Lecture 8

Research Technologies in Developmental Biology

Chapter 5 in Larsen's *Human Embryology* (4th edition)
Chapter 4 in Scott Gilbert's *Developmental Biology* (8th edition)

Dr Annemiek Beverdam – School of Medical Sciences, UNSW
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Animal models in Developmental biology

Lineage Tracing and Transplantation Studies

Gene and protein expression analysis methods

Methods to study gene/protein function in embryos

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Introduction

Developmental Biology Research is driven by two questions:

1. How does development occur at tissue, cellular and molecular level?

2. How does development go wrong and result in birth defects?

Experimental questions:

How do embryonic cells/tissues develop, what do they give rise to?

Where is a gene/protein expressed during development?

What does the gene/protein do during development?

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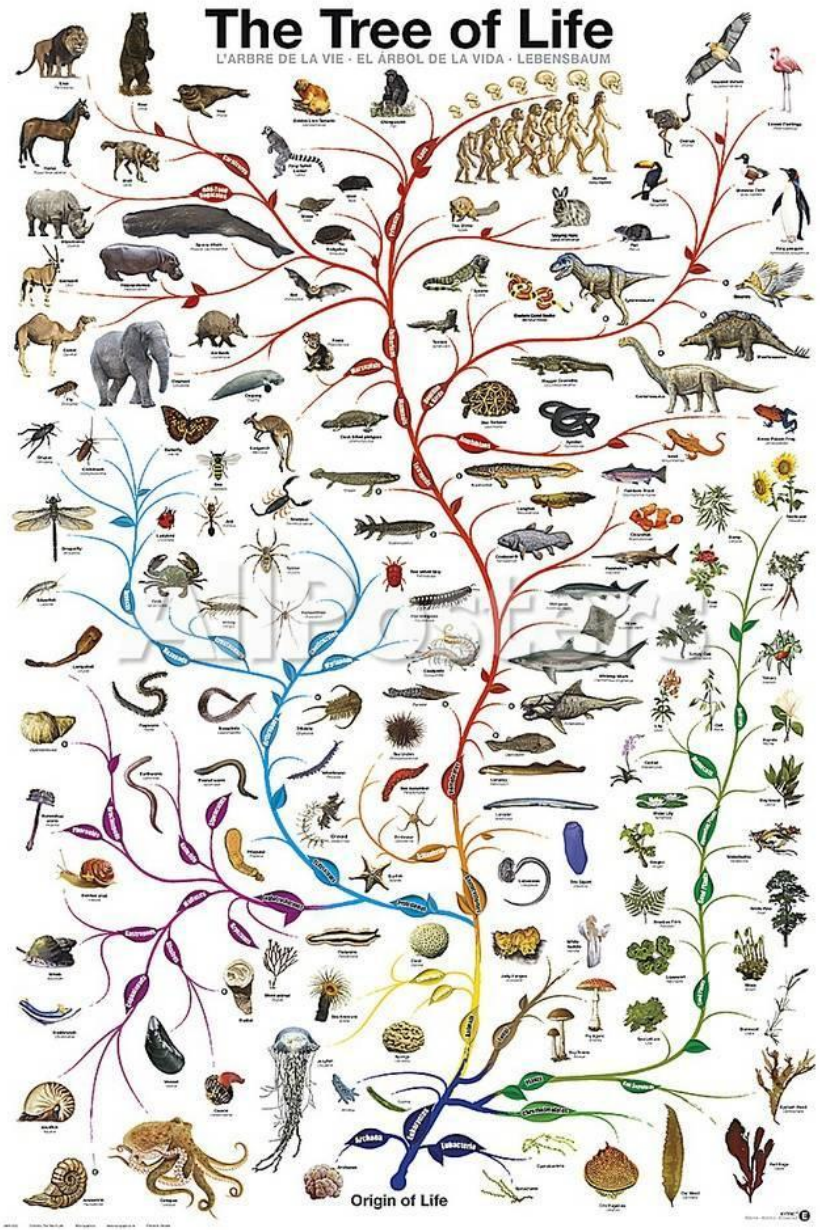
Animal Models in Developmental Biology Research

Why do we use animals in research?

People get sick
We want to make them better

We do not want to experiment on human embryos

Animal Models in Developmental Biology Research



Animal models have a similar genetic make-up

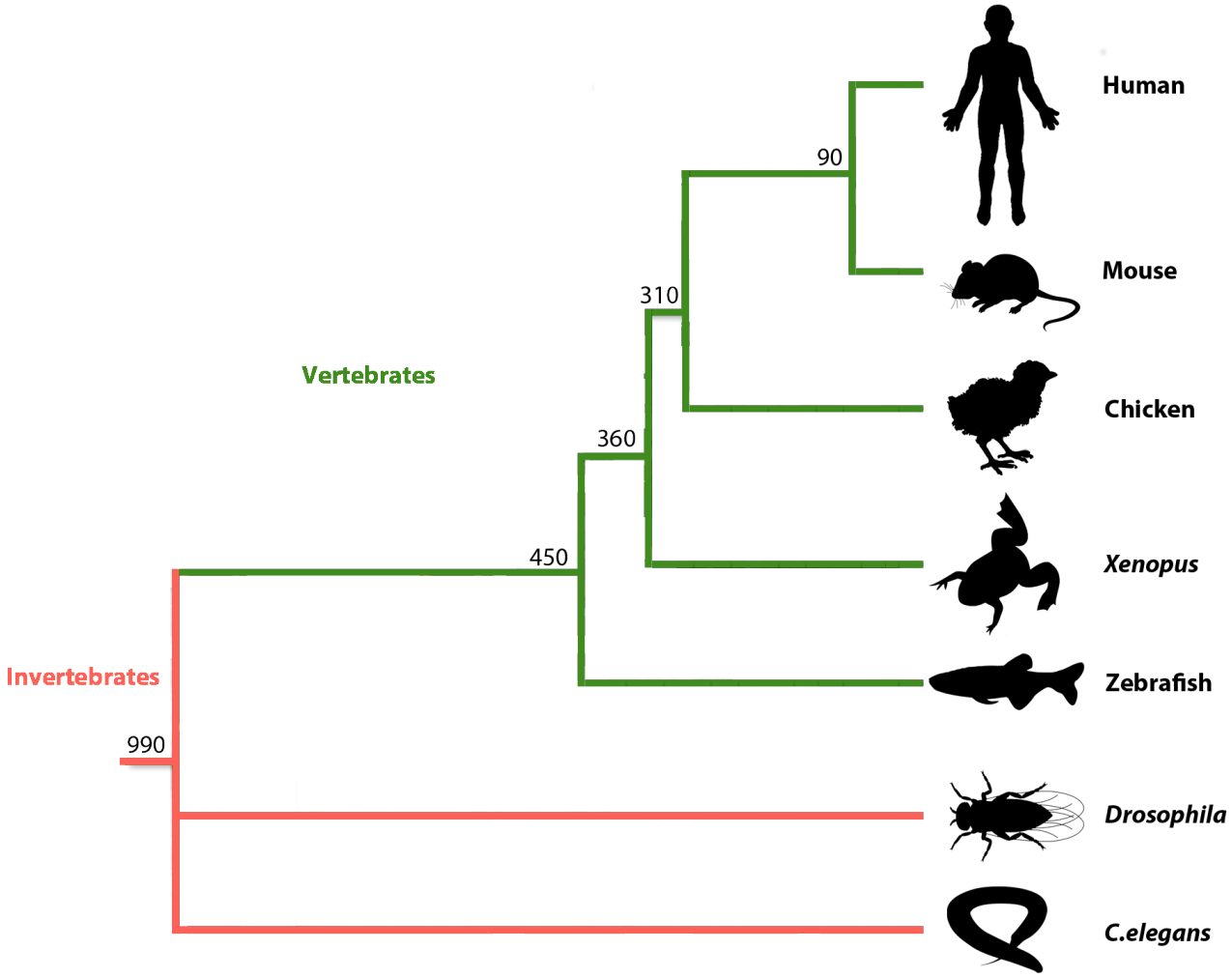
All life on earth is related

At the very start of life, life forms were simple single cell organisms (bacteria, protozoa, algae)

These gradually evolved into more complex multicellular organisms over time

All life forms on earth share common genetic information

Animal Models in Developmental Biology Research



Animal models have a similar genetic make-up

Species that diverged more recently share more common genetic information with us compared to those whose evolution diverged longer ago.

Animal Models in Developmental Biology Research:

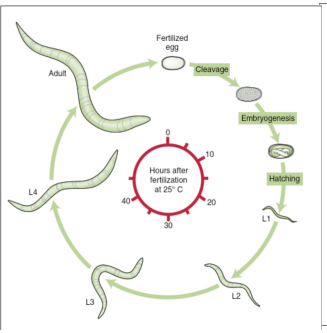
Early embryogenesis very similar across vertebrate species

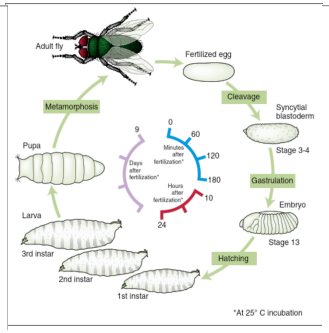
Development diverges during fetal development

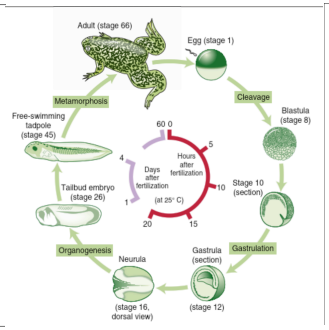
Animal Models in Developmental Biology Research:

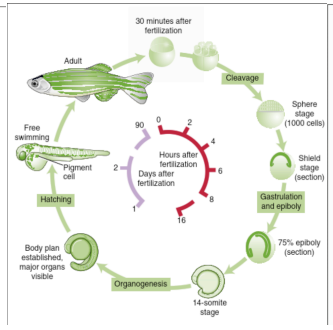
Common animal models in Developmental Biology Research:

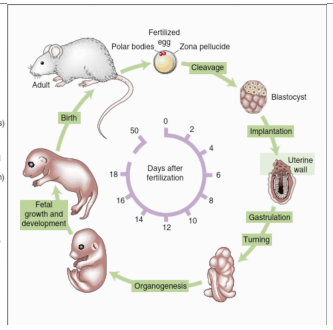
C. elegans and Planarians
Drosophilia
Xenopus leavis and Xenopus tropicalis
Zebrafish
Chicken
Mouse

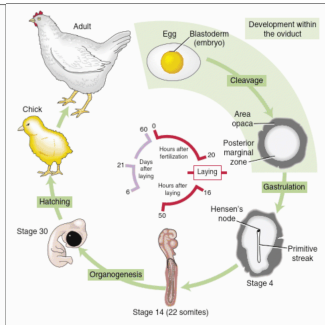












Animal Models in Developmental Biology Research:

Category:	C. elegans	Drosophila	Zebrafish	Xenopus	Chicken	Mouse
Broodsize	250-300	80-100	100-200	500-3000+	1	5-8
Cost per embryo	low	low	low	low	medium	high
High-throughput multiwell-format screening	good	good	good	good	poor	poor
Access to embryos	good	good	good	good	poor	poor
Micro-manipulation of embryos	limited	limited	fair	good	good	poor
Genome	known	known	known	known	known	known
Genetics	good	good	good	fair	none	good
Knockdowns (RNAi, morpholinos)	good	good	good	good	limited	limited
Transgenesis CRSPR	good	good	good	good	poor	good
Evolutionary distance to human	very distant	very distant	distant	intermediate	intermediate	close

Color code: green, best in category; red, worst in category.
Adapted from Wheeler & Brändli 2009 Dev Dyn 238:1287-1308.

