Techniques used in Developmental Biology

Example: Fate Mapping

Fate Mapping – Vital Dye (1929)

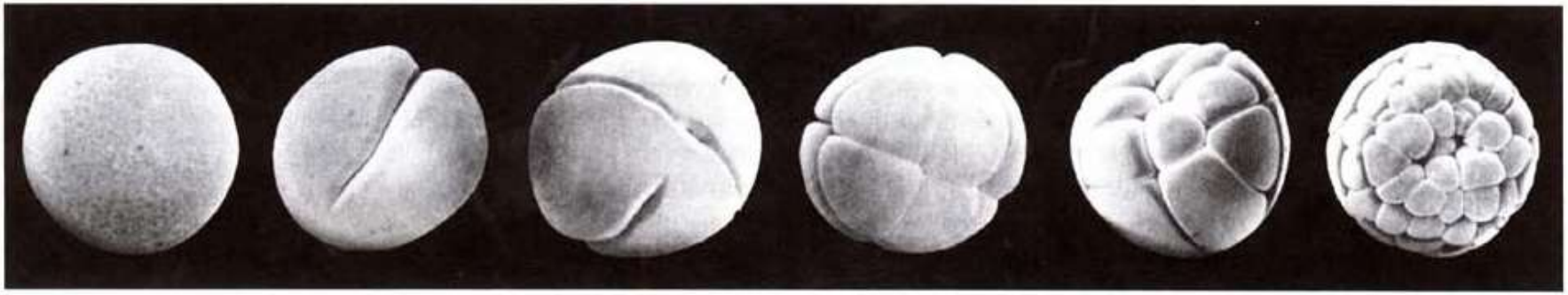


Fig. 2.5 Cleavage of the *Xenopus* embryo. The *Xenopus* embryo undergoes successive cleavages at intervals of about 20 minutes. Photographs courtesy of R. Kessel, from Kessel, R.G. *et al.*: 1974.

Walter Vogt

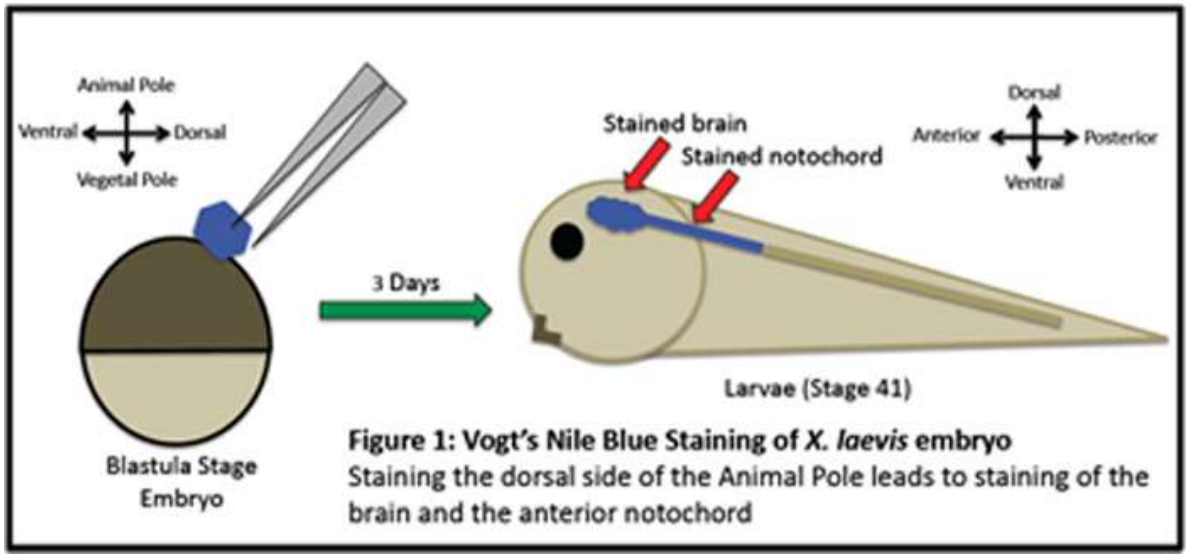
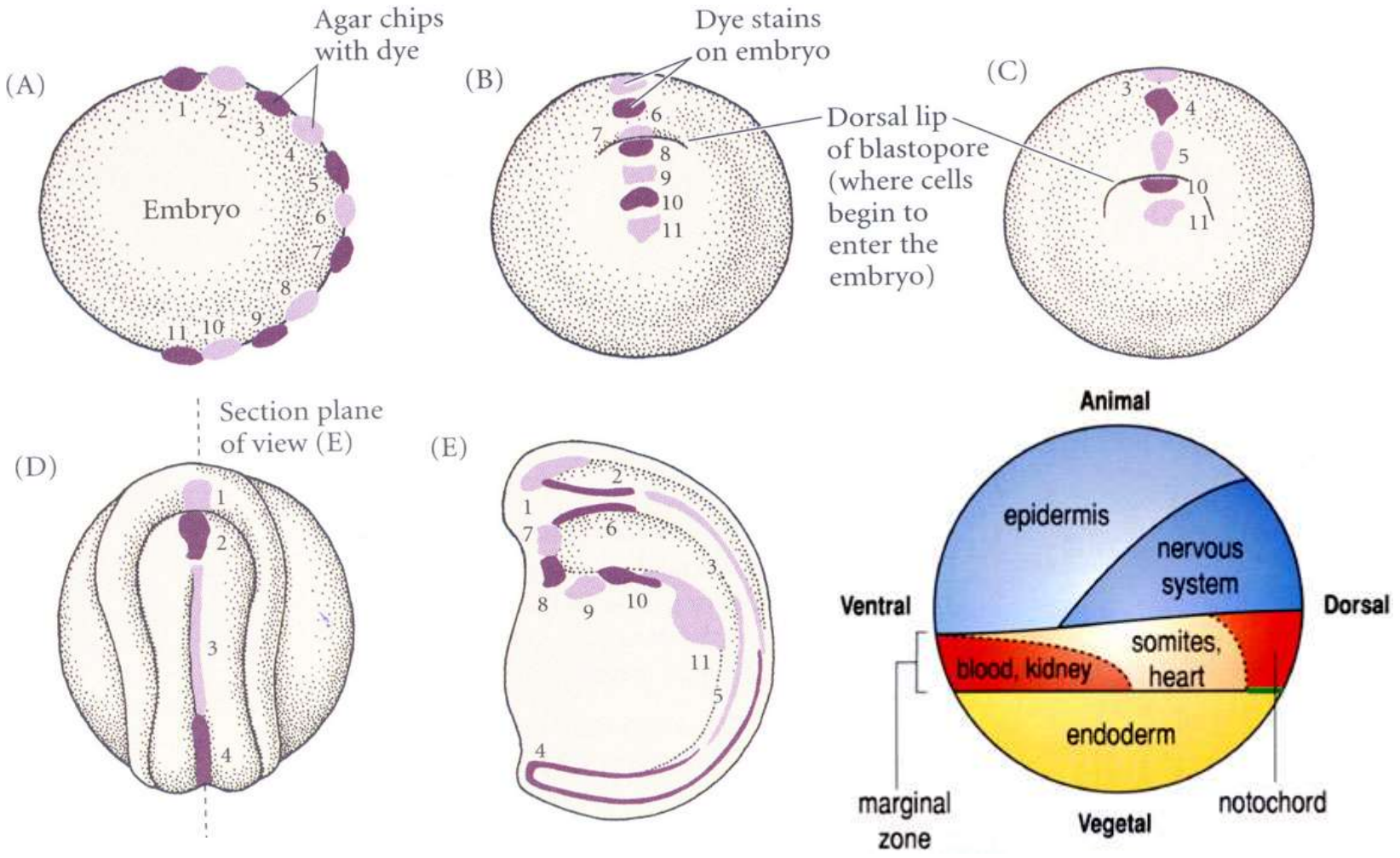


