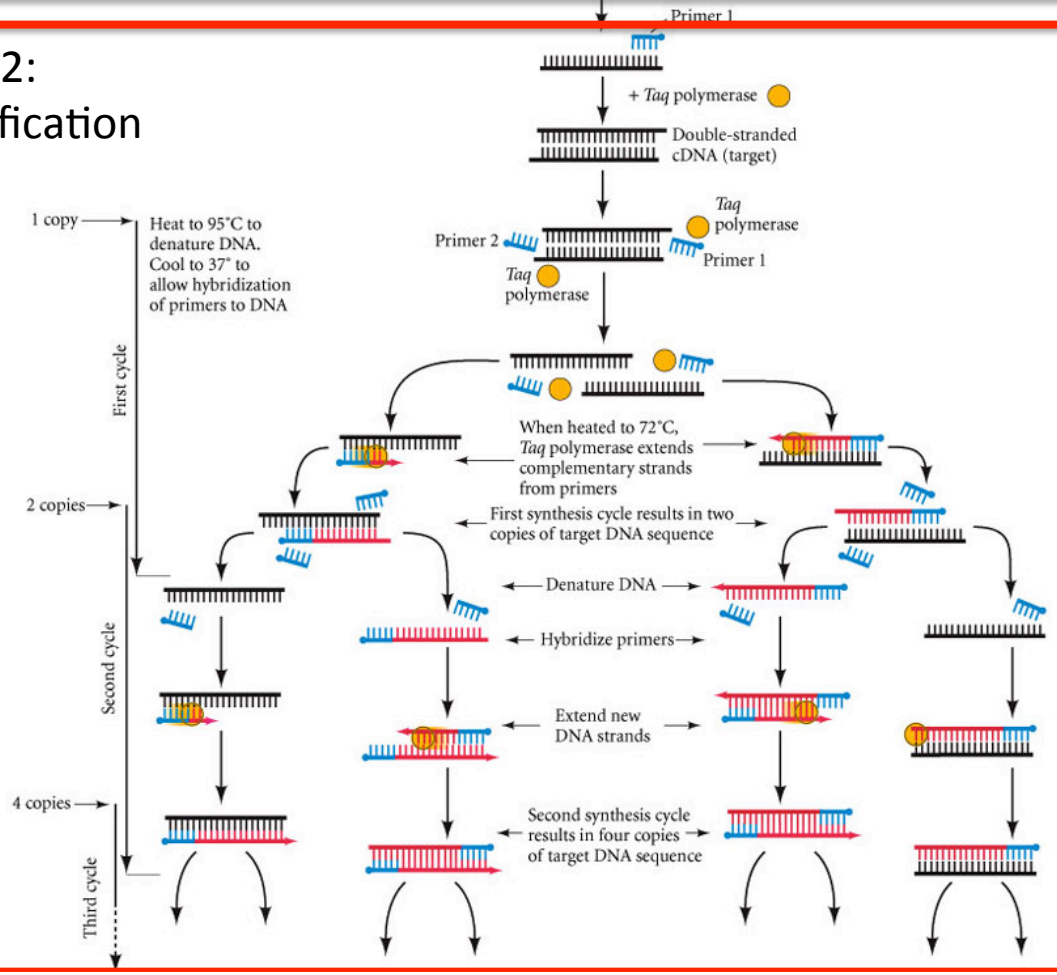
Gene expression analyses

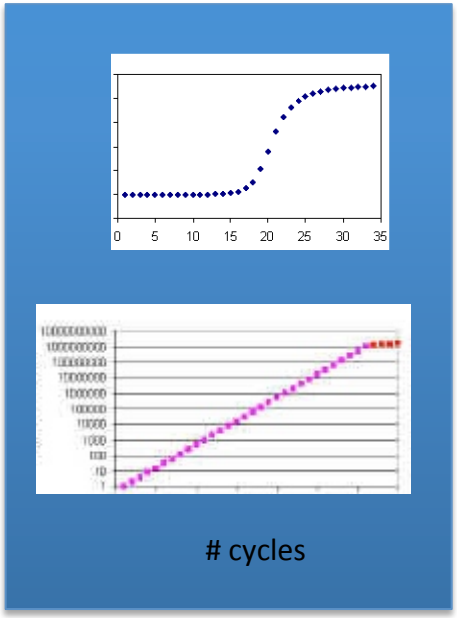
RT PCR

Step 1
Reverse transcription,
Generation of cDNA



Step 2:
PCR amplification

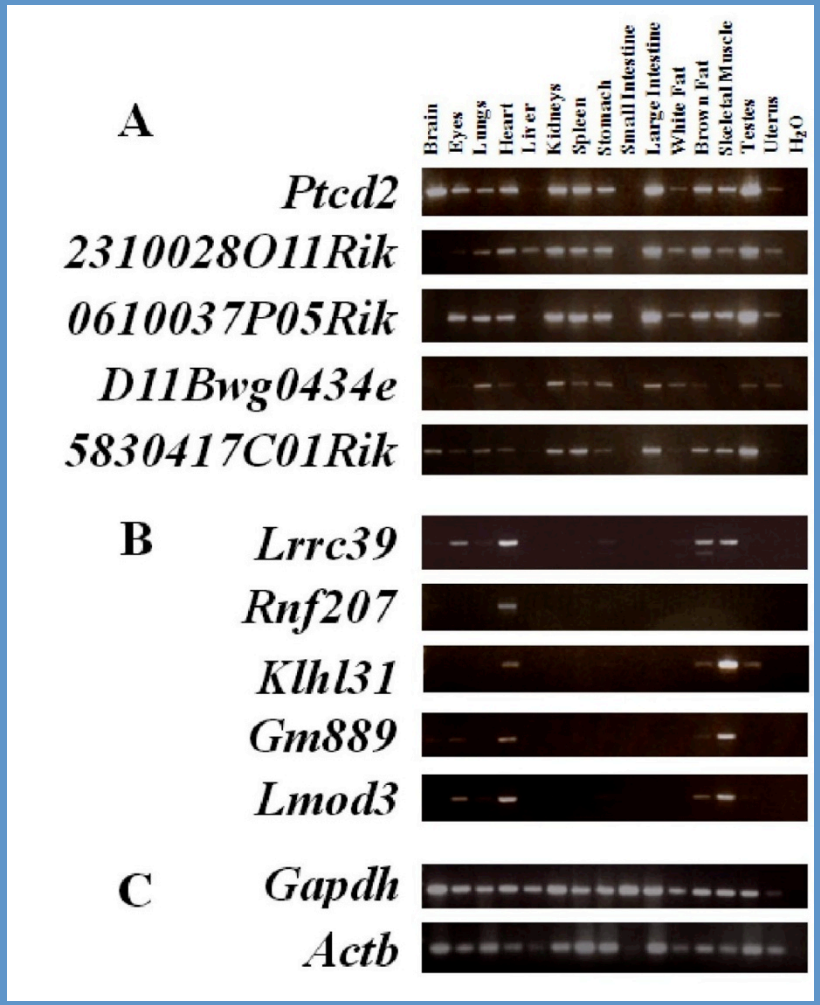




Gene expression analyses

RT PCR

Example experimental results

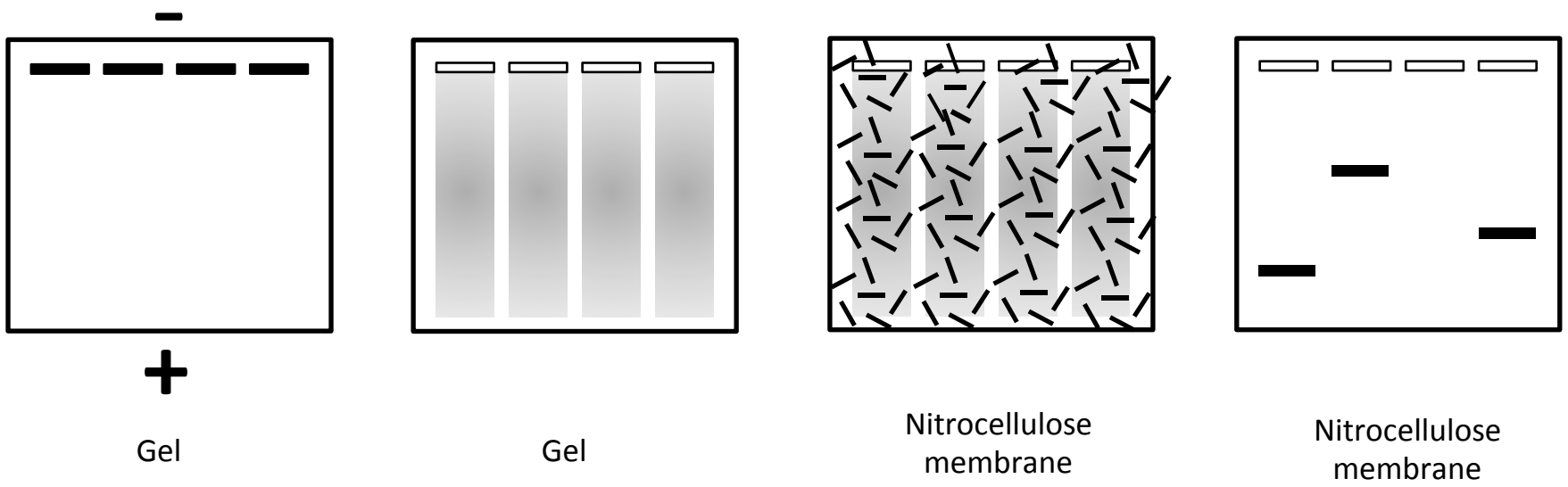


Gene expression analyses

Northern Blotting

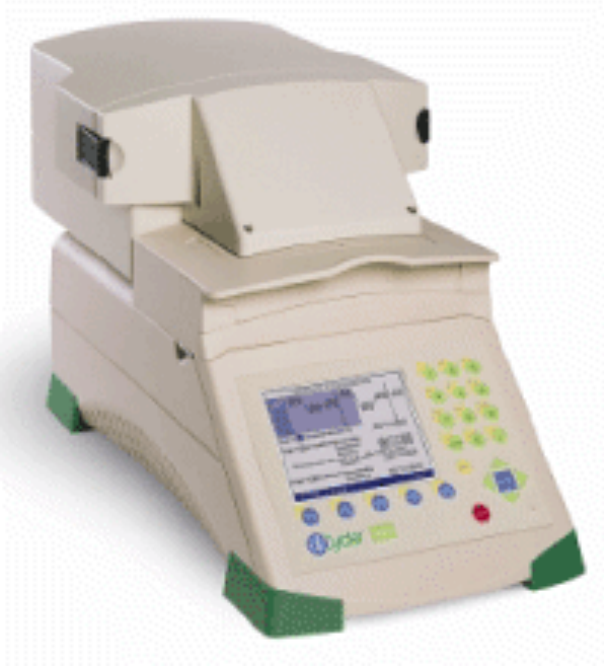
Northern blotting (RNA blotting):

- Isolate RNA from tissue of interest
- Run RNA on denaturing gel and blot onto nitrocellulose membrane
- Make radioactively labeled antisense cDNA probe
- Hybridize cDNA probe to RNA on membrane
- Visualize probe binding using photographic film