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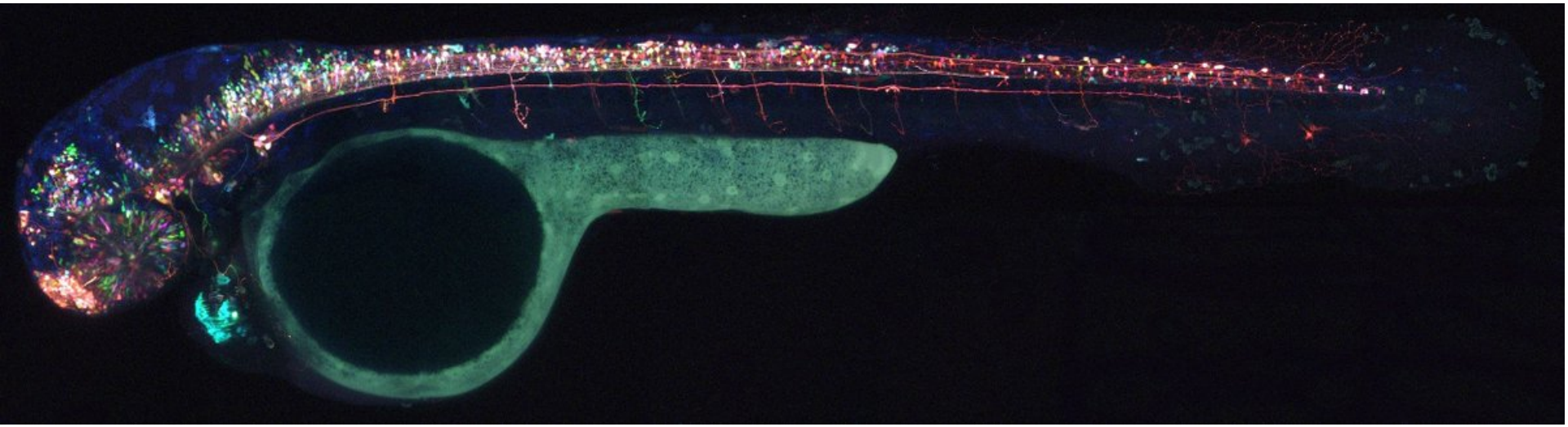
Lineage Tracing and Transplantation Studies

In **lineage tracing**, a single cell is marked in such a way that the mark is transmitted to the cell's progeny resulting in a set of labeled clones.

Lineage tracing provides information about the number of progeny of the founder cell, their location, and their differentiation status.

- Injection of visible dyes into cells/tissues (DyI)
- Genetic labelling of cells/tissues (Rainbow)

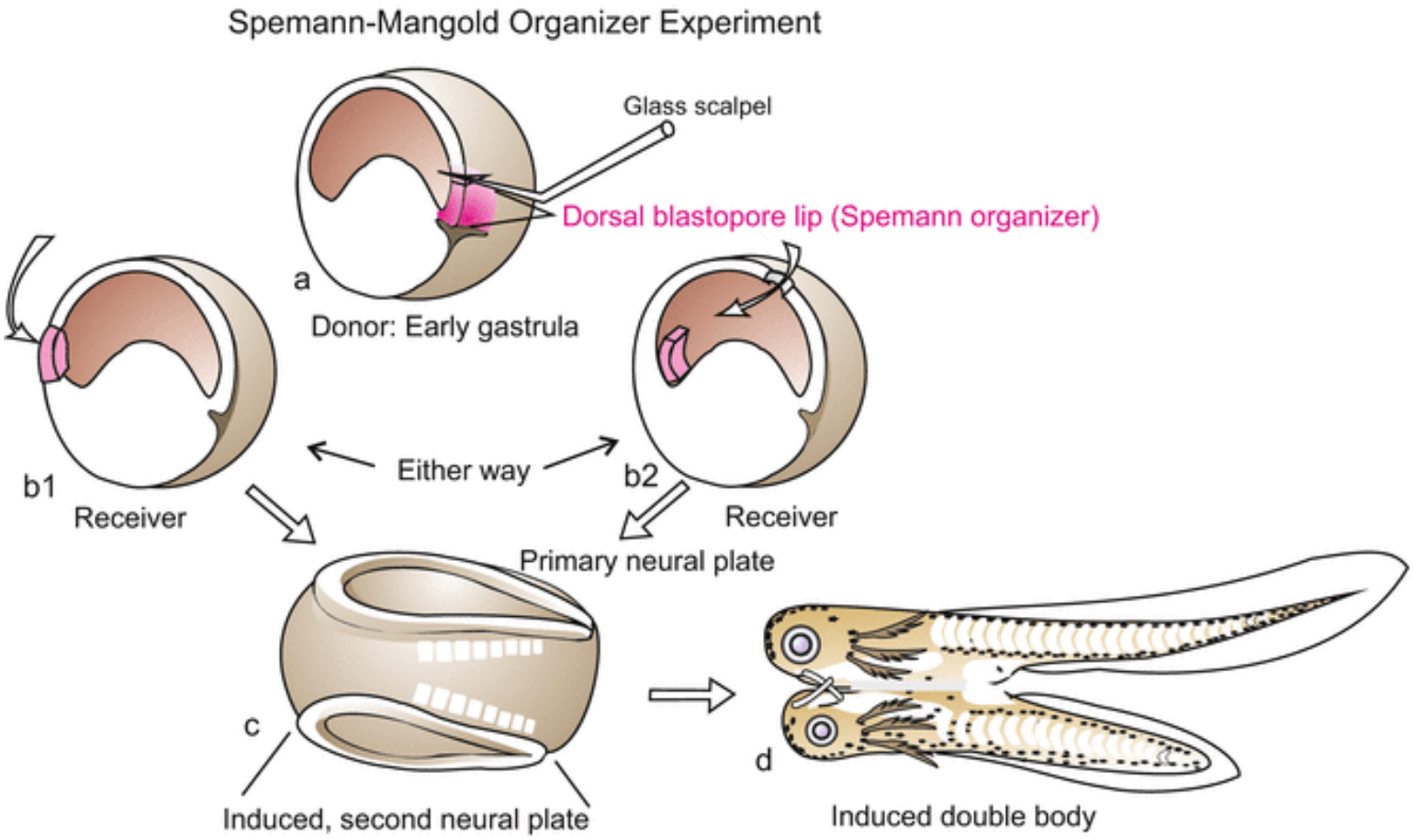


Lineage Tracing and Transplantation Studies

Transplantation studies:

What does the node do?

Transplantation of the node in gastrula Xenopus leavis embryos
Induction of second body axis
(Spemann and Mangold, 1924)



Lineage Tracing and Transplantation Studies

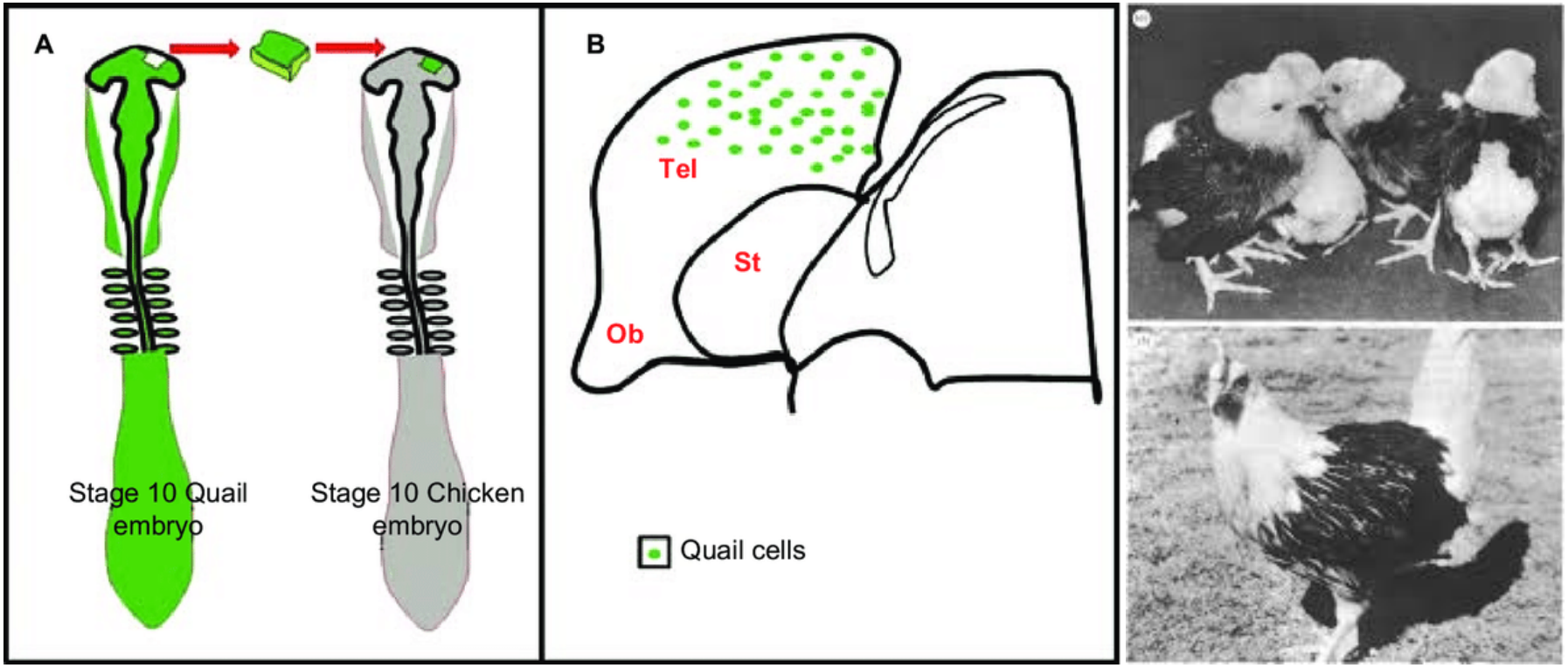
Transplantation studies

What does this neural crest give rise to?