Figure 1: Vogt's Nile Blue Staining of *X. laevis* embryo
Staining the dorsal side of the Animal Pole leads to staining of the brain and the anterior notochord

Nile Blue (尼罗蓝) Staining



Disadvantage: Dye could diffuse ⟶ low resolution

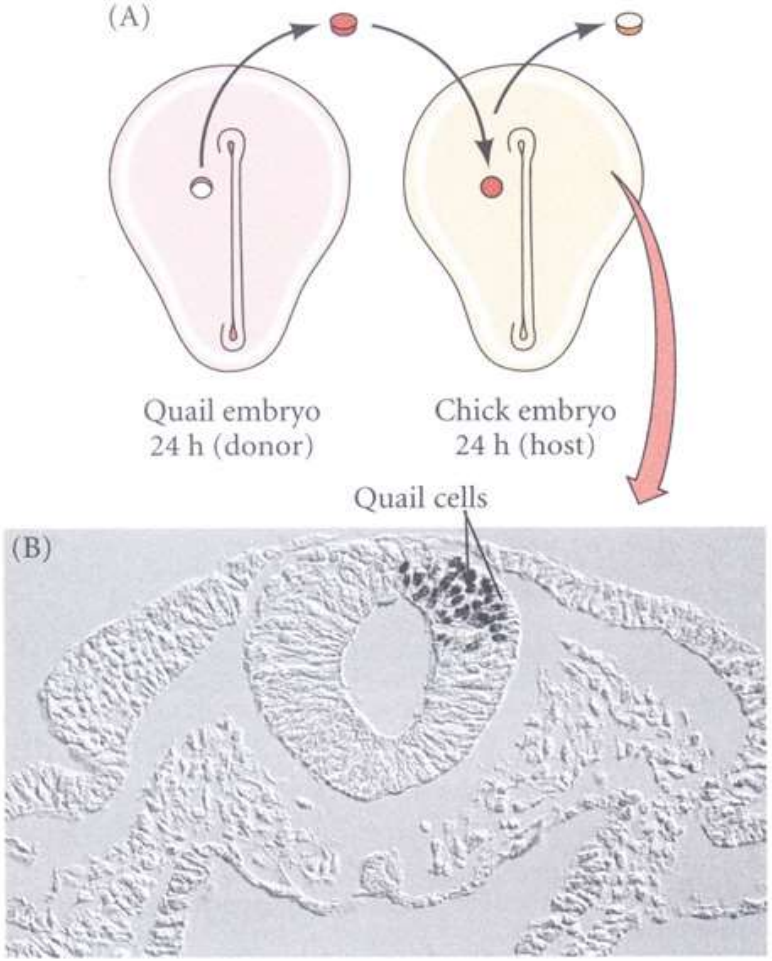
Fate Mapping – Cell Transplantation (1969)

Nicole Le Douarin

chick

quail

Fate Mapping – Cell Transplantation (1969)