Gene expression analyses
quantitative real time RT PCR

- Used for
  - Quantitative gene expression (both relative and absolute),
  - Genotyping,
  - miRNA analysis
  - SNP analysis,
  - Pathogen detection
- Measures PCR amplification as it occurs
- More sensitive than conventional RTPCR



Gene expression analyses
quantitative real time RT PCR

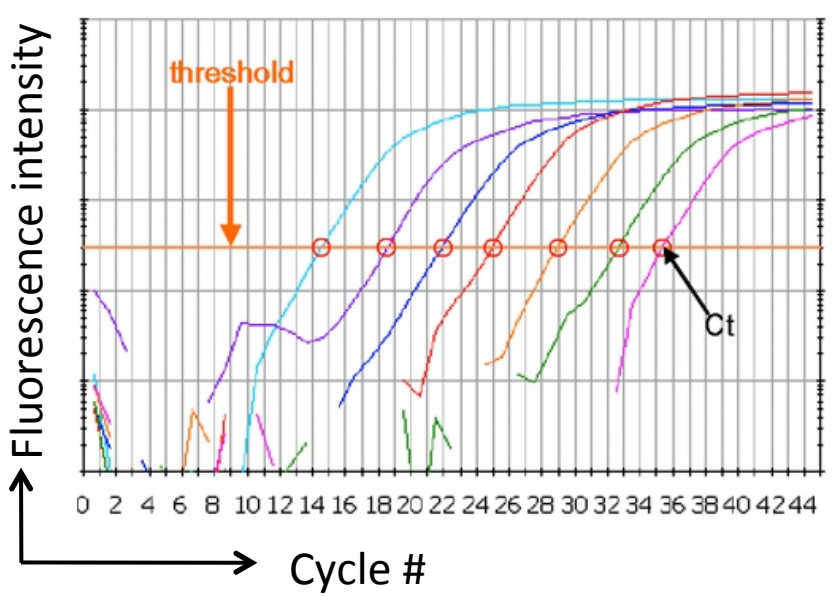
Method:

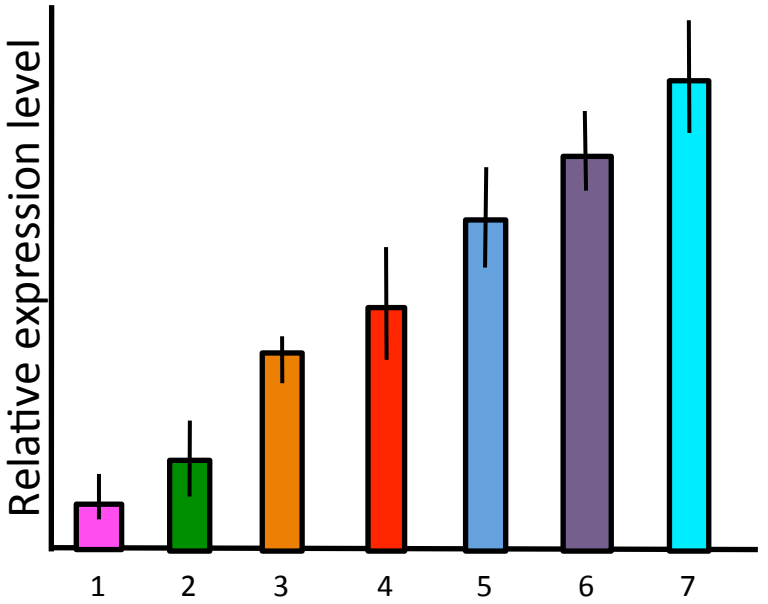
- Isolate RNA
- Make cDNA with reverse transcriptase
- Carry out PCR with primers to amplify genes of interest and intercalating fluorescent dye SYBR Green or Taqman probes
- Detect fluorescent signal during linear amplification phase as measure for amount of PCR product made

Gene expression analyses

quantitative real time RT PCR

Fluorescent signal intensity (Ct) is measure for amount of product



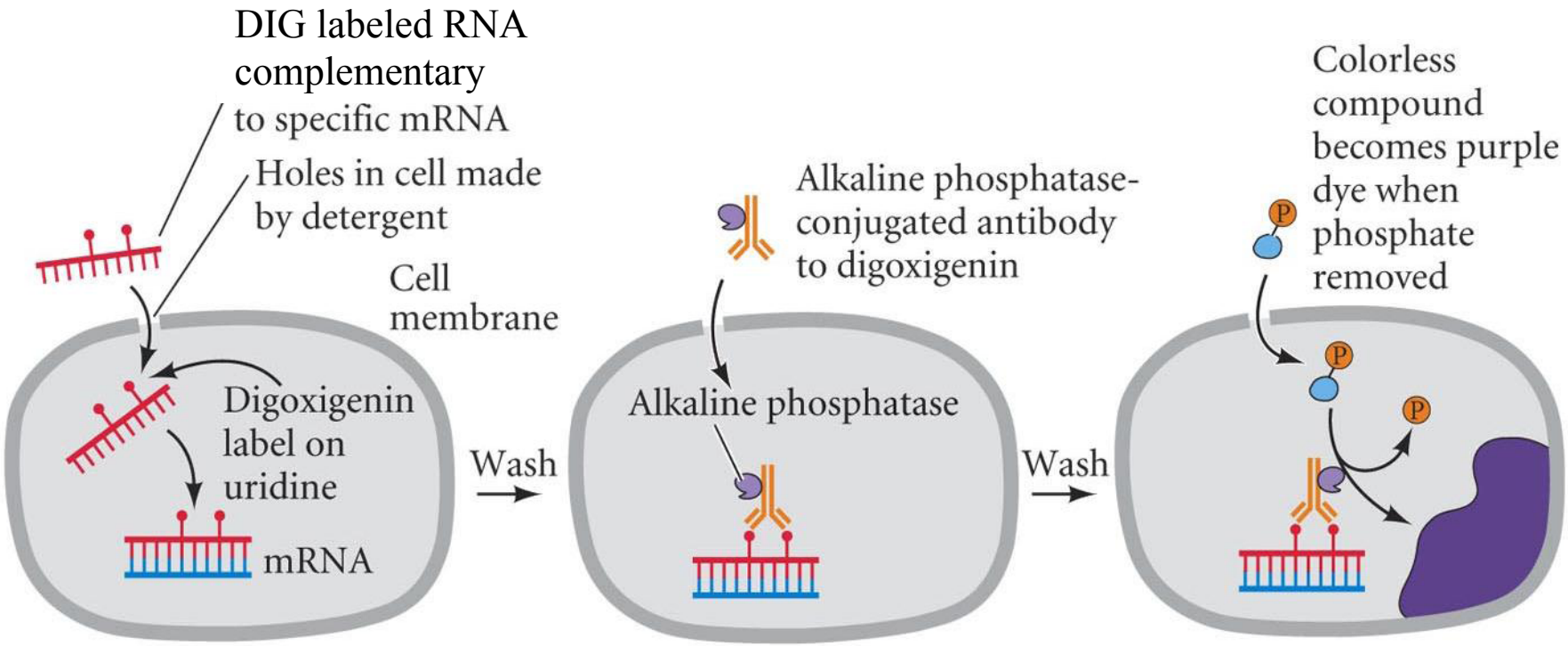


- Quantification of expression levels of:
  - different genes within one RNA sample
  - the same gene in different samples (against a reference ‘house hold gene’)
- Relative vs absolute quantification (against a known standard)

Gene expression analyses

in situ hybridization

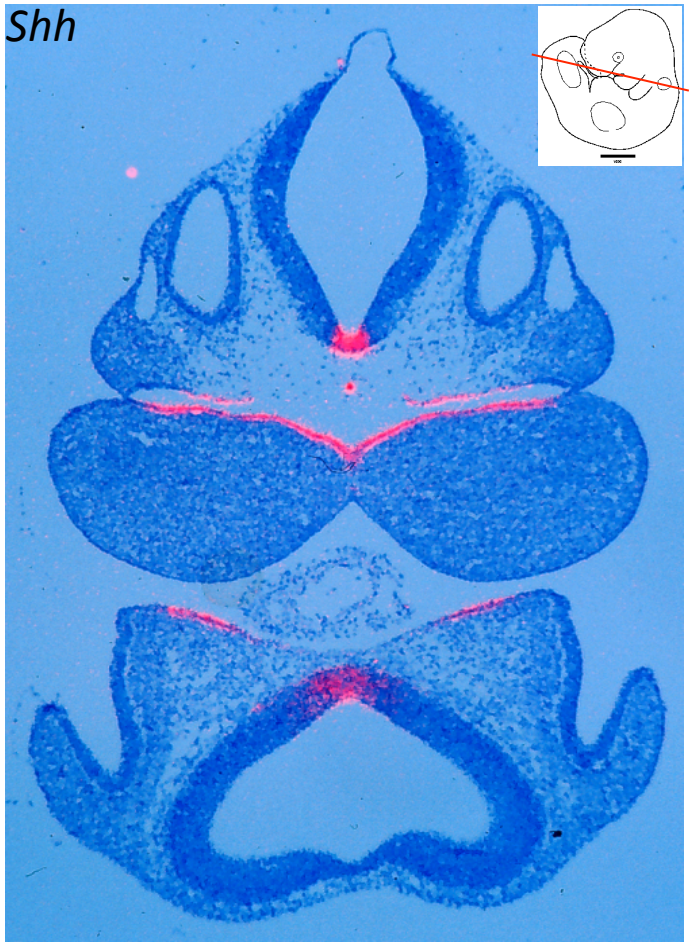
Prepare labeled antisense RNA probe:
- Digoxigenin (DIG)
- Radioactive label



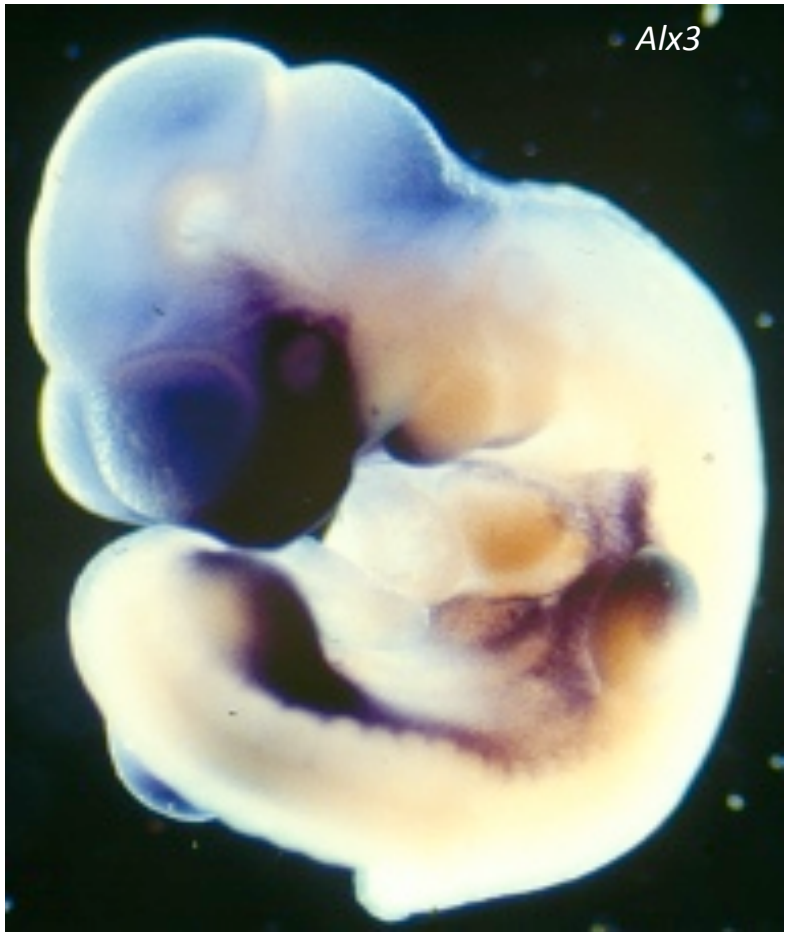
Gene expression analyses

in situ hybridization

On sections:



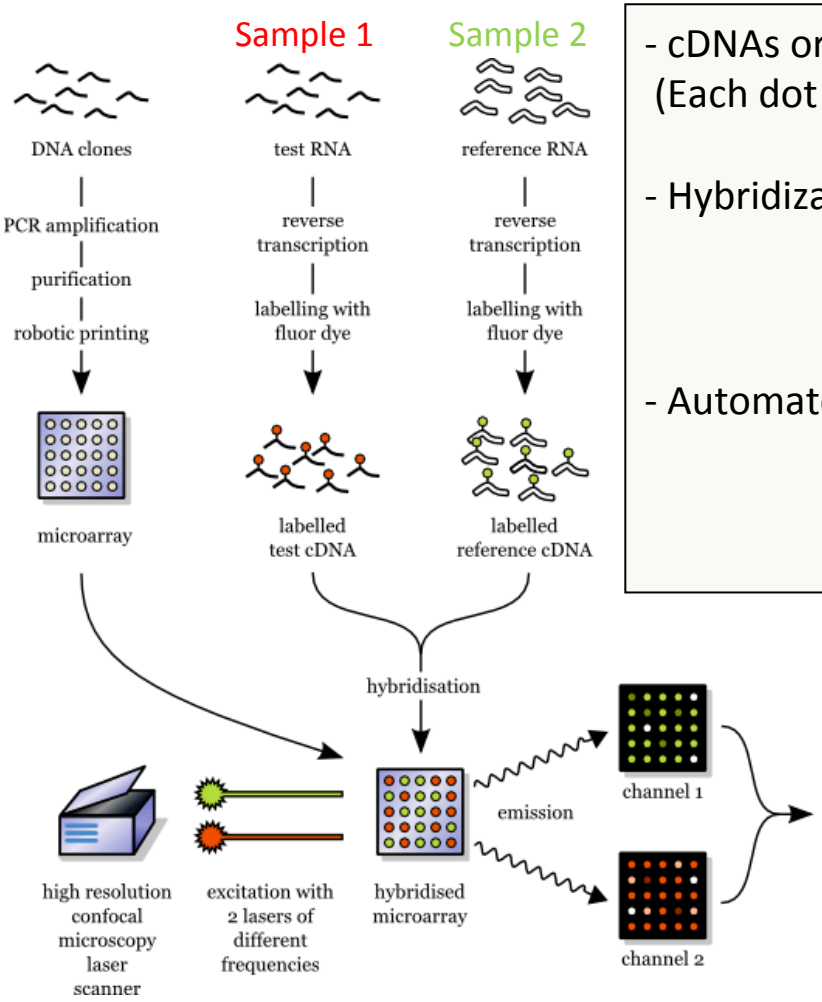
On whole embryos/whole tissues:



Gene expression analyses

Expression microarrays

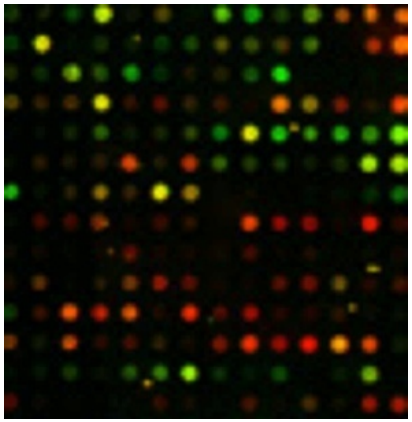
Compare the expression levels of thousands of genes between samples



- cDNAs or gene-specific oligos spotted onto glass slides (arrays).
  (Each dot represents 1 gene)

- Hybridization with 2 fluorescently labeled probes:
  - Probe 1: sample 1 labeled with red label
  - Probe 2: sample 2 labeled with green label

- Automated signal quantification:
  - Red signal: gene only expressed in sample 1
  - Green signal: gene only expressed in sample 2
  - Yellow signal: gene expressed in both sample 1 and 2



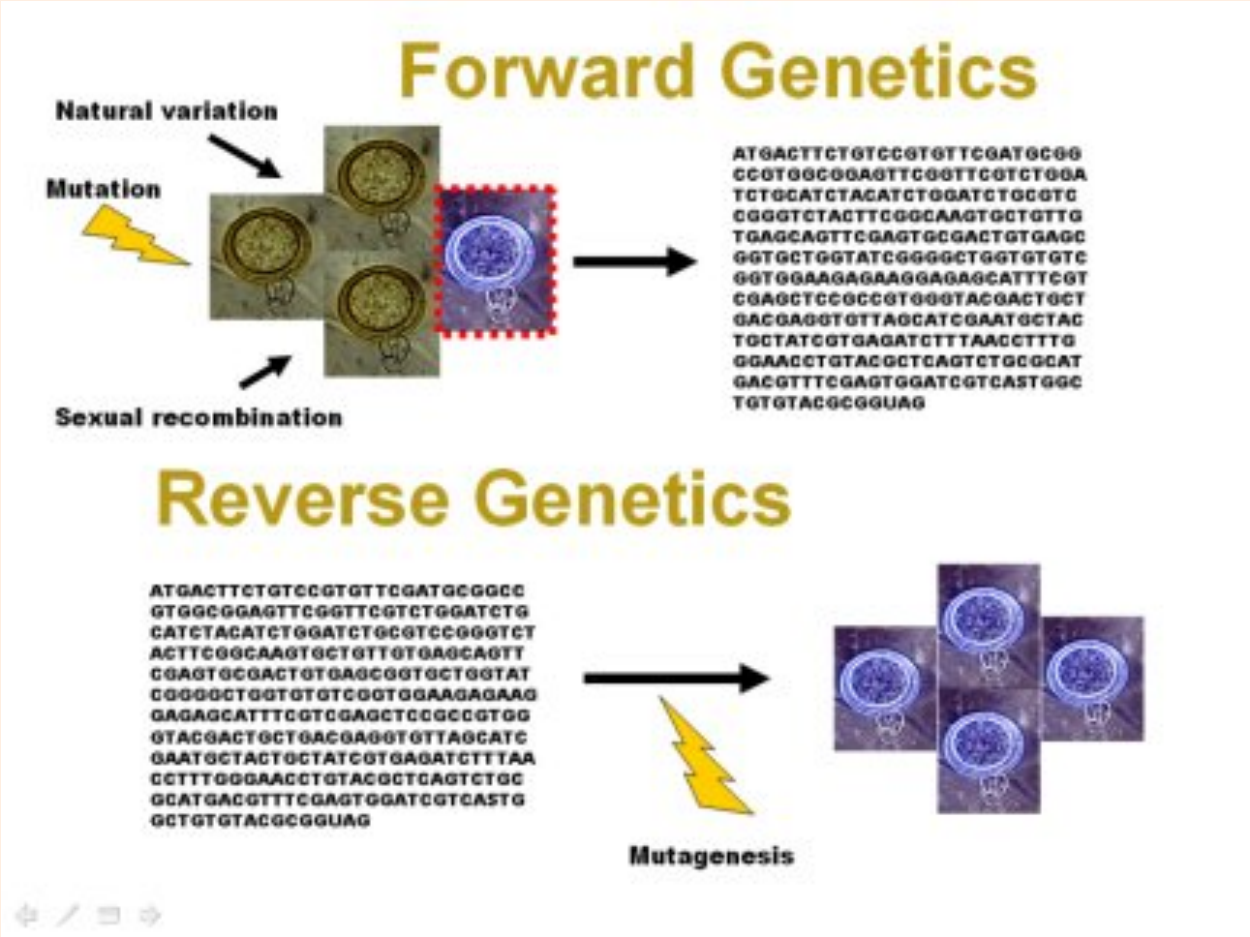
Expression analyses

overview

Method	Detection of	Quantitative	Spatial information	Results within
Protein gel Western blot	Protein	Limited	No/Little	2 Days
IHC/IF	Protein	Limited	Yes	2 Days
Proteomics 2D protein gels	Proteome	Yes	No/Little	Depends
RT PCR	RNA	No	No/Little	1 Day
Real Time PCR	RNA	Yes	No/Little	1 Day
Northern blotting	RNA	Limited	No/Little	Few days
Section *in situ* hybridization	RNA	Limited	Yes	Few weeks
Whole mount *in situ* hybridization	RNA	Limited	Yes	1 Week
Expression microarray	Transcriptome	Yes	No/Little	Depends

Methods of studying gene function

Forward genetics: phenotype -> gene
Reverse genetics: gene -> phenotype



(Random) gene mutagenesis
Caenorhabditis elegans
Drosophila melanogaster
Zebrafish
(Mouse)

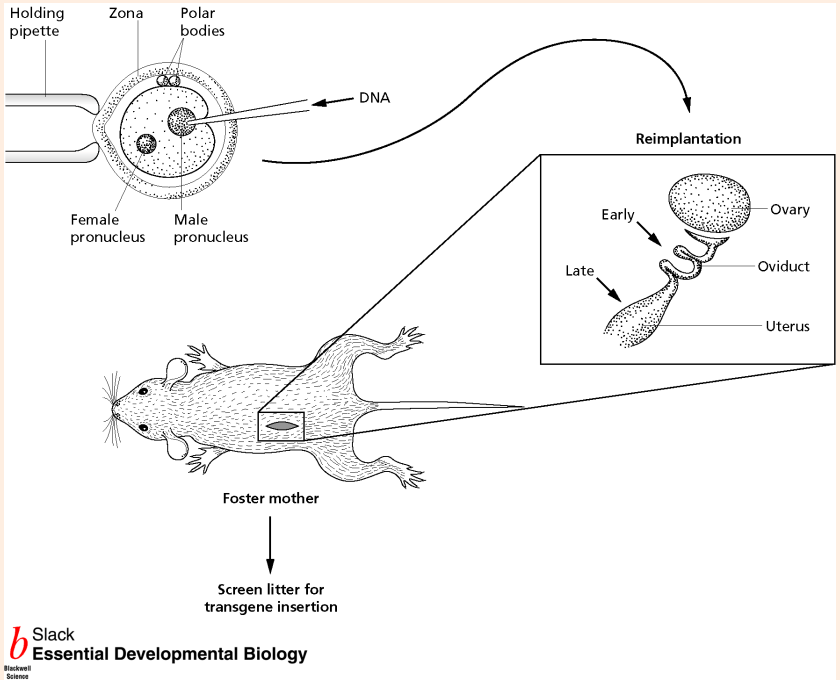
Gene gain- or loss-of-function
Caenorhabditis elegans
Drosophila melanogaster
Zebrafish
Mouse