Transplantation of neural tube tissue from quail to chick embryos

Investigate the donor tissue derivatives

(LeDouarin et al)



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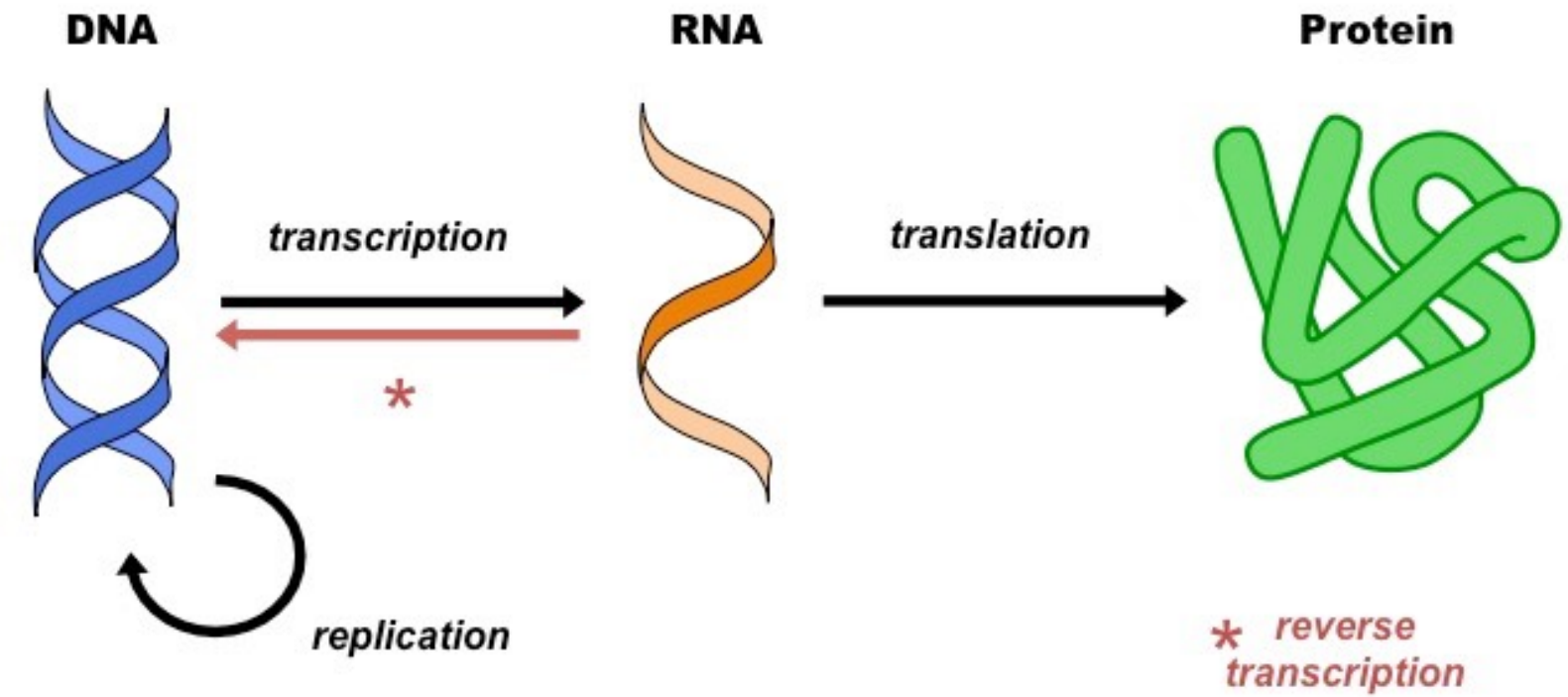
Gene and protein expression analysis methods

Flow of genetic information

Central dogma of molecular biology



Gene and Protein Expression Analysis

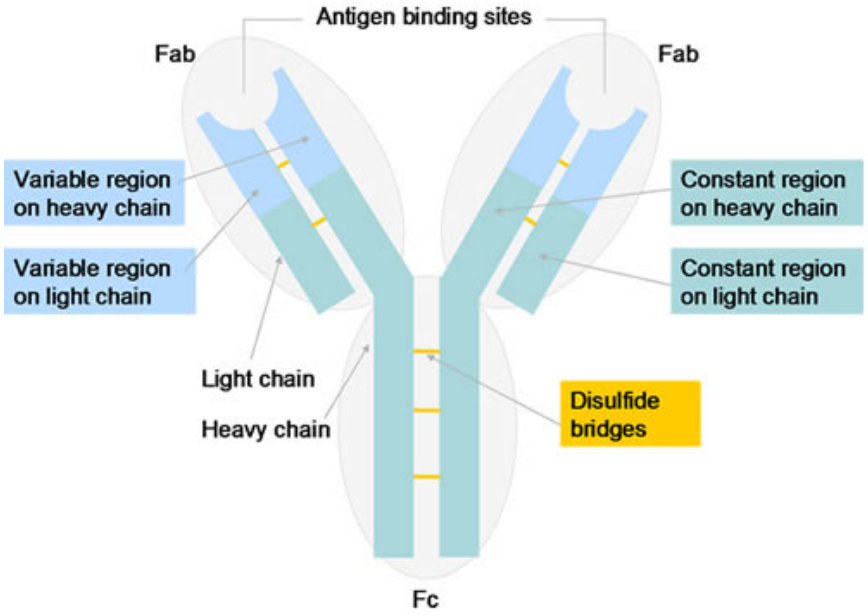
Detection of **protein** expression:
- Antibodies
- Immunodetection

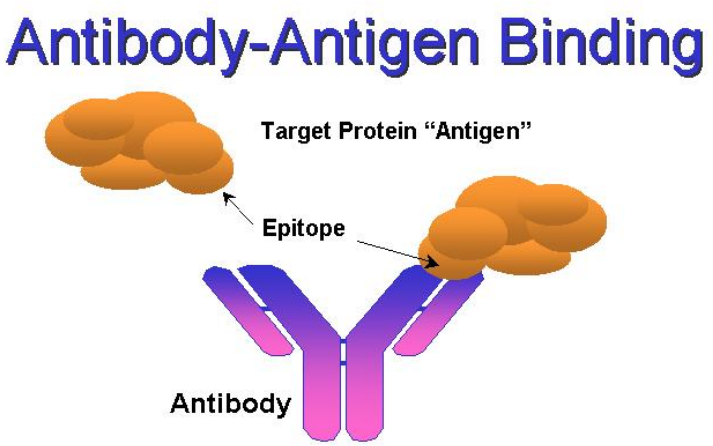
Detection of **RNA** expression:
- RTPCR
- Quantitative RT PCR
- In situ hybridization
- RNA sequencing

Gene and protein expression analysis methods

Antibodies – not only for immunity!

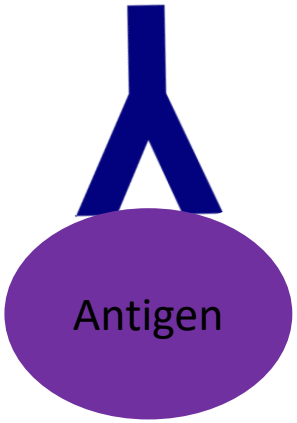
An **antibody**, also known as an **immunoglobulin**, is a large, Y-shaped protein produced mainly by the immune system to identify and neutralize pathogens such as bacteria and viruses



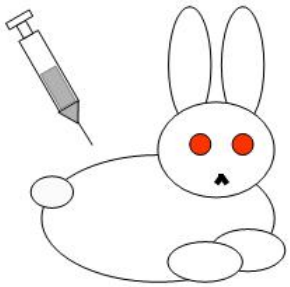


Gene and protein expression analysis methods

We can produce antibodies binding defined antigens at large scale

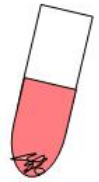


immunization



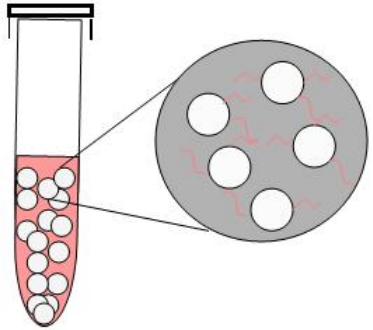
A solution containing a specific antigen is injected into a rabbit; the rabbit is immunized.

collect serum

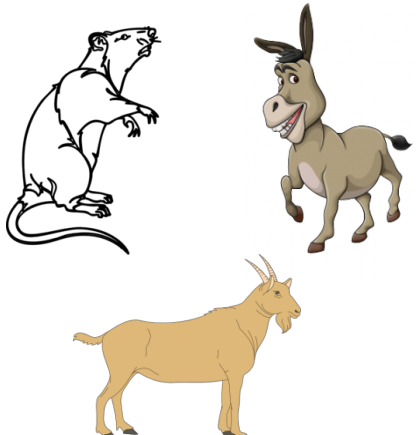


Antiserum is taken from the rabbit; the supernatant contains the antibodies of interest.