Chick embryo with region of quail cells on the neural tube

chick–quail chimera

Resolution at cellular level

Fate Mapping – Fluorescent Dye (1980)

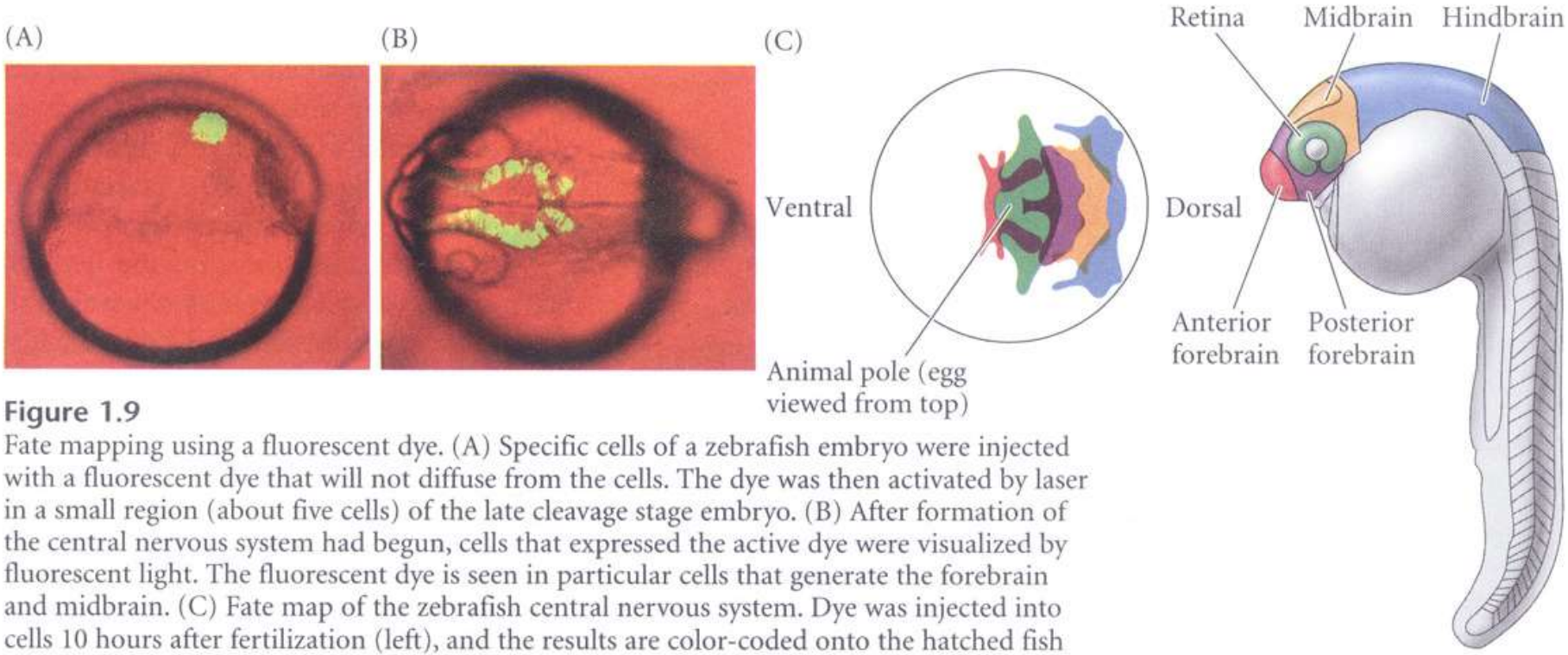
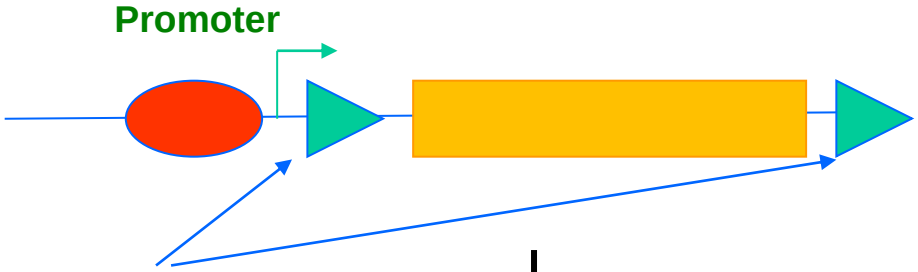


Figure 1.9
Fate mapping using a fluorescent dye. (A) Specific cells of a zebrafish embryo were injected with a fluorescent dye that will not diffuse from the cells. The dye was then activated by laser in a small region (about five cells) of the late cleavage stage embryo. (B) After formation of the central nervous system had begun, cells that expressed the active dye were visualized by fluorescent light. The fluorescent dye is seen in particular cells that generate the forebrain and midbrain. (C) Fate map of the zebrafish central nervous system. Dye was injected into cells 10 hours after fertilization (left), and the results are color-coded onto the hatched fish (right). Overlapping colors indicate that cells from these regions of the 6-hour embryo contribute to two or more regions. (A, B from Kozlowski et al. 1998; photographs courtesy of E. Weinberg. C after Woo and Fraser 1995.)