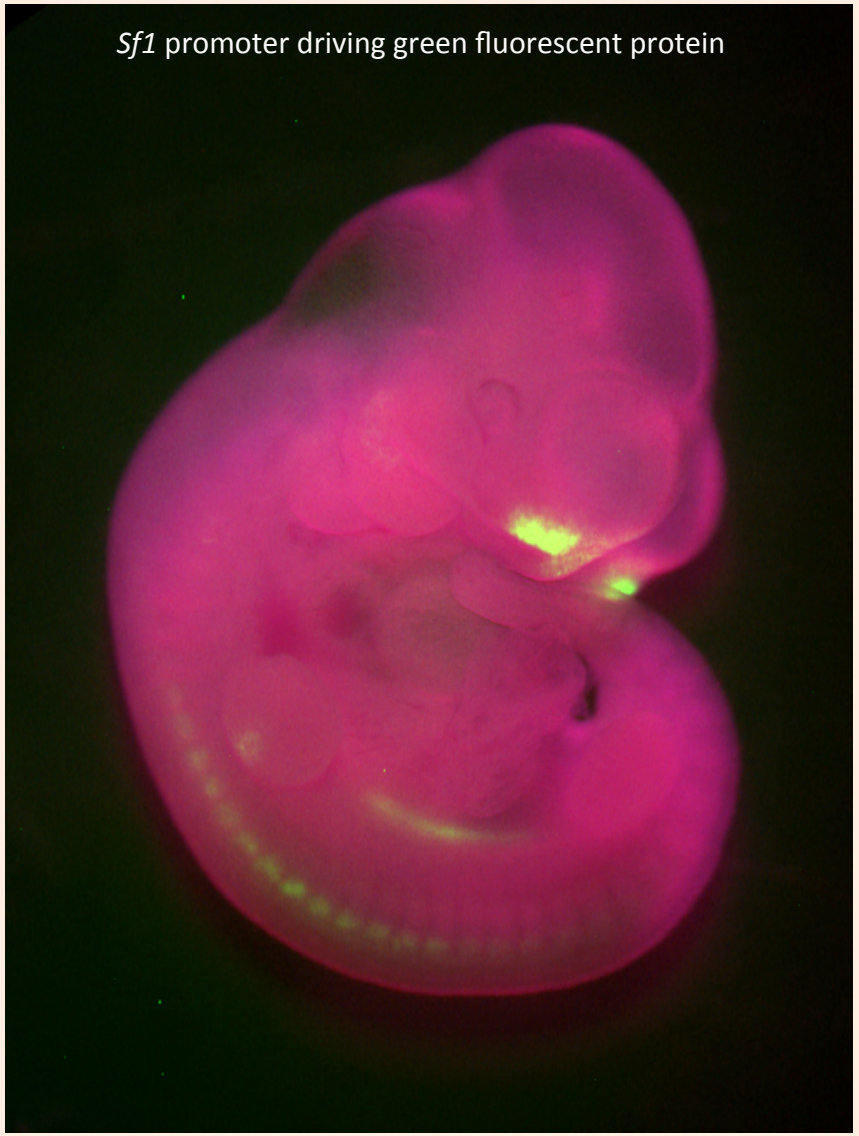
Gain-of-function transgenesis

Generation of Transgenic mice:

1. Generate transgenic construct

   NB! promoter + cDNA
2. Inject transgene into zygotes
3. Transgene is integrated into genome
4. Transfer zygotes to pseudopregnant mouse
5. Transgene is expressed by mouse (embryo)