purification

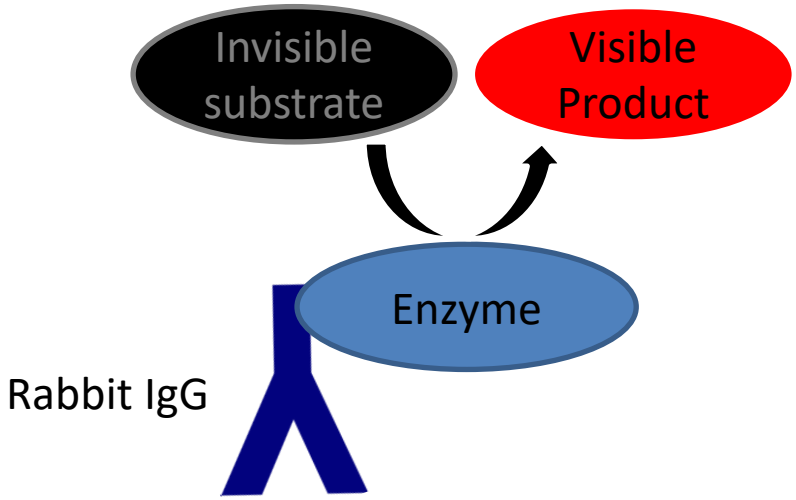


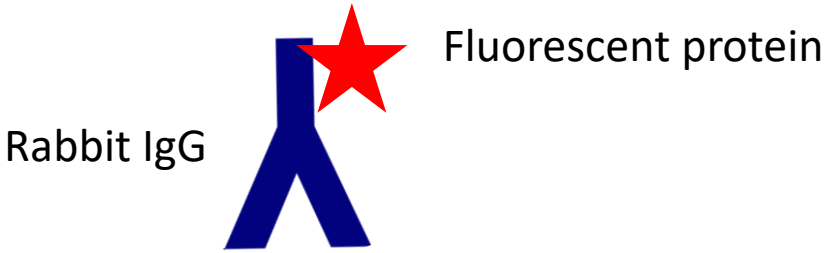
Antibodies are incubated with sepharose beads that is conjugated to the original antigen.



Gene and protein expression analysis methods

We can modify antibodies – add conjugates to Fc portion IgG

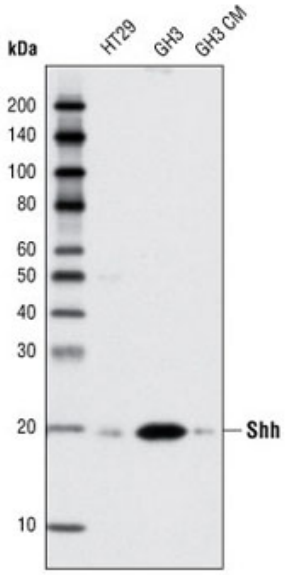




Gene and protein expression analysis methods

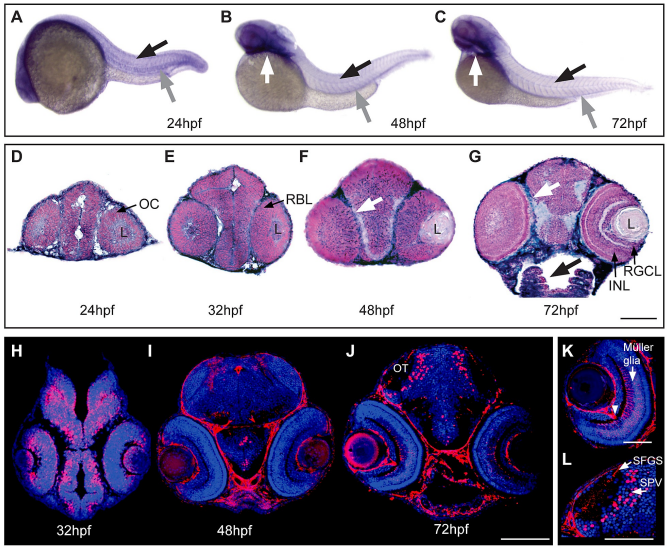
Immunodetection

1. Cell or tissue lysates



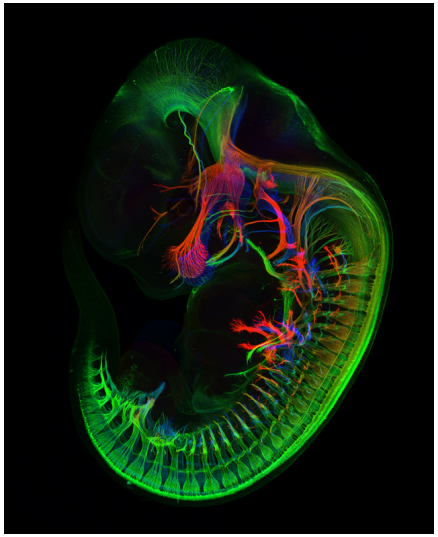
Quantitative
Rapid
Limited spatial information

2. Tissue/embryo sections



Limited quantitative information
2 days plus imaging
Excellent spatial information

3. Whole embryos/tissues

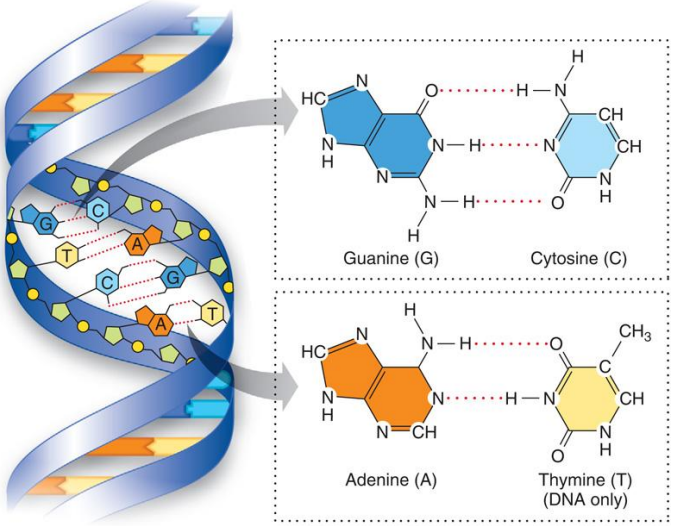


Limited quantitative information
Weeks plus imaging
Excellent spatial information

Gene and protein expression analysis methods

RNA detection methods are based on nucleotide base pairing

Base Pairing in the genome
DNA:DNA



DNA structure with base pairs: G with C and A with T

Base Pairing in transcription
DNA:RNA

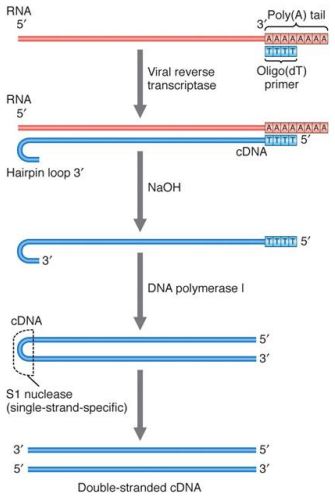
Complementary base pairing

DNA Base	Complementary RNA Base
G	C
C	G
A	U
T	A

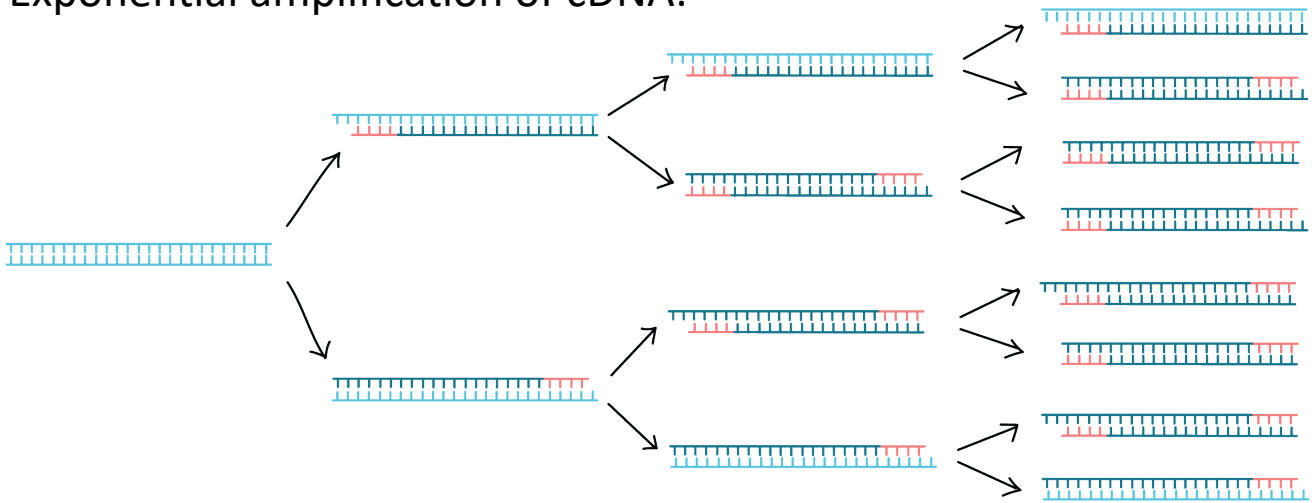
Gene and protein expression analysis methods

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

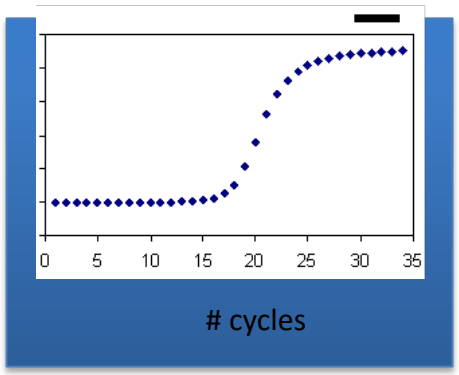
Step 1:
Reverse transcription:
Template: RNA
Product: double stranded cDNA



Step 2:
Exponential amplification of cDNA:

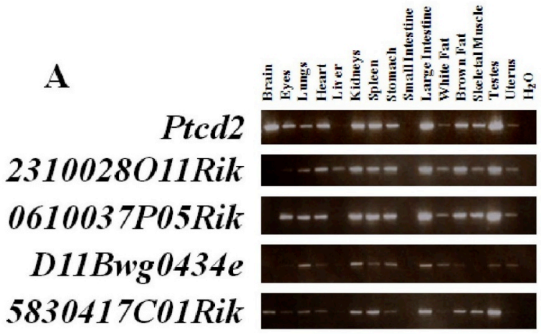


Transfer Product to DNA gel



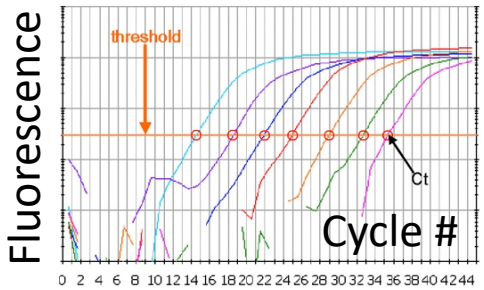
Gene and protein expression analysis methods