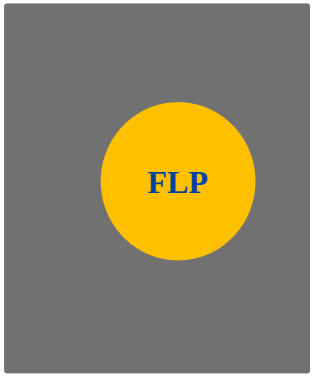
Fate Mapping – Genetic cell lineage tracing (1990)

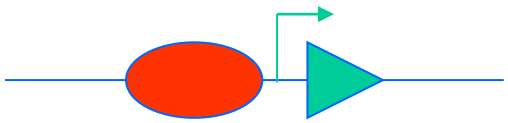
FLP – FRT system (Drosophila)

y- ; actin – FRT – y+ – FRT

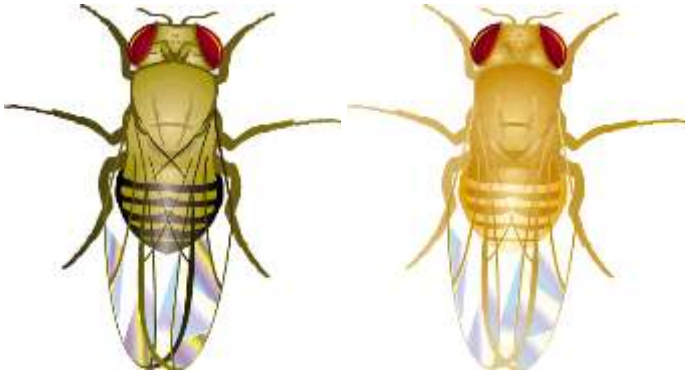


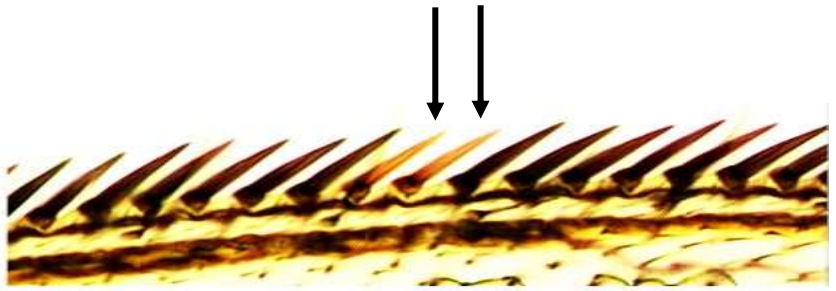
hs-FLP (Flipase)