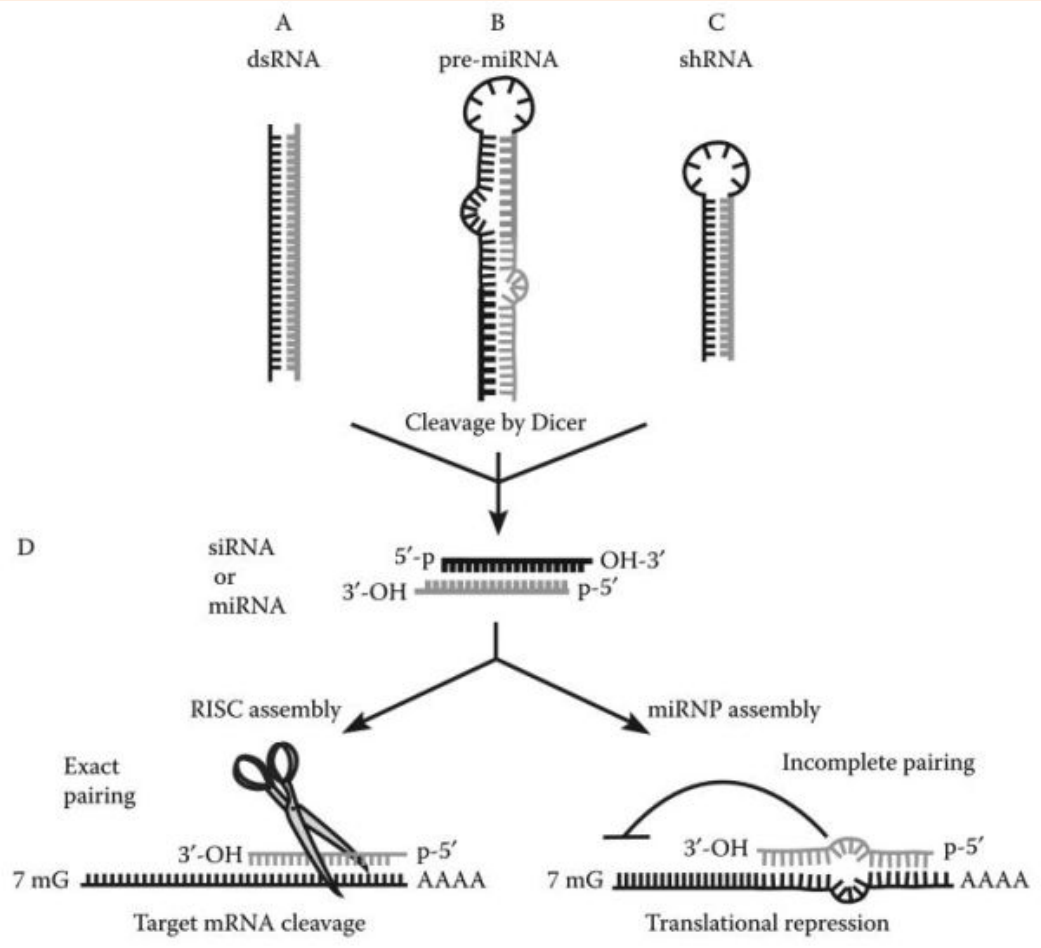


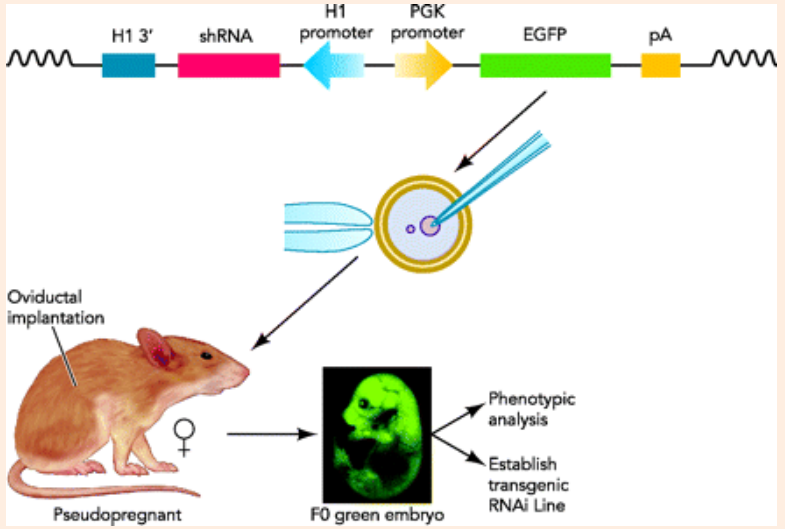


Loss-of-function transgenesis

RNA interference

Overexpression of short hairpin RNAs (shRNA) that silence genes of choice

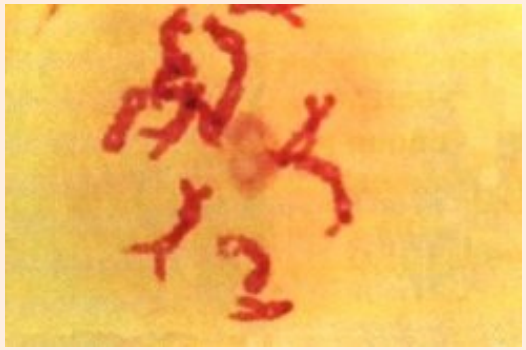


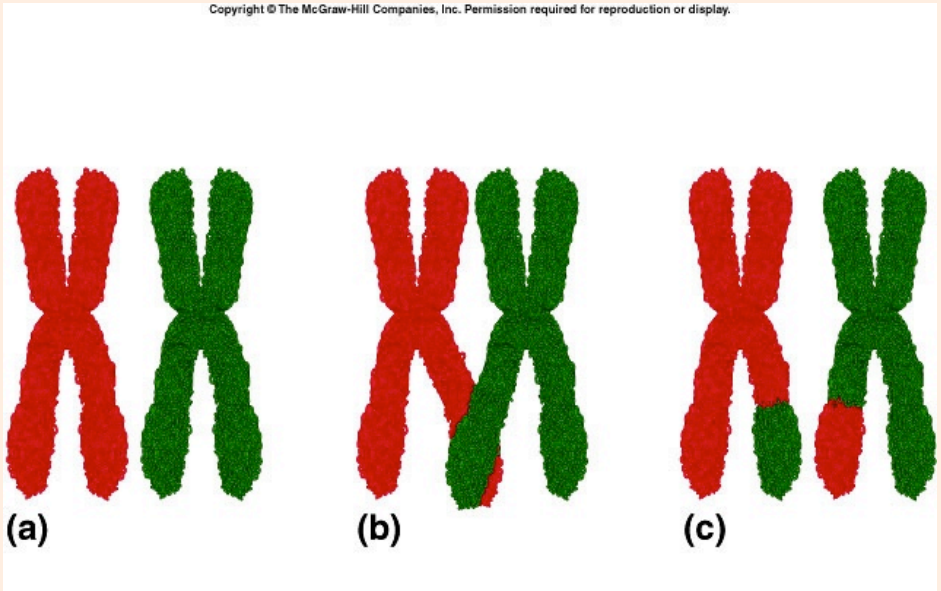


Knock out technology

Crossing over is a natural process that happens during meiosis

Knock out technology =
directed homologous recombination in omnipotent ES cells





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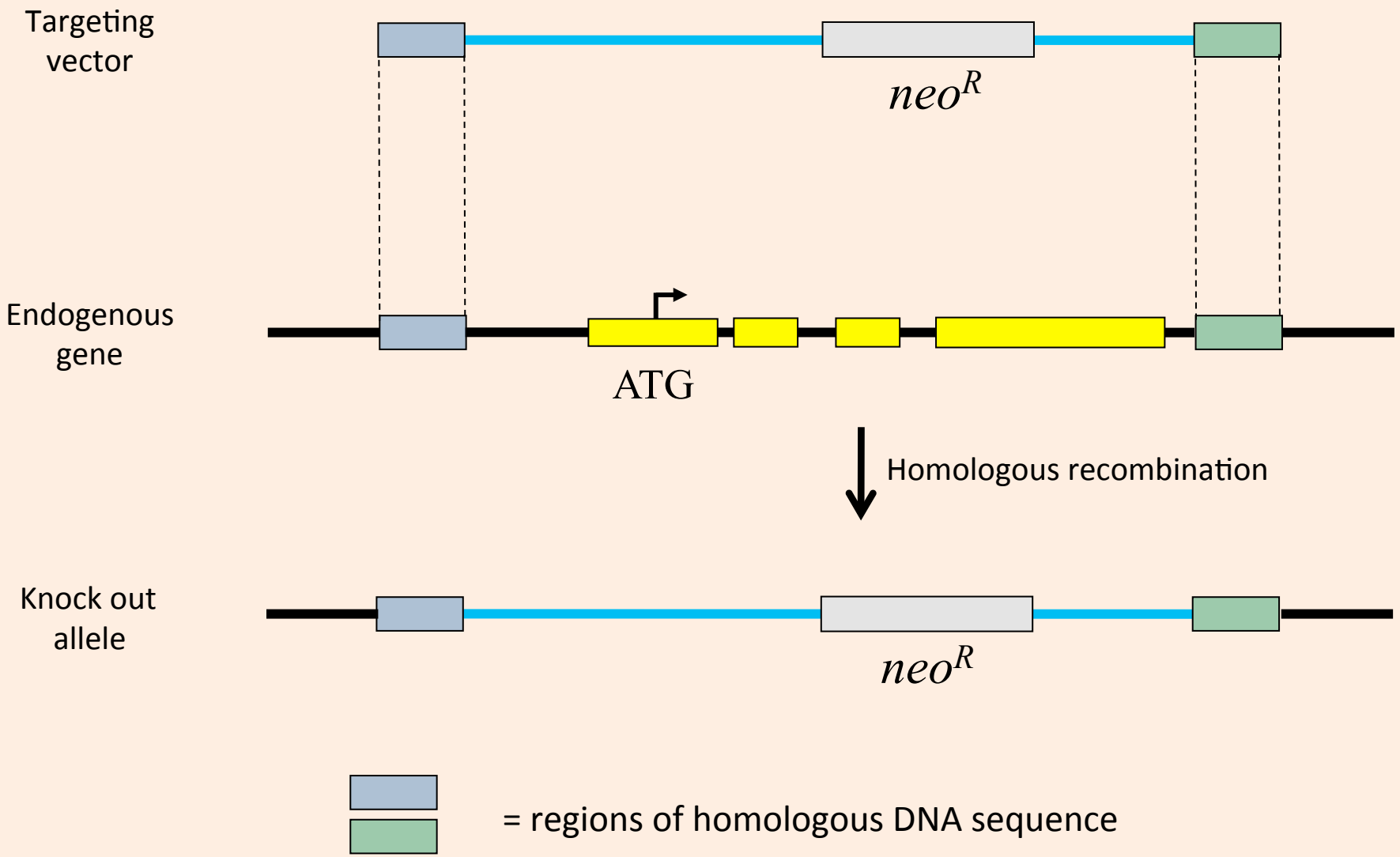

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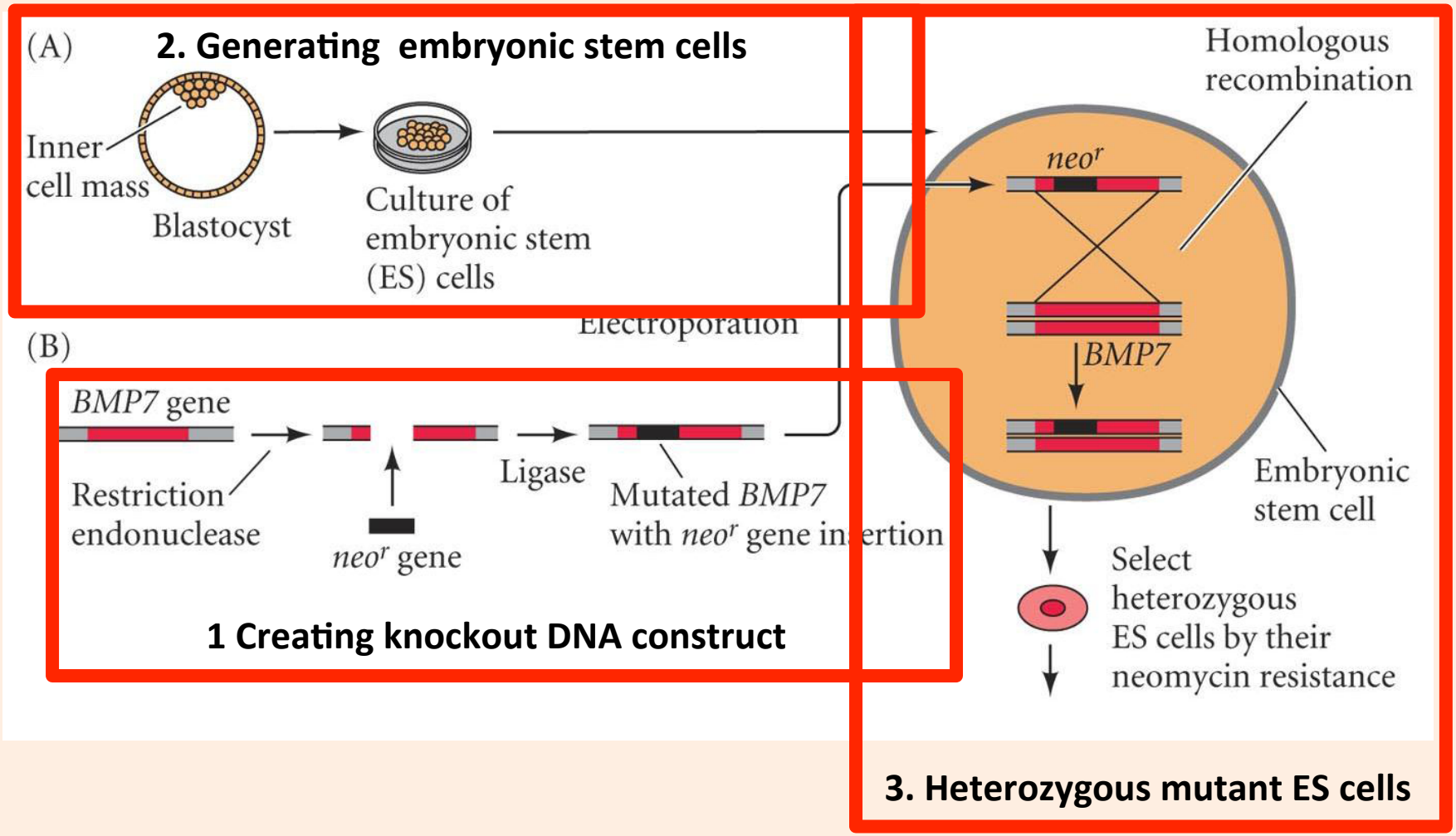
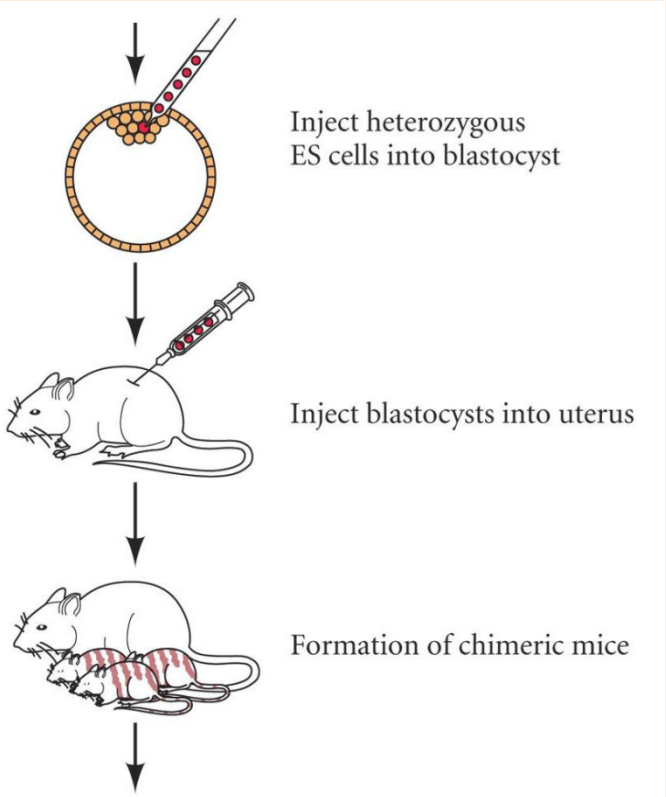