1. RT PCR



Not quantitative (Y/N answer)
Rapid (2-3 hours)
No spatial expression information

2. Quantitative real time RT PCR



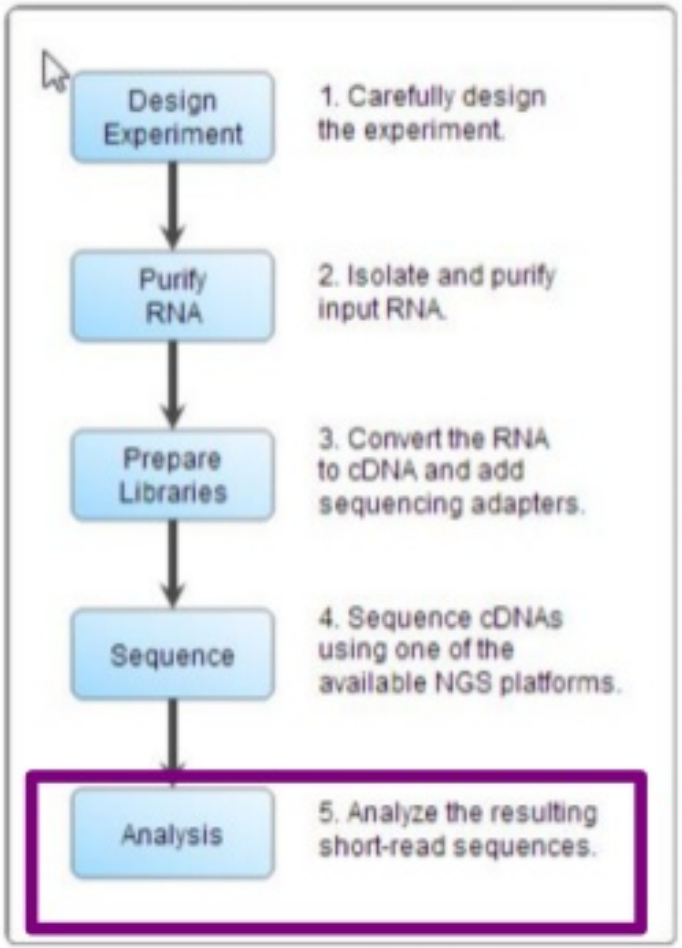
Quantification of RNA expression levels:

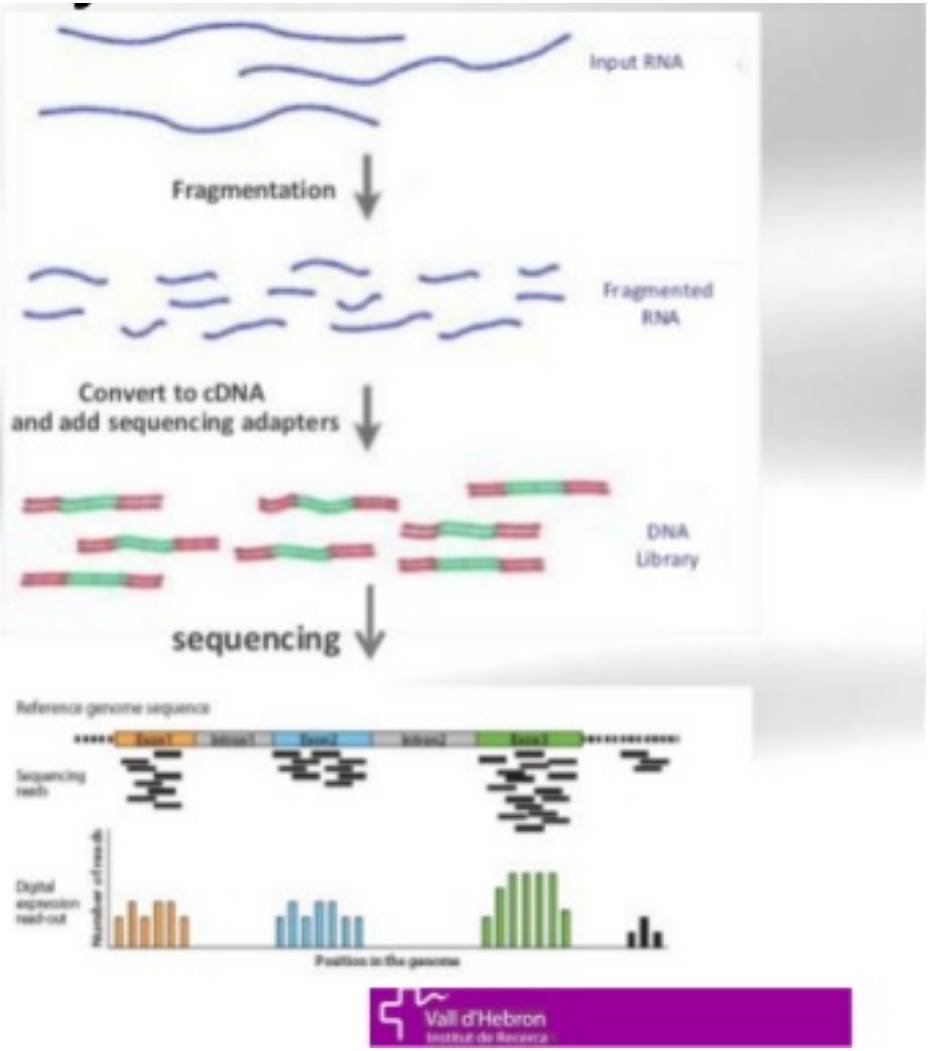
- different genes within one sample
- the same gene in different samples (against a reference gene)
- against a known spiked standard

Rapid
No spatial information

Gene and protein expression analysis methods

RNA sequencing: analyse and compare the transcriptome of thousands of genes





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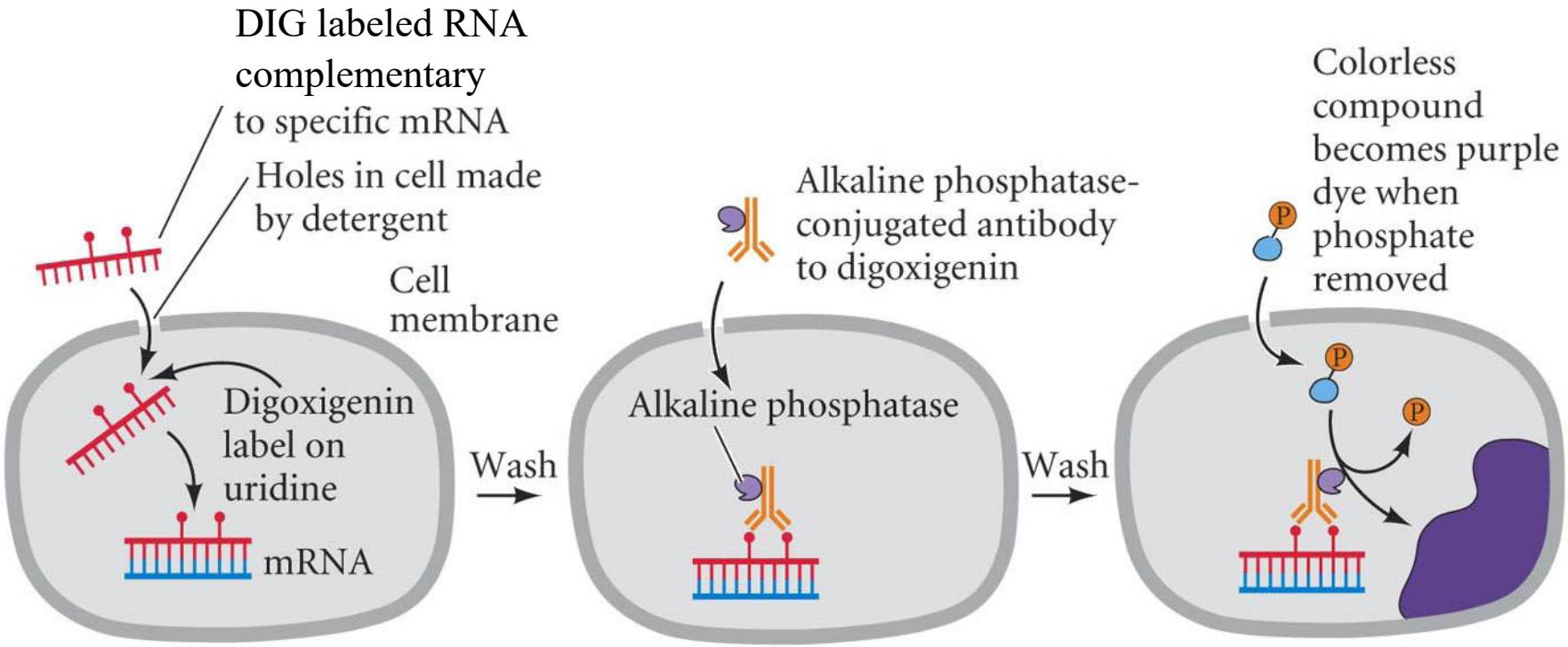
Gene and protein expression analysis methods

in situ hybridization

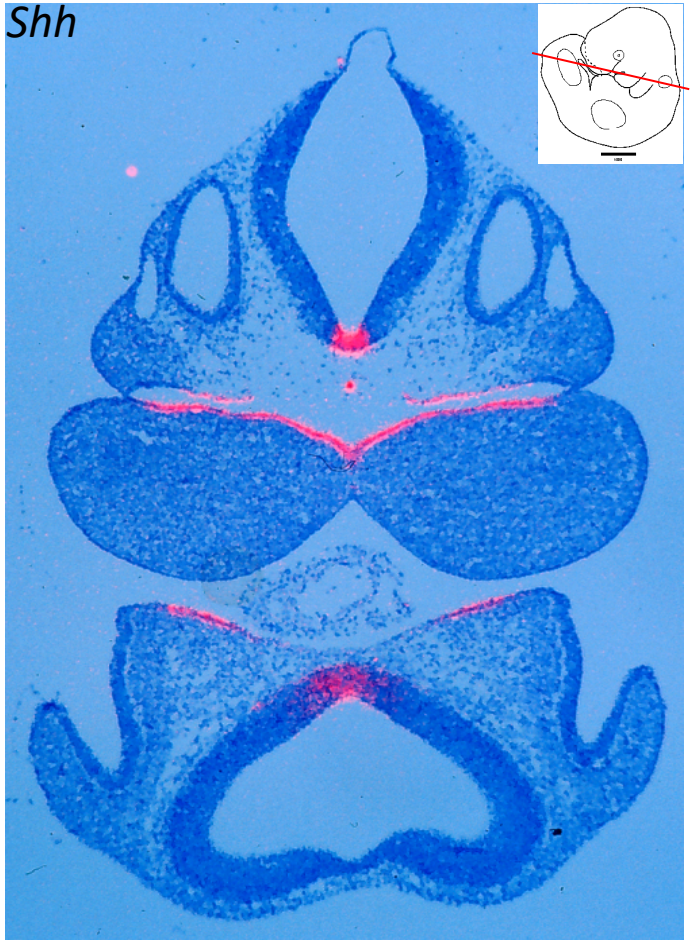
Labeled antisense RNA probe
Generate probe RNA:mRNA hybrids
Detection of conjugates on probe RNA