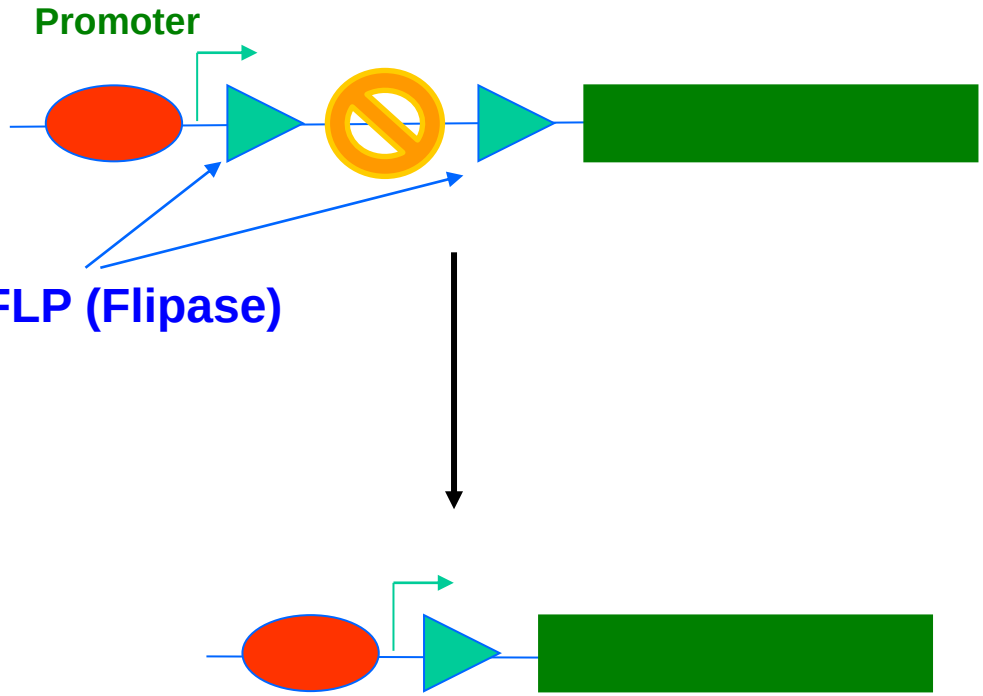


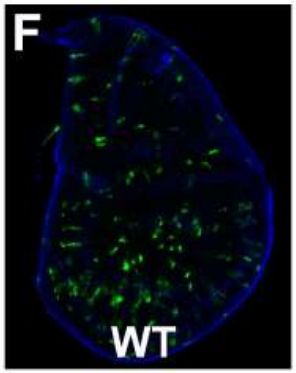
y- ; act – FRT

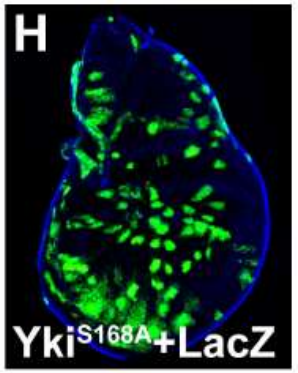




act – FRT – STOP– FRT – GFP

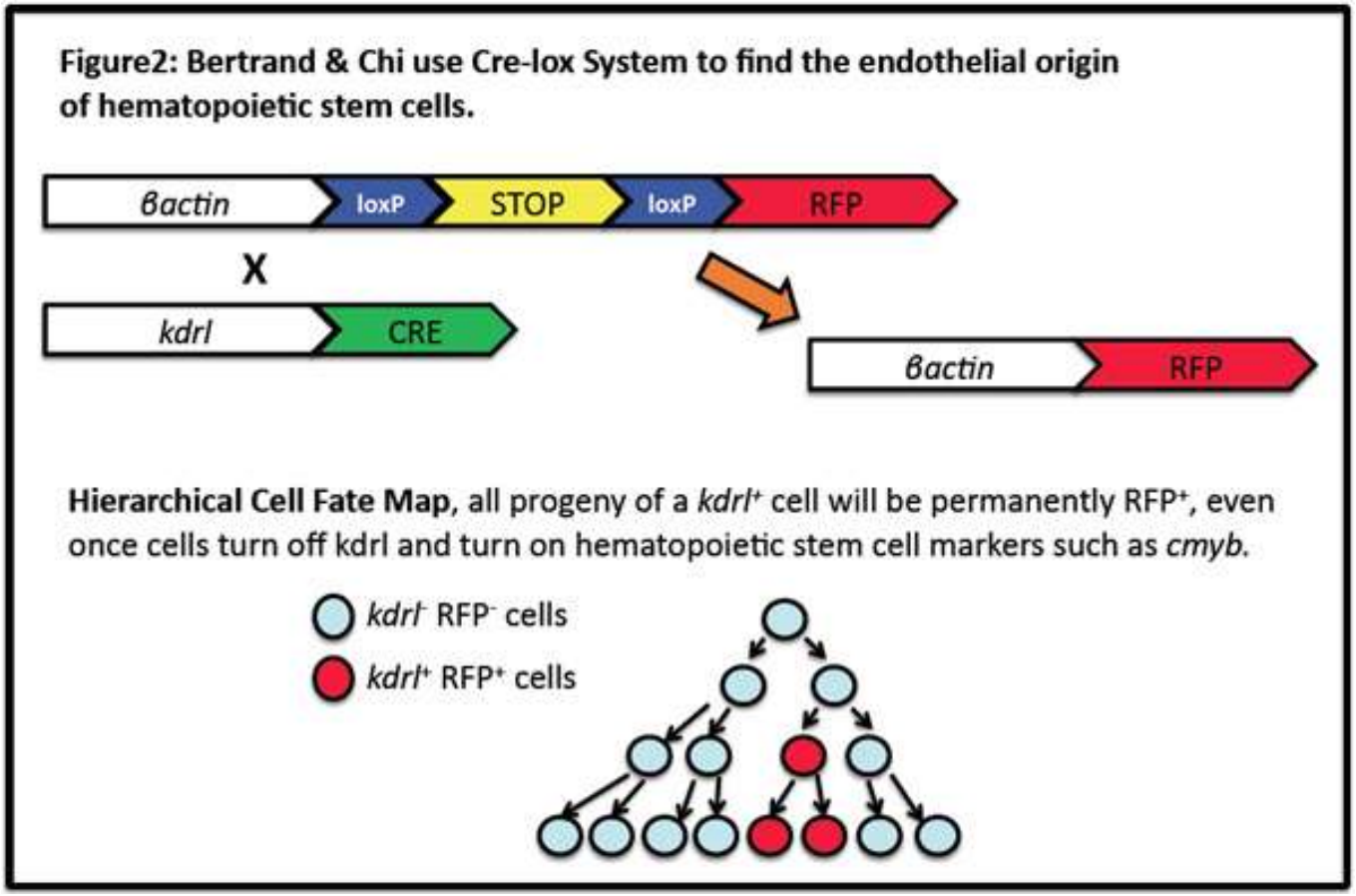






Fate Mapping – Genetic cell lineage tracing (2000)

Cre – LoxP system (mammal)



To study the expression a gene

mRNA - Transcription

Protein - Translation

Detection of gene expression

mRNA

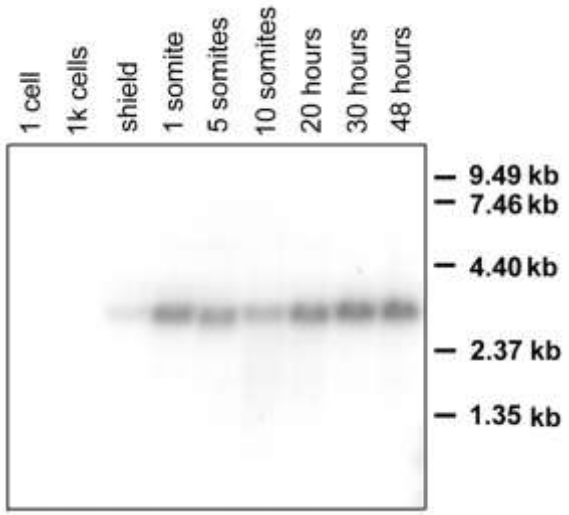
Northern blot

in situ hybridization

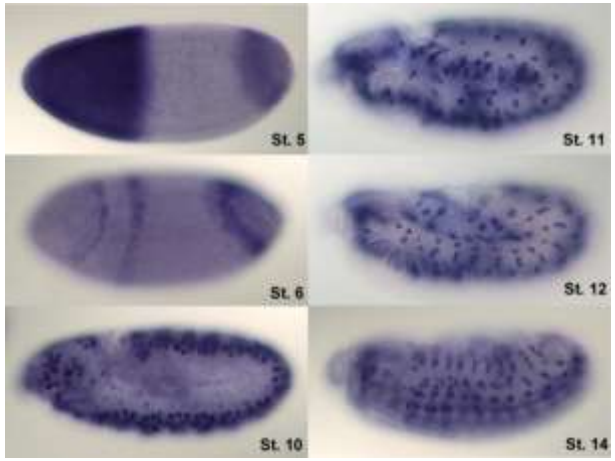
RT-PCR

DNA Microarray (GeneChip)