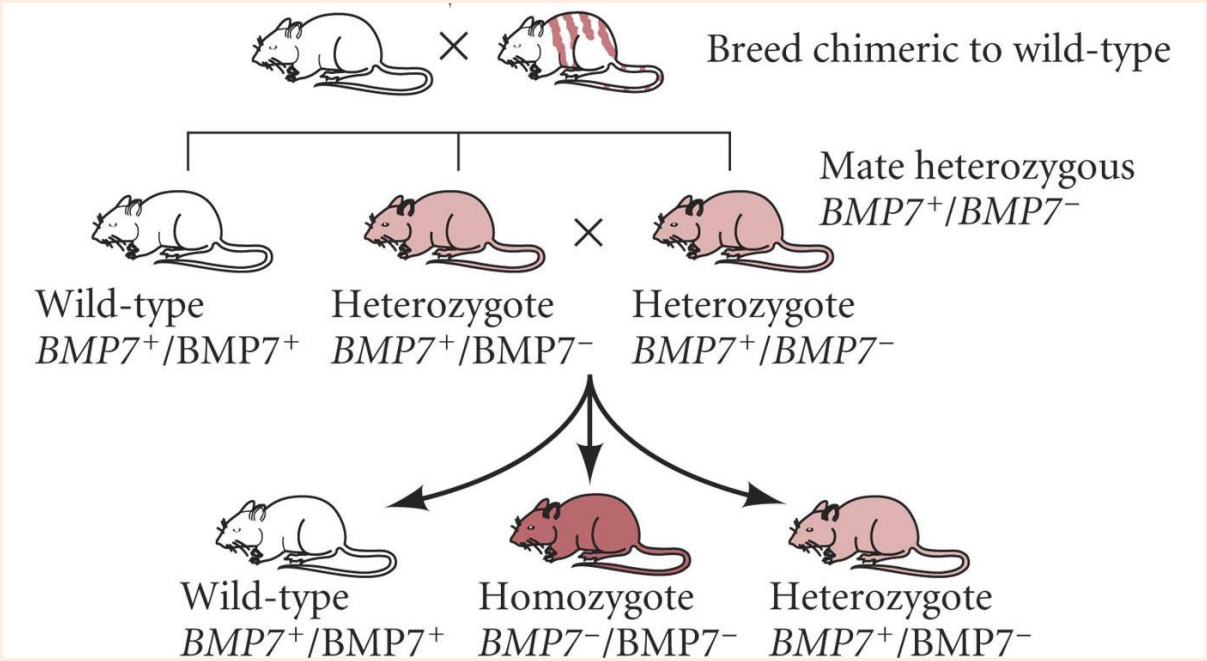
Knock out technology

Heterozygous mutant
Embryonic stem cells



Chimeric mice

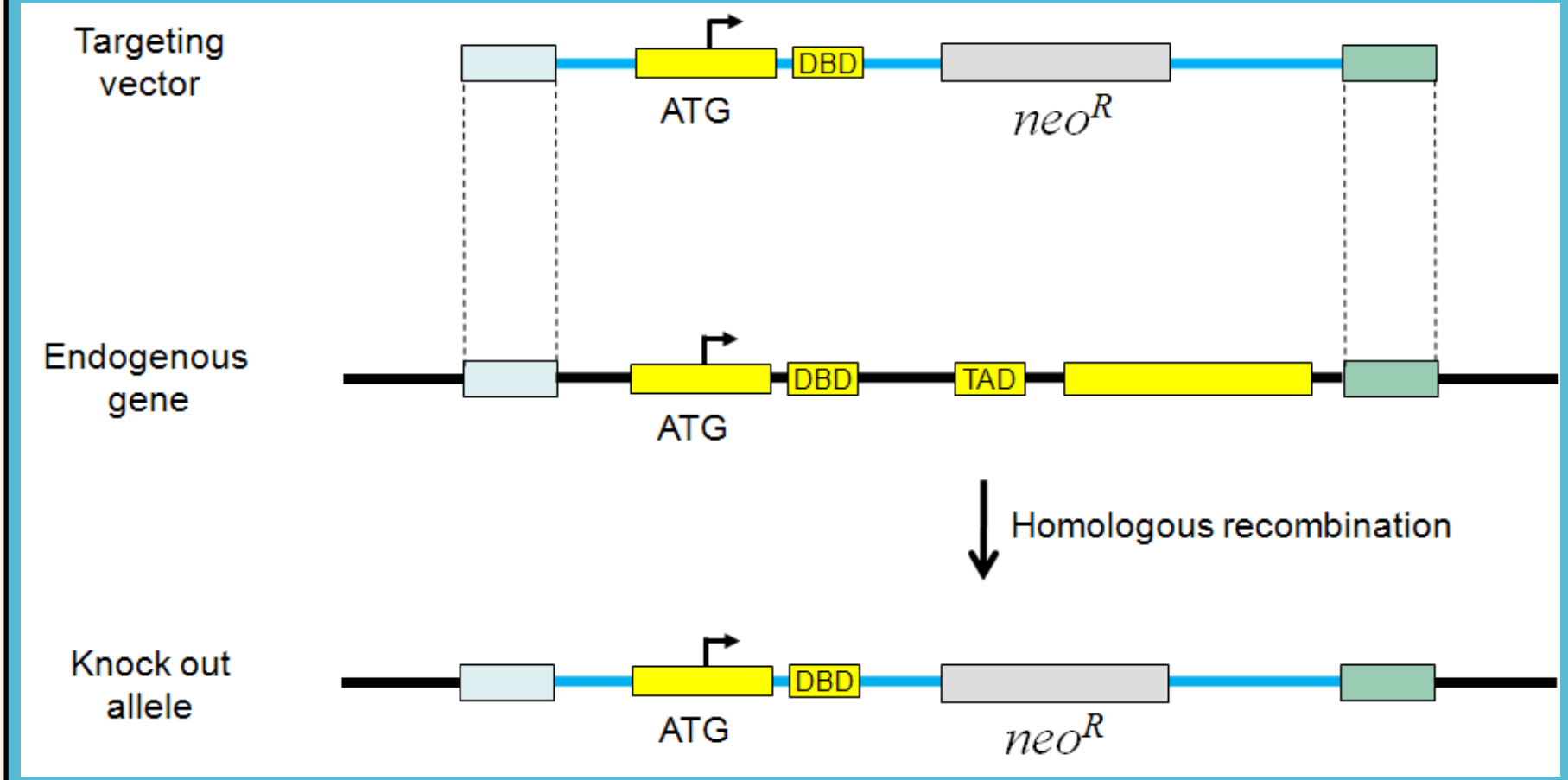
Genetic crosses to obtain
Homozygous mutant mice



Knock out technology

Engineering of targeting vectors

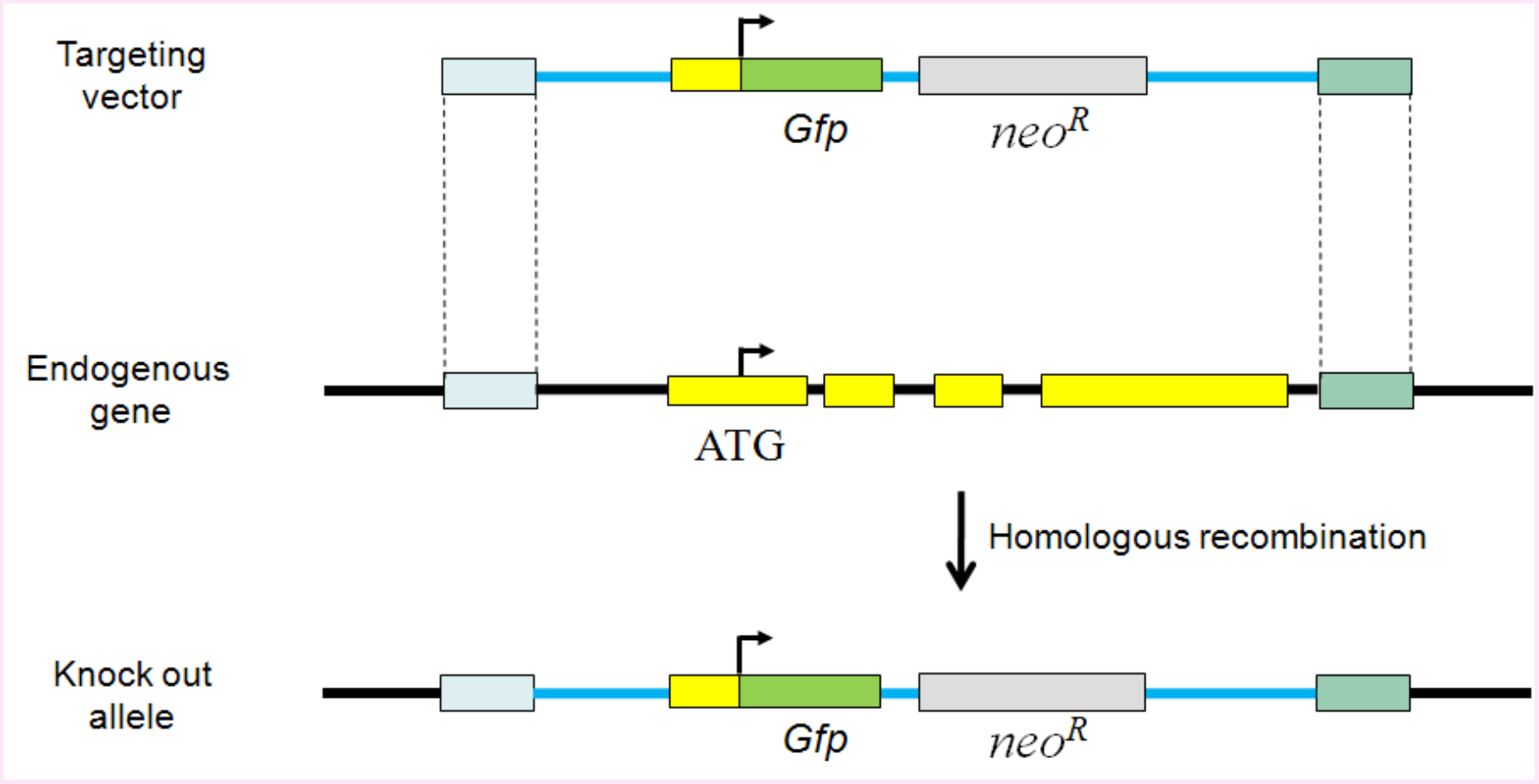
Generating dominant negative proteins



Knock out technology

Engineering of targeting vectors

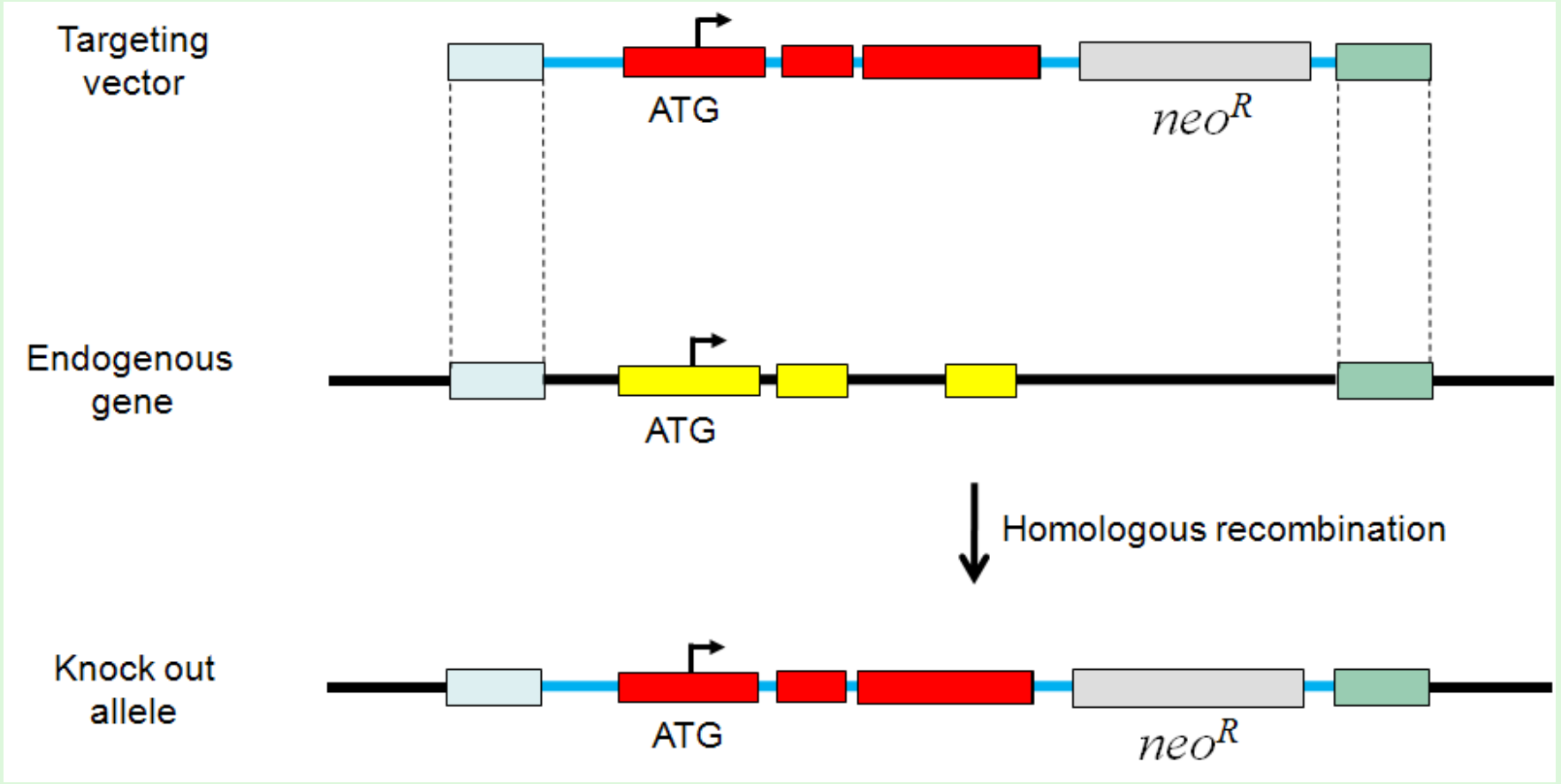
Expressing reporter genes from mutant locus