Gene and protein expression analysis methods

in situ hybridization

On sections:



Radioactively labelled probe

On whole embryos/whole tissues:

Alkaline phosphatase labelled probe

Gene and protein expression analysis methods

Summary

Method	Detection of	Quantitative	Spatial information	Results within
Protein gel Western blot	Protein	Yes	No/Little	2 Days
Section immunostaining	Protein	Limited	Yes	2 Days
RT PCR	RNA gene	No	No/Little	1 Day
Real Time PCR	RNA gene	Yes	No/Little	1 Day
RNAseq	RNA Transcriptome	Yes	No/Little	Weeks
in situ hybridization	RNA Gene	Limited	Yes	1-2 Weeks

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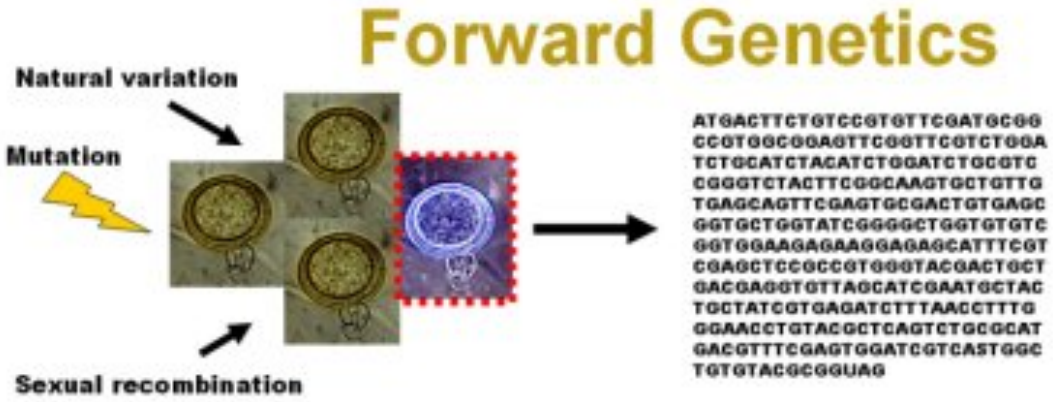
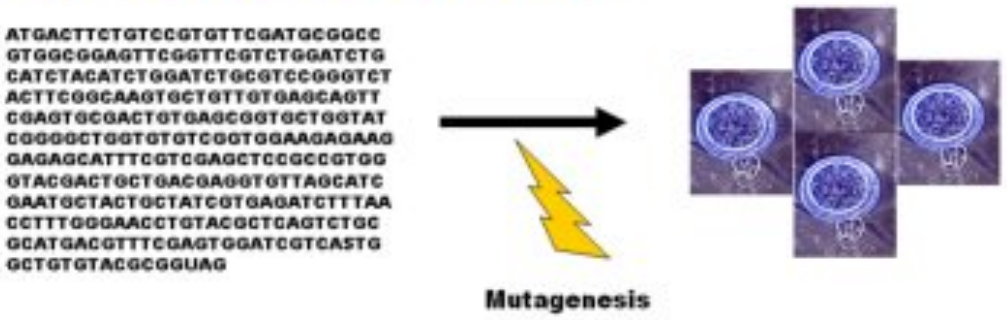
Methods of studying gene/protein function

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



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



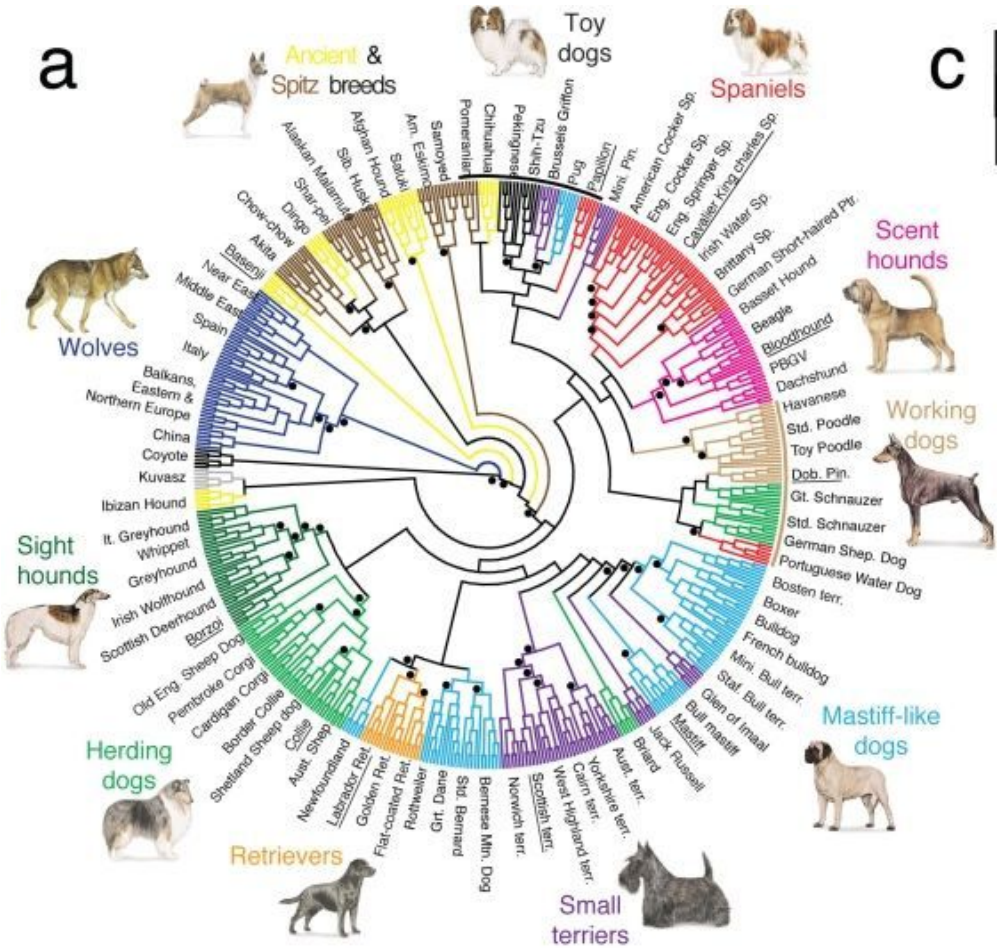
Gene gain- or loss-of-function
Caenorhabditis elegans
Drosophila melanogaster
Zebrafish
Mouse

Methods of studying gene/protein function

Forward Genetics:

Accidental mutagenesis

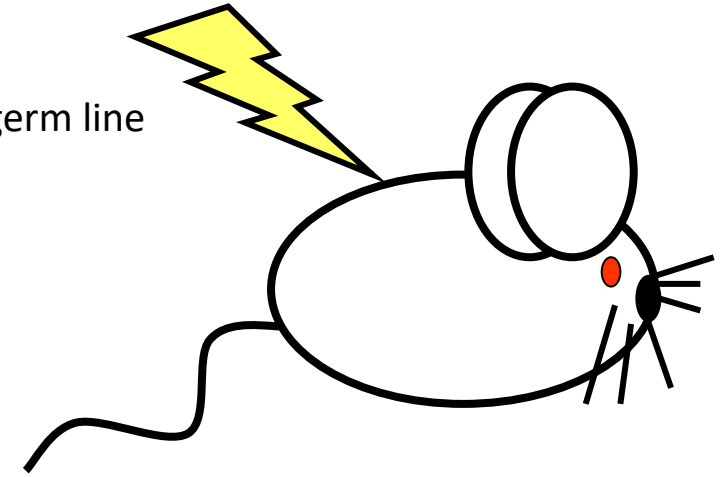
Mutations that have been maintained by breeding strategies to preserve desired phenotypical features



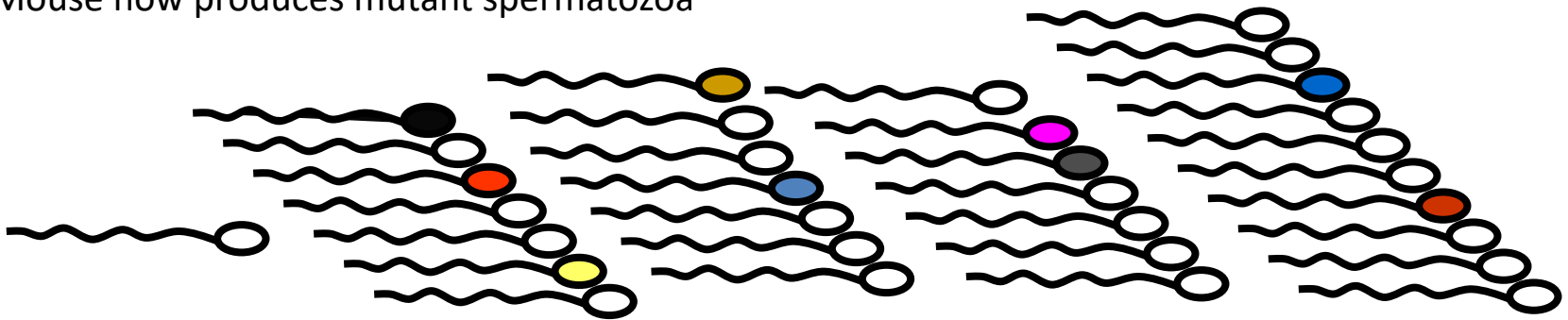
Methods of studying gene/protein function