Northern blot

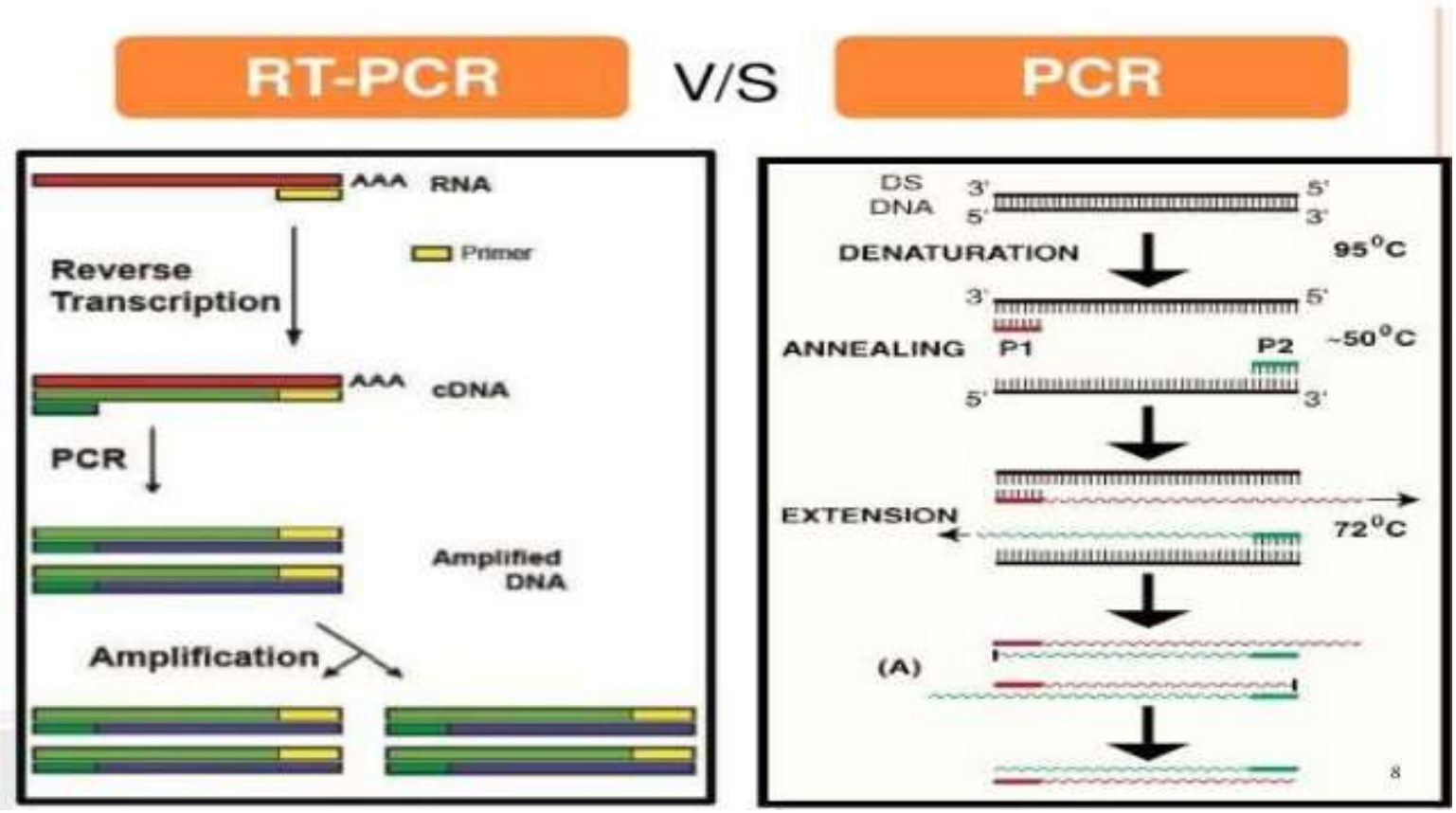


in situ hybridization



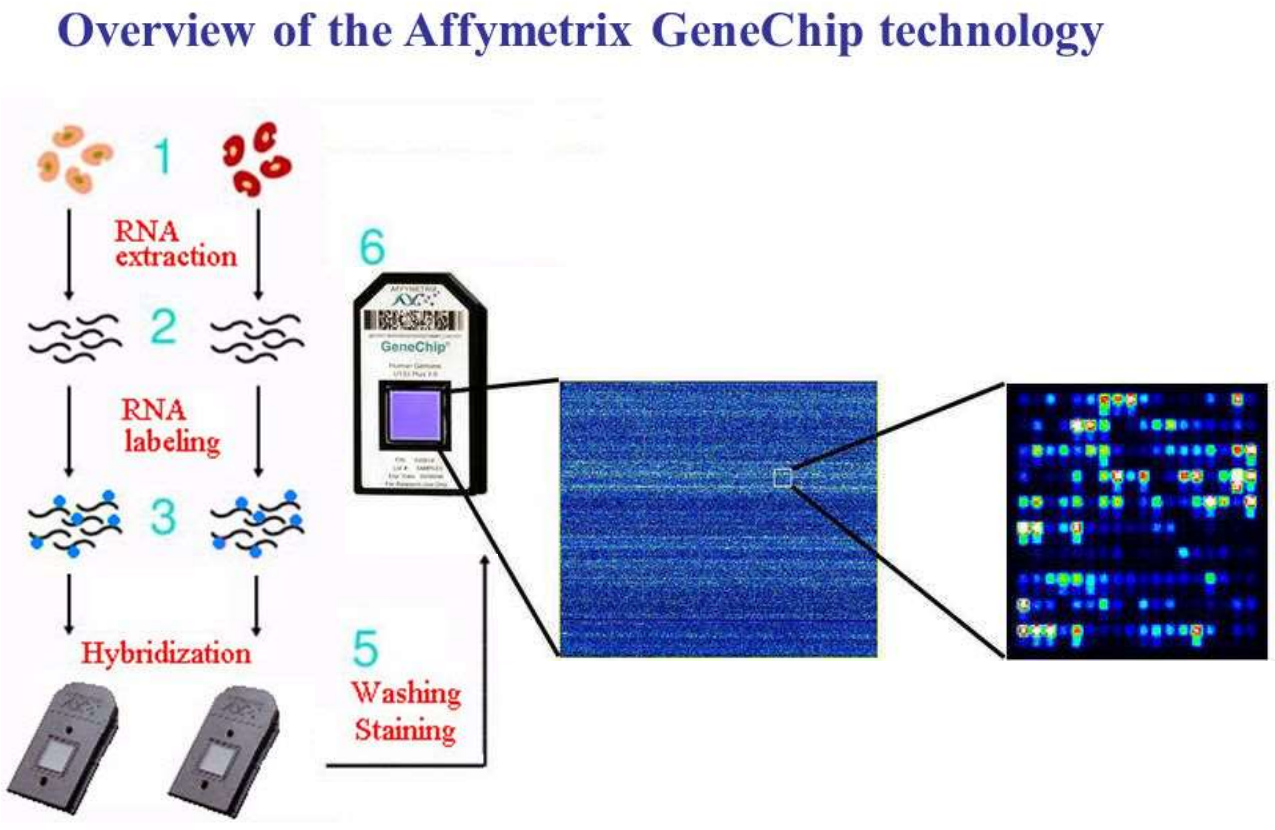
RT-PCR

(reverse transcription-polymerase chain reaction)