Knock out technology

Engineering of targeting vectors

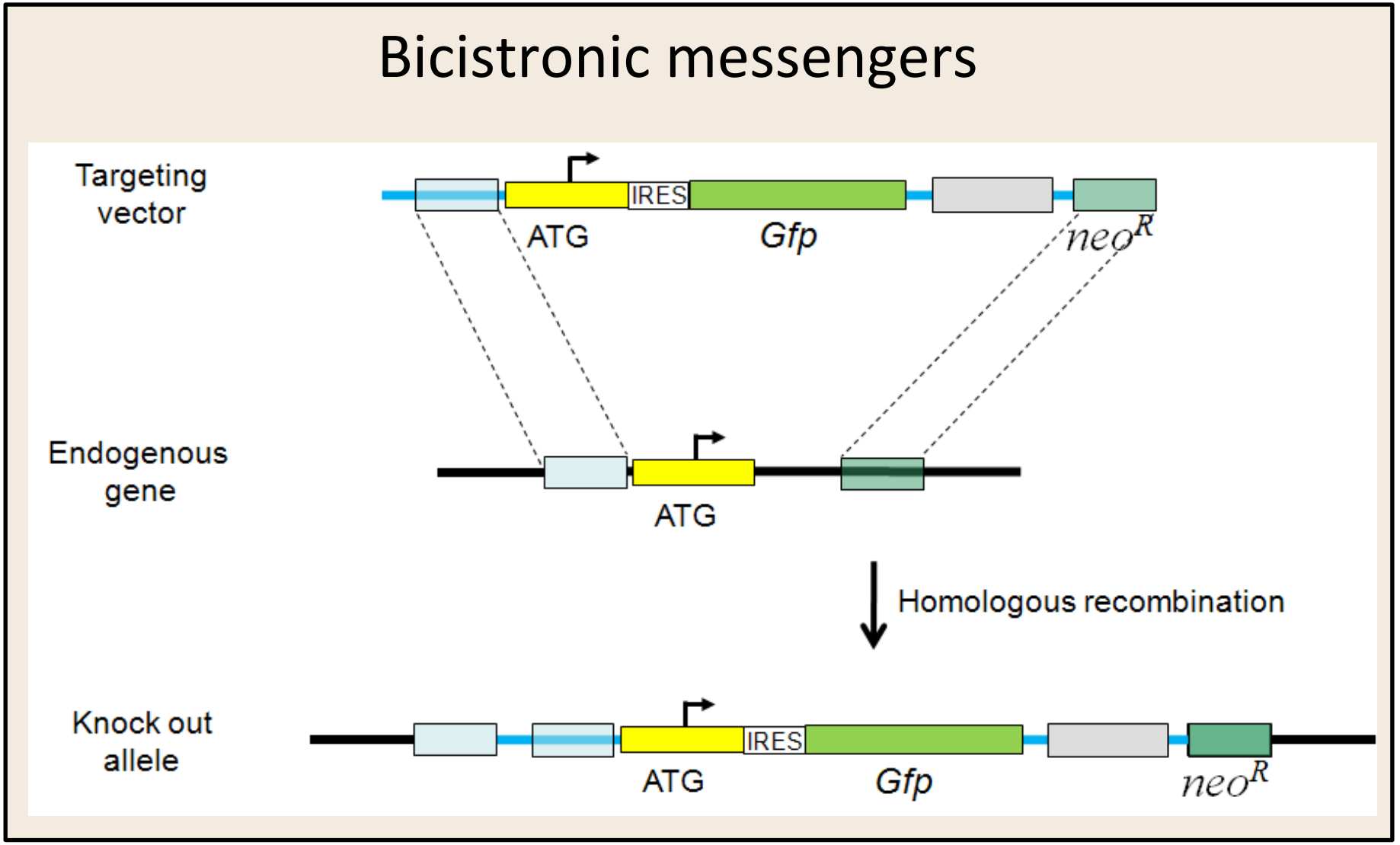
Gene swapping



Knock out technology

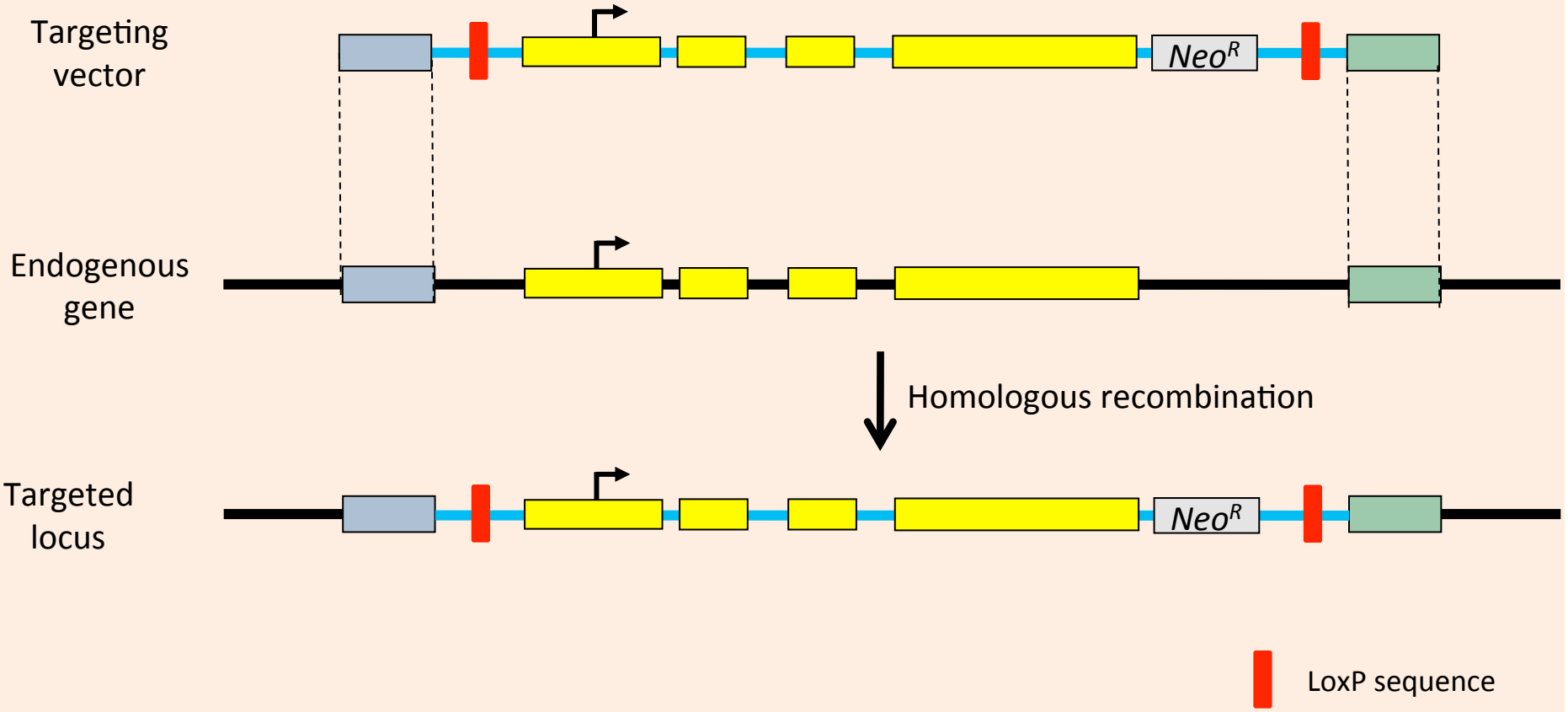
Engineering of targeting vectors

Bicistronic messengers

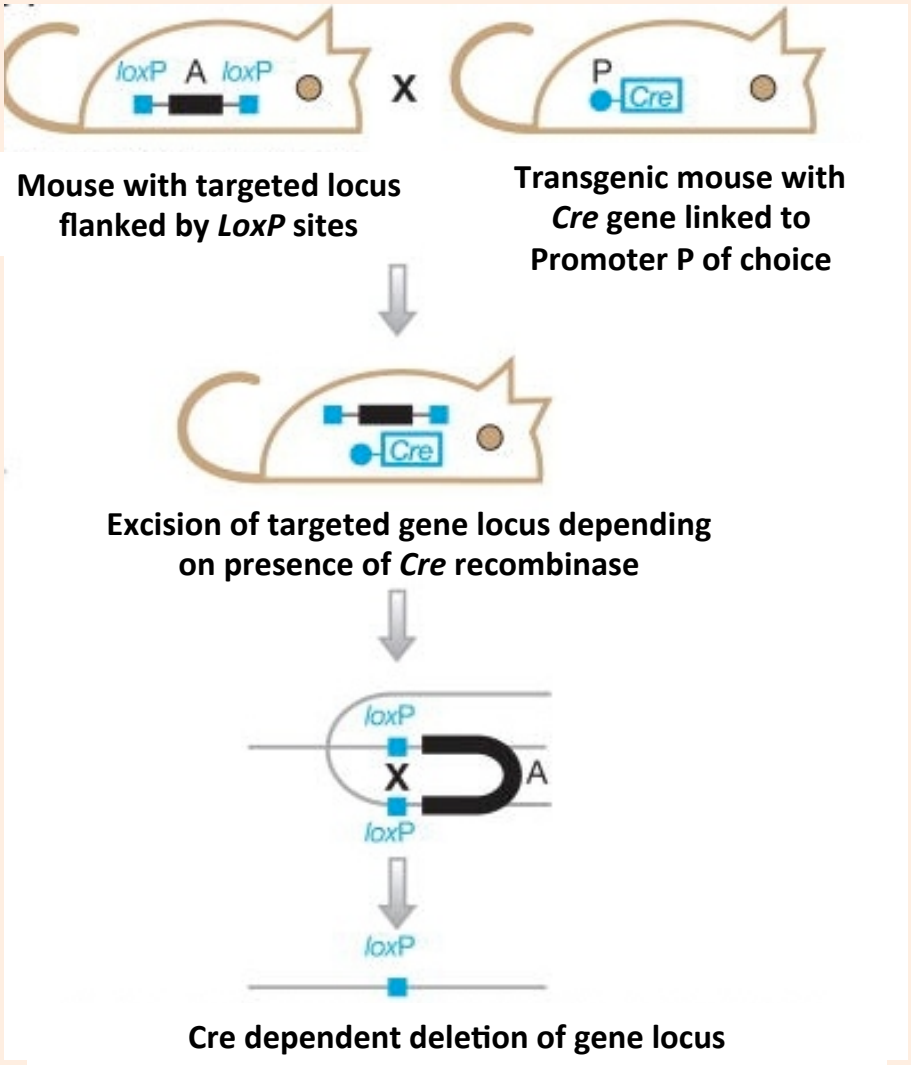


Conditional knock out technology

Conditional LOF mutants: excision of gene dependent on presence of
- Loxp sites in gene locus
- Cre recombinase