Forward Genetics:
Induced random mutagenesis
Mutagen introduced in male germ line (ENU)
Breed with female

Mutagen hits the germ line



Mouse now produces mutant spermatozoa



Methods of studying gene/protein function

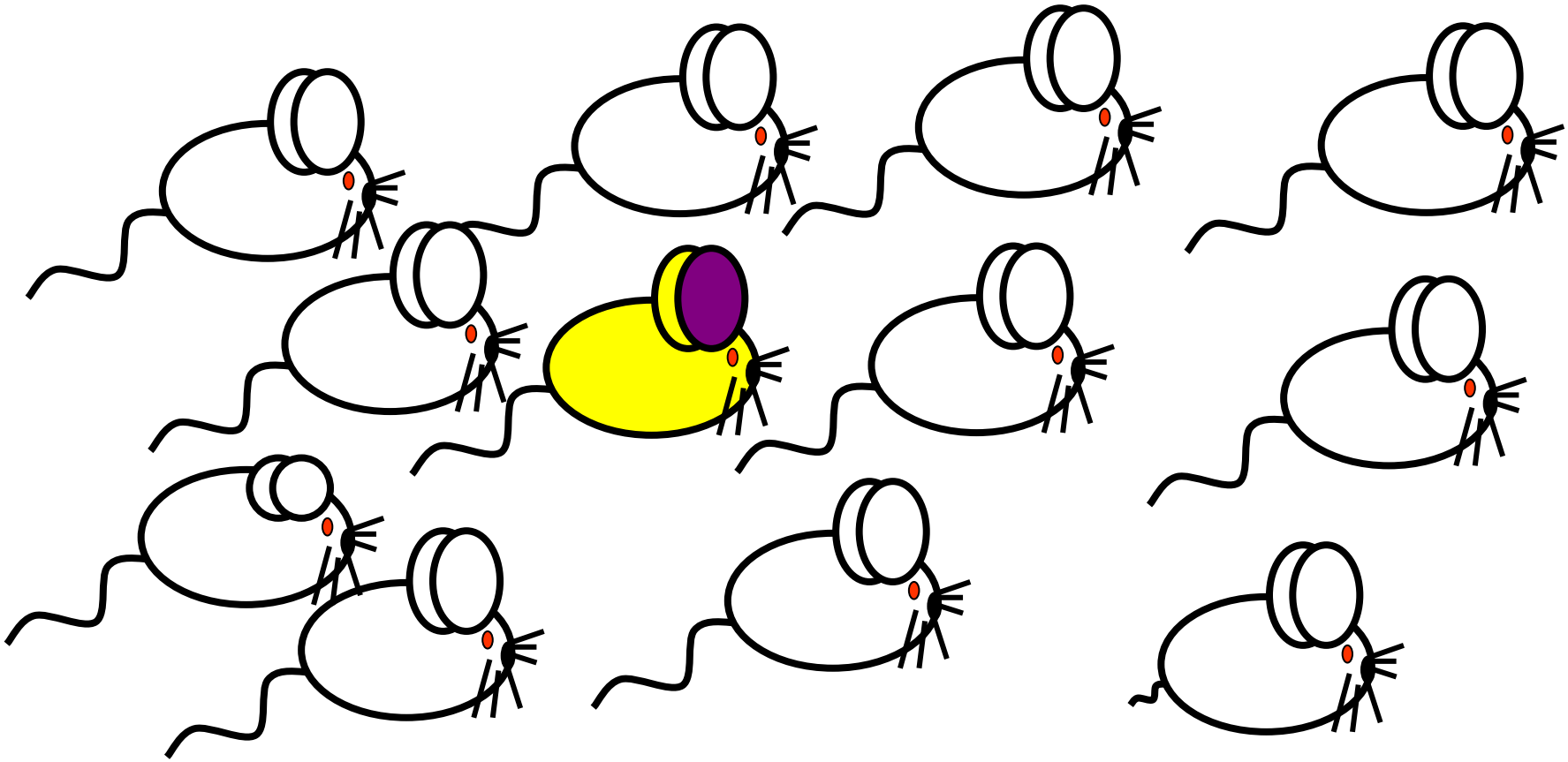
Forward Genetics:

Induced random mutagenesis

Screen offspring for desired abnormalities

Identify mutated gene

Lengthy process (months to years depending on species)



Methods of studying gene/protein function

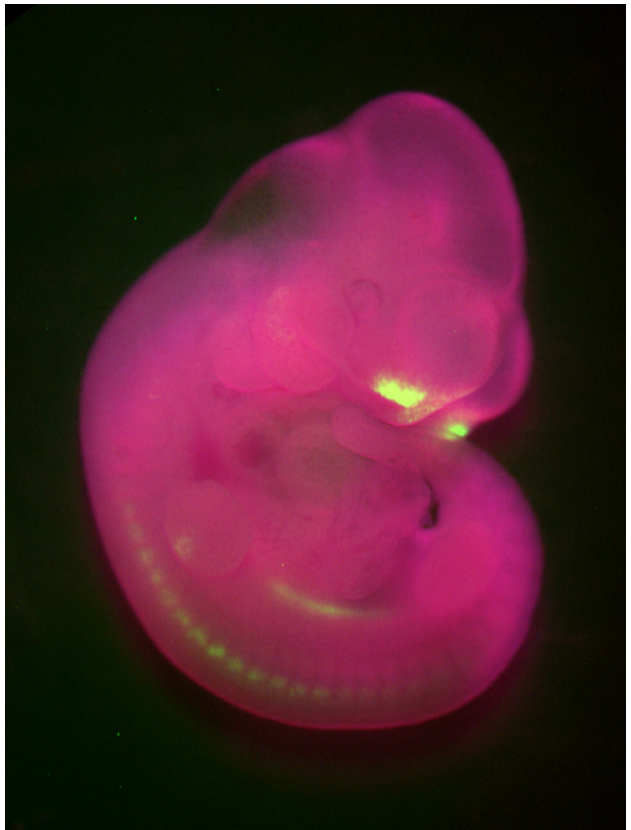
Reverse Genetics: Transgenesis

Gain- or loss of function

Introduction of transgenic constructs into the genome of zygotes

promoter + cDNA/shRNA/Fluorescent marker

Process takes days, weeks, or months depending on species



Methods of studying gene/protein function

Reverse Genetics: Knock Out Technology

Based on homologous recombination

Occurs in gametes during meiosis

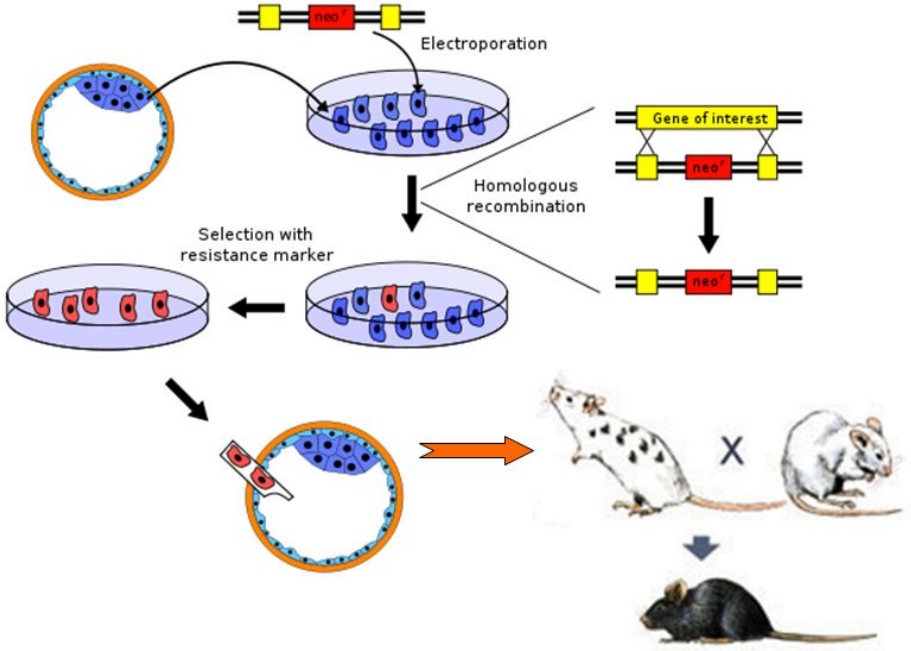
Inactivate gene in genome of omnipotent embryonic stem (ES) cells