DNA Microarray

（GeneChip）



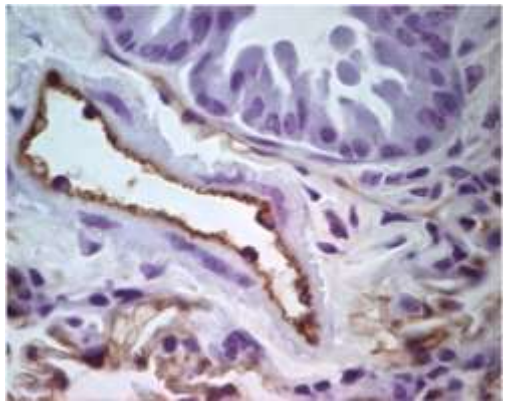
Detection of gene expression

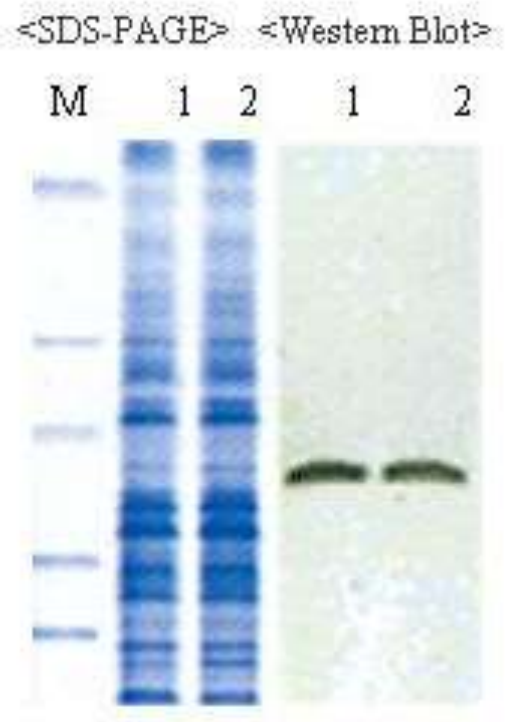
Protein

Protein gel electrophoresis (SDS-PAGE)

Western blot

Immunostaining

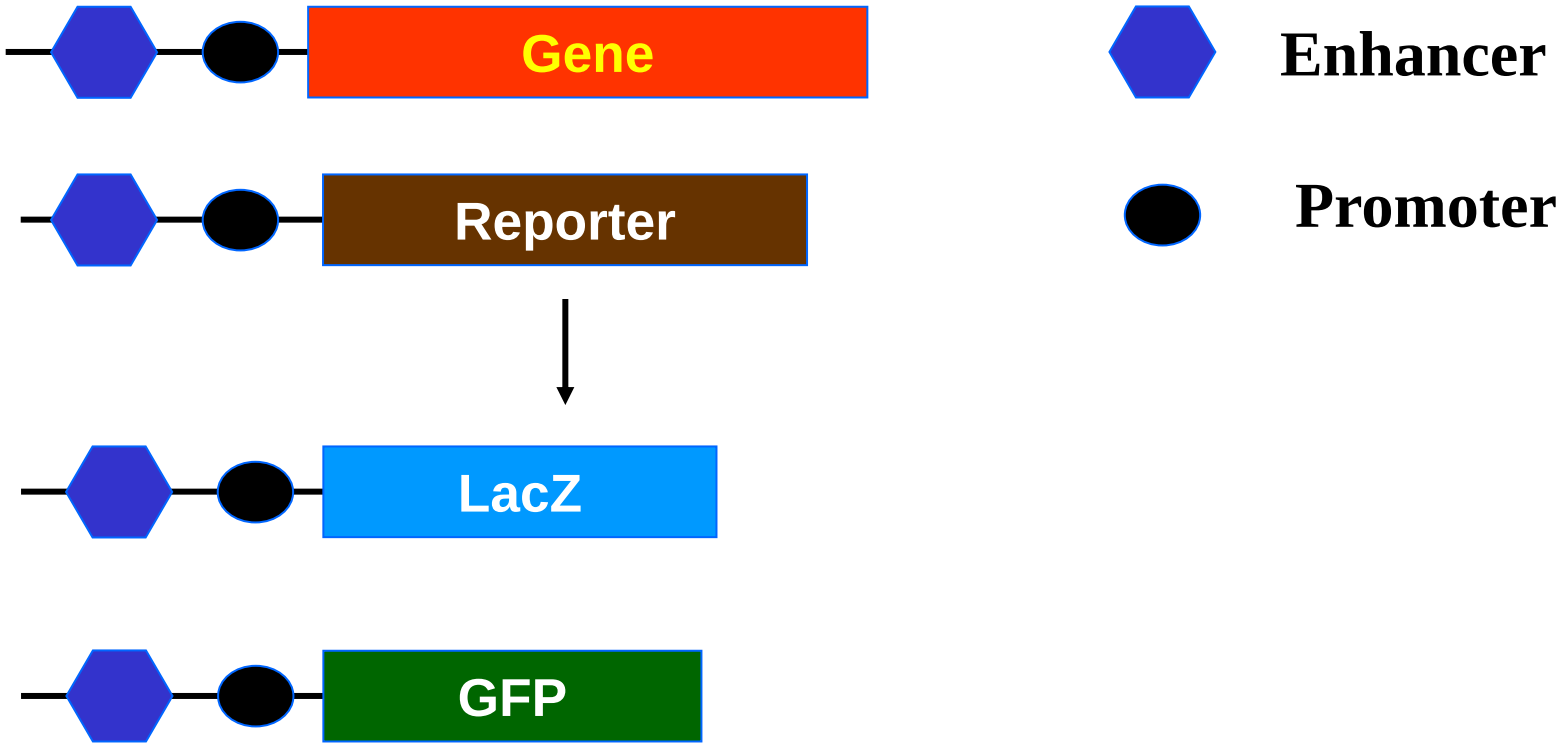




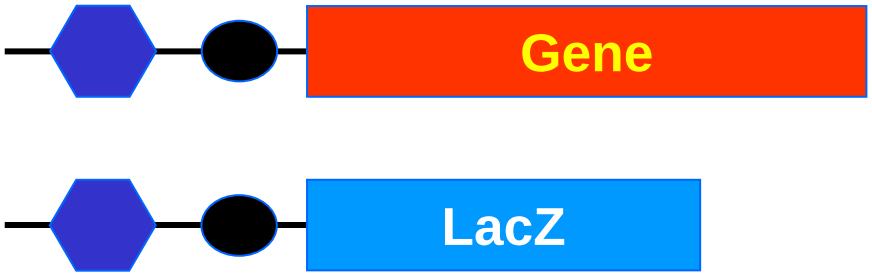
Expression of a gene is controlled

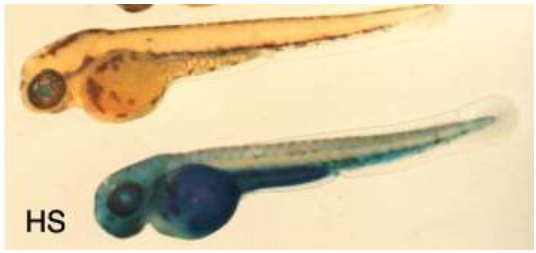
by

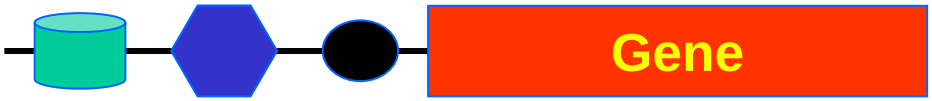
Enhancer & Promoter

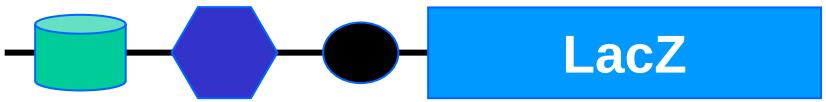


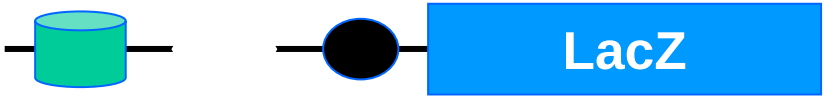
Detection of gene expression