Conditional knock out technology



Advantages:

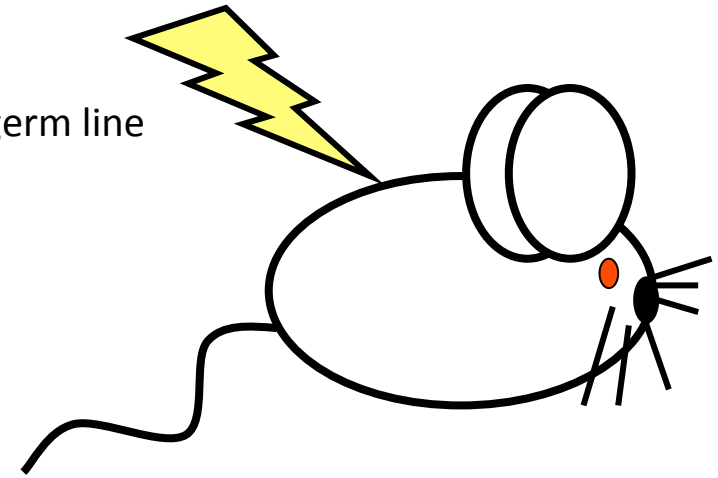
Cell/tissue type specific
Timing specific
Inducible

Random mutagenesis screens

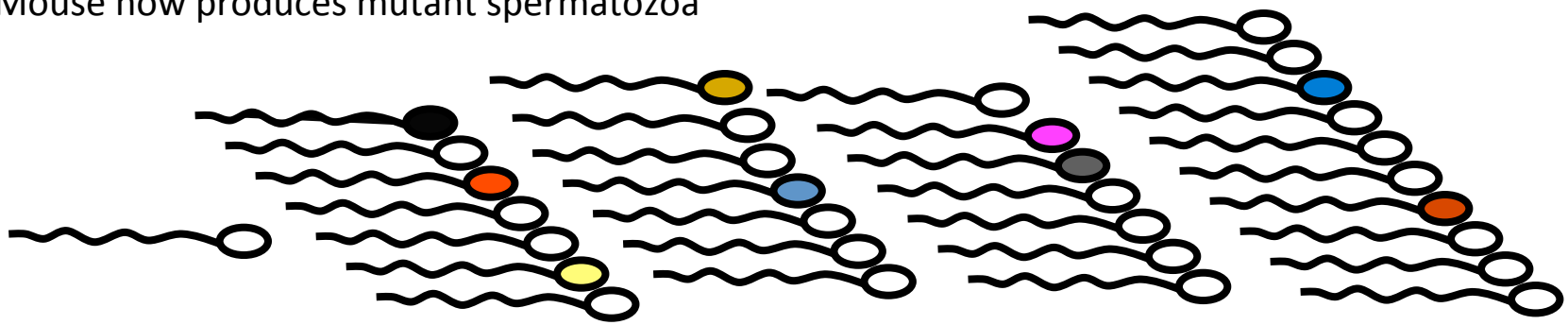
You want to identify new genes that are involved with a certain process

The male animal is subjected to a mutagen, e.g. radiation, or chemical mutagens such as ethylnitrosurea (ENU) or ethylmethyl sulphate (EMS).

Mutagen hits the germ line



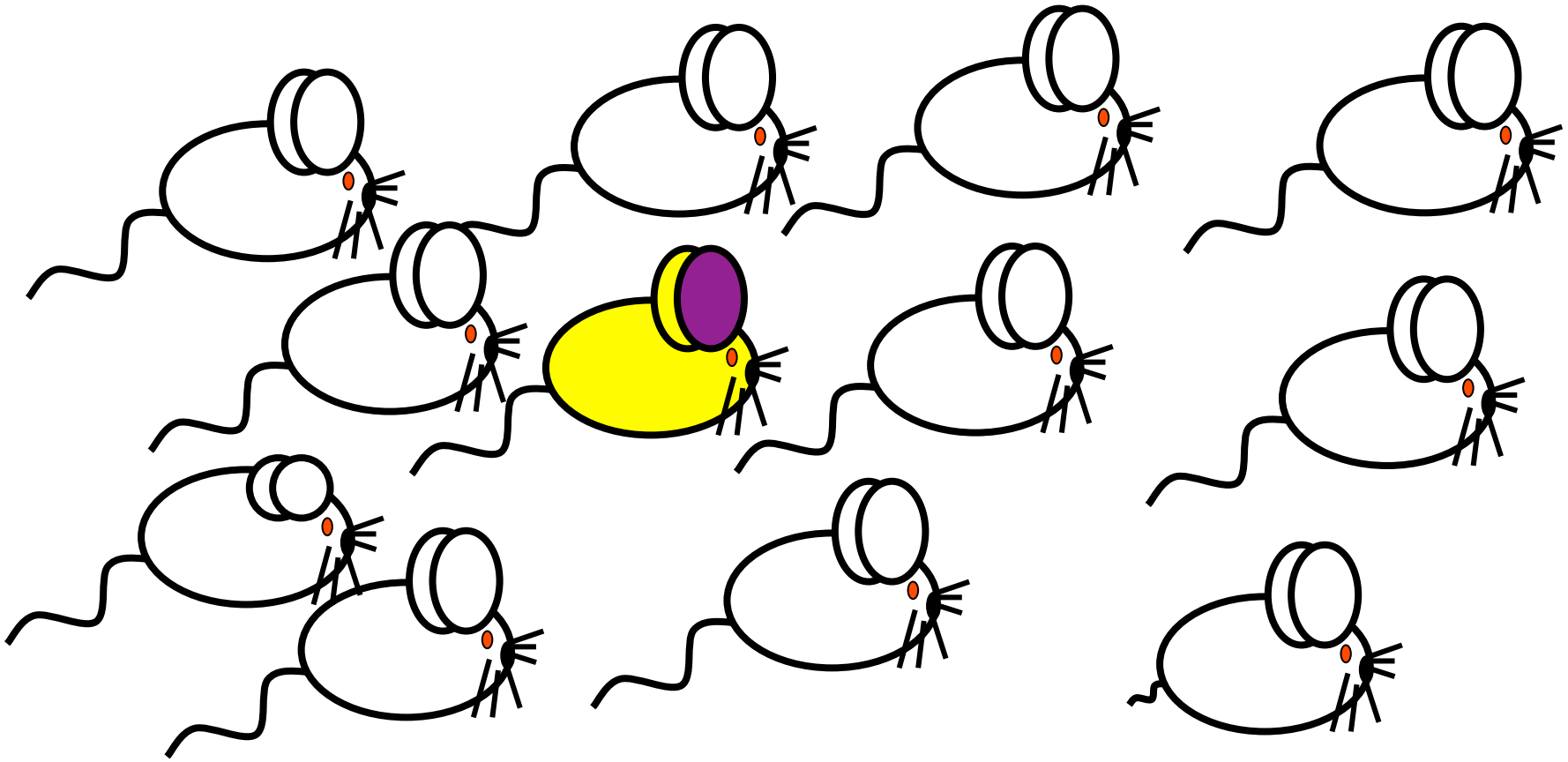
Mouse now produces mutant spermatozoa



Random mutagenesis screens

A screen for dominant mutations:

Mate mutated mice with wildtype females.
Screen babies for phenotypes.
Those that are heterozygous for a dominant gene will show a phenotype.

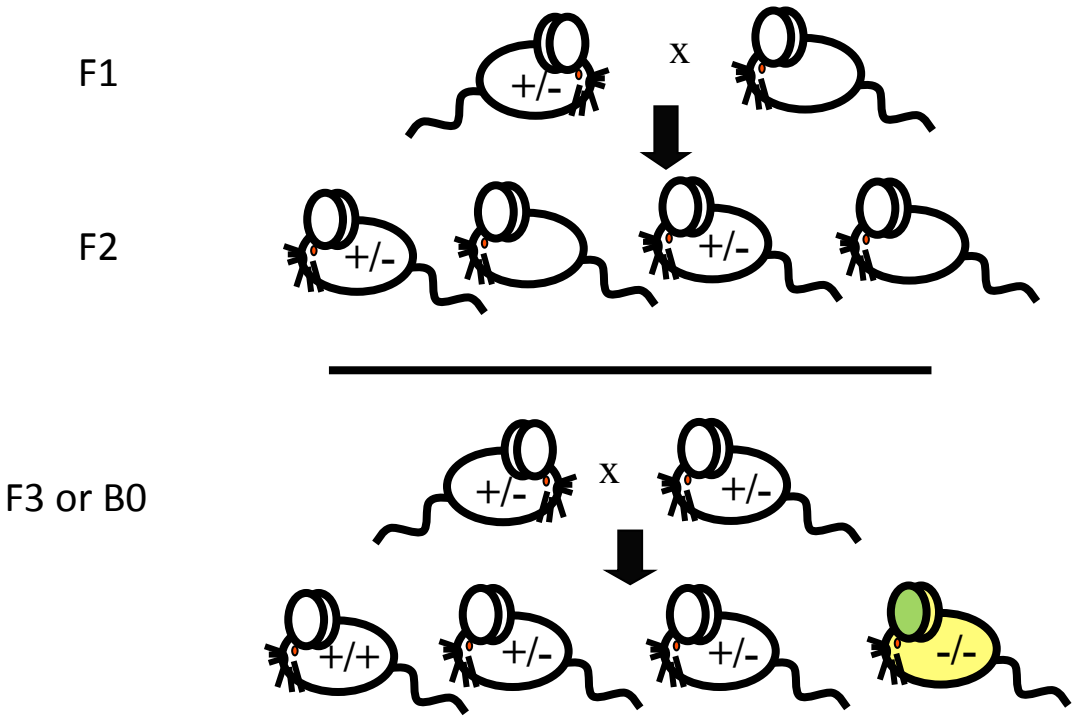


Random mutagenesis screens

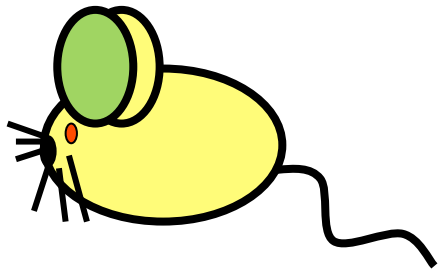
A screen for recessive mutations:

Some of the F1 progeny of the mutagenised mice might LOOK normal, but be heterozygous for a recessive mutation.

Have to breed a litter of progeny then do brother-sister matings to get -/- mice.



Random mutagenesis screens



Select mice with desired phenotypes

Start identification of mutated genes:
Combination of genetic linkage mapping and sequencing

Manipulation of gene function in the mouse

Transgenesis: - gain of function transgenesis
- reporter overexpression
- loss of function transgenesis

Mutagenesis: - Conventional knock out mice
- Conditional knock out mice
- Random mutagenesis

ANAT2341 – Lab 4

The mouse as model for human disease

Why mice?

Gene and protein expression analysis methods

Methods to study gene function in mice *in vivo*

Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au