Transfer mutant ES cells to host blastocysts: production of chimeras

ES cells give rise to all cell types in chimeras, including to gametes

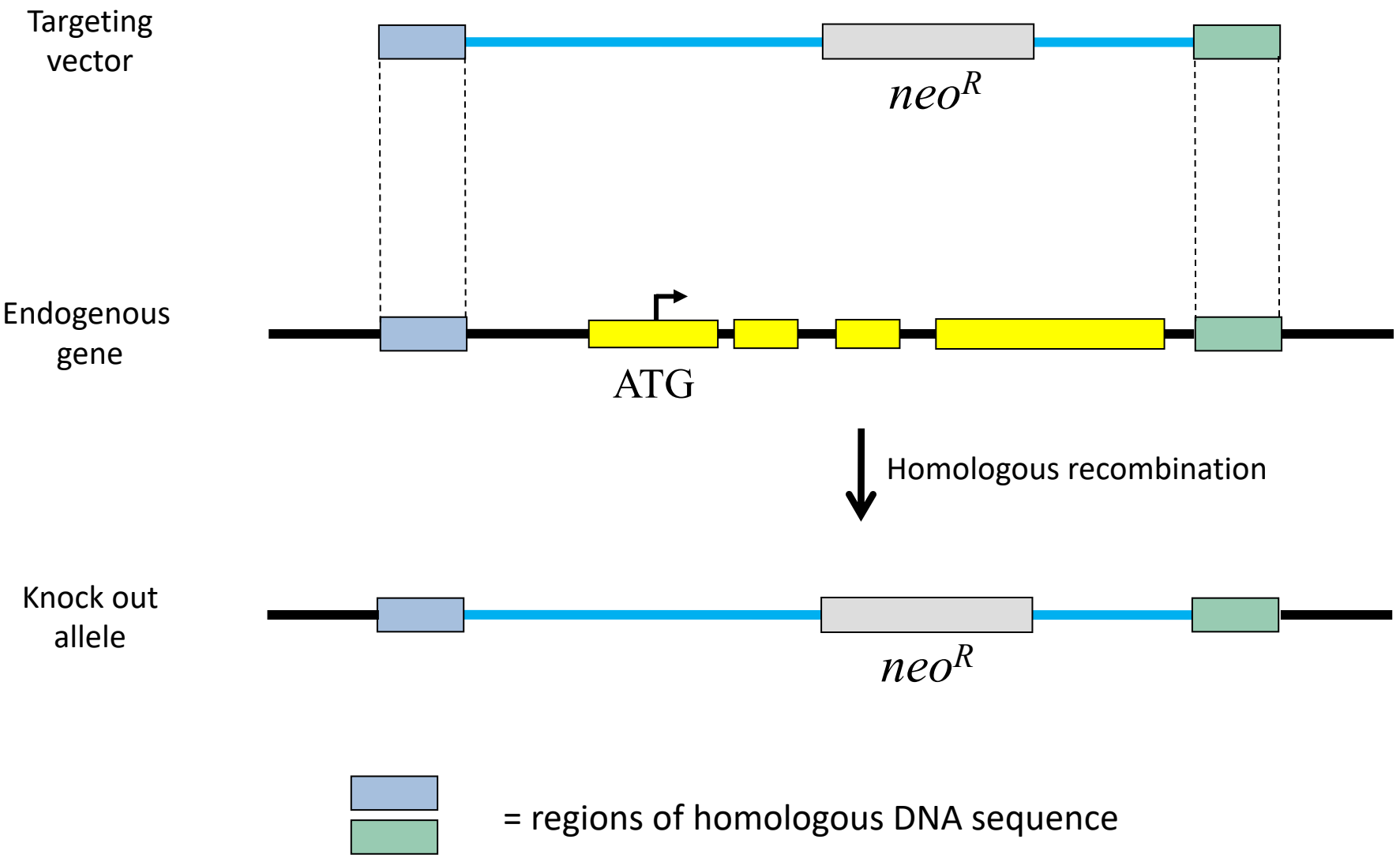
Breed to homozygosity

Done in mice only: process takes 1-2 years



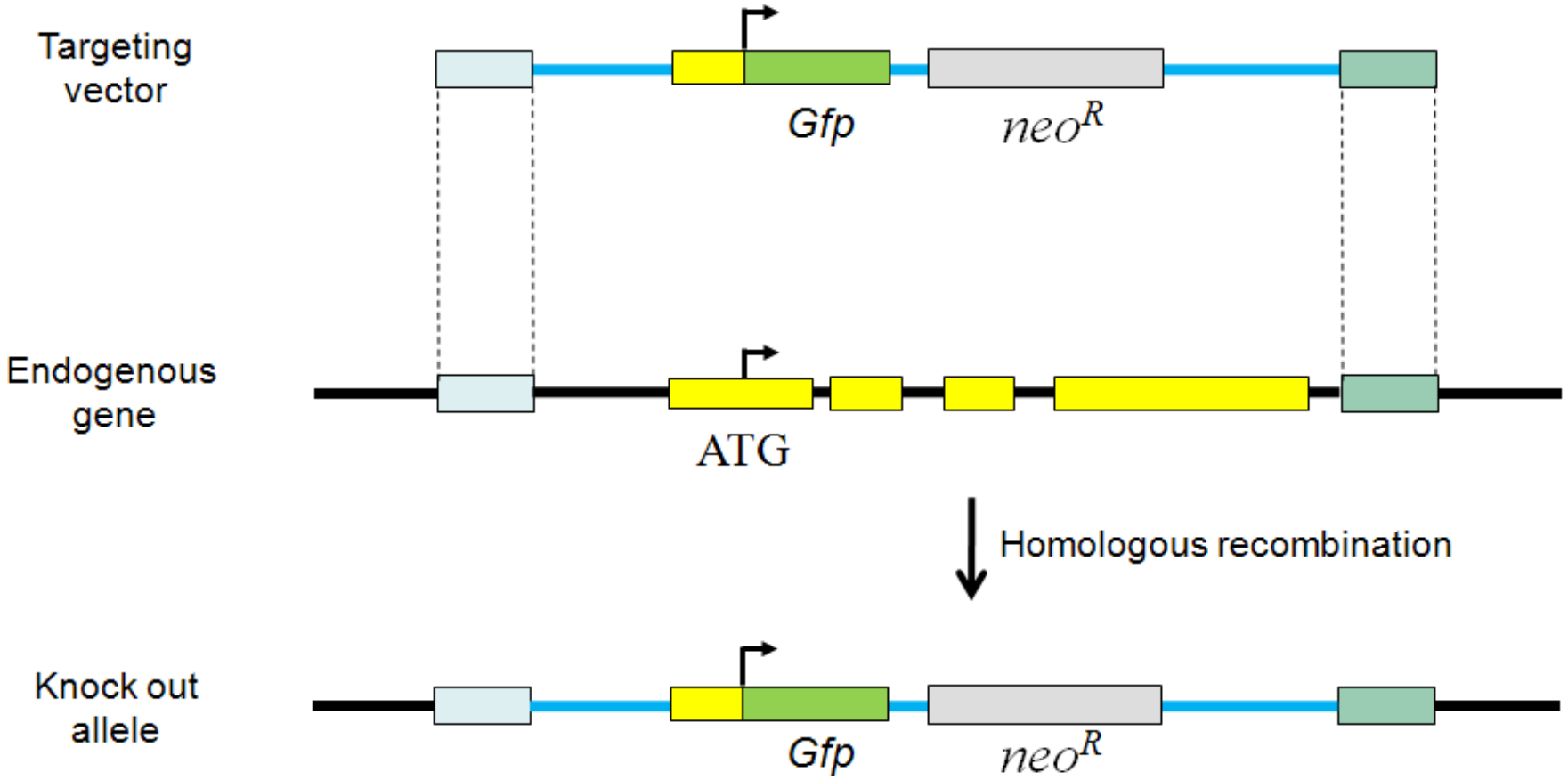
Methods of studying gene/protein function

Reverse Genetics: Knock Out Technology: excision of gene allele



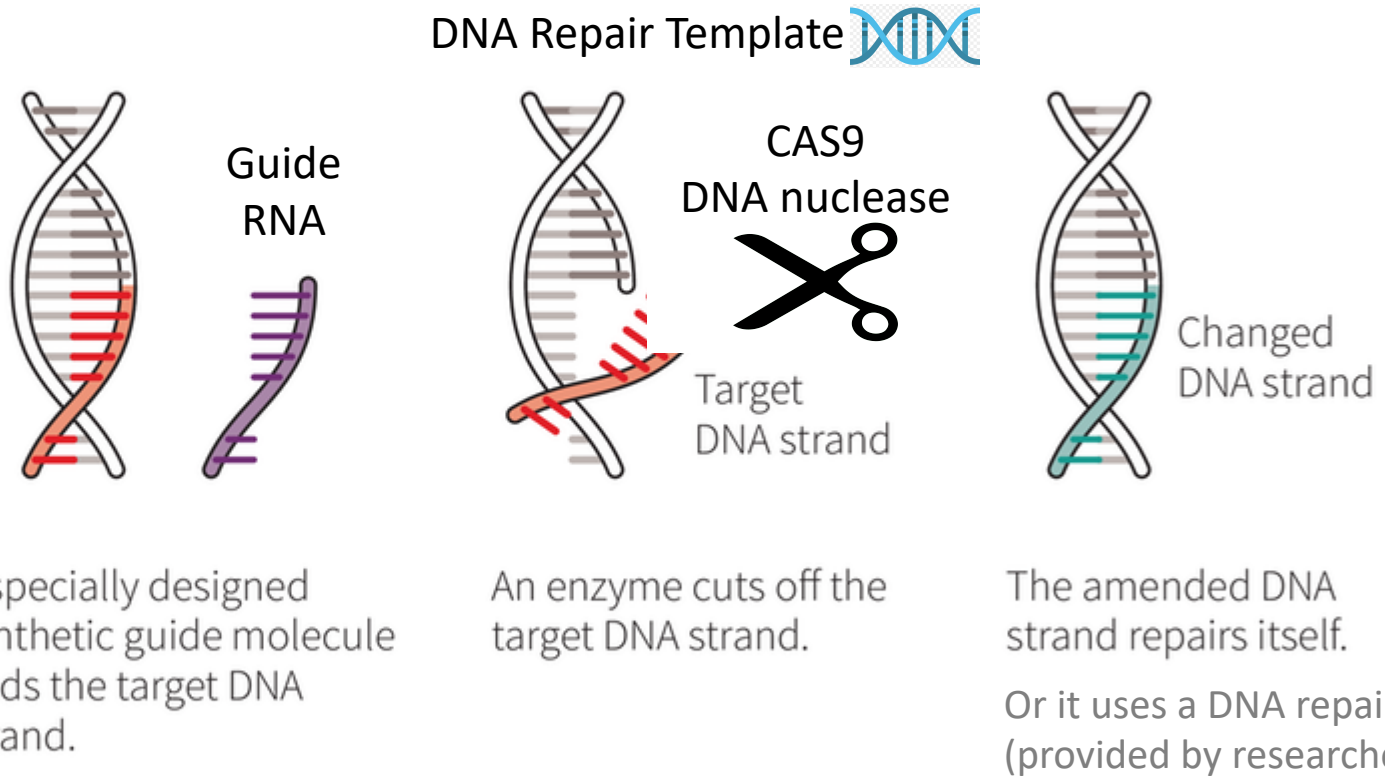
Methods of studying gene/protein function

Reverse Genetics: Knock Out Technology: Insertion of reporter gene into gene allele



Methods of studying gene/protein function

Reverse Genetics: CRSPR/CAS9 Genome Engineering: "Find and Replace"



Introduction of point mutations or engineered changes (DNA repair template)

Mutation of one or multiple genes

In ES cells or in zygotes/embryos of many species

Very quick relative to homologous recombination-based knock out technologies

Lecture 8

Research Technologies in Developmental Biology

Introduction

Animal models in Developmental biology

Lineage Tracing and Transplantation Studies

Gene and protein expression analysis methods

Methods to study gene/protein function in embryos

Chapter 5 in Larsen's *Human Embryology* (4th edition)
Chapter 4 in Scott Gilbert's *Developmental Biology* (8th edition)

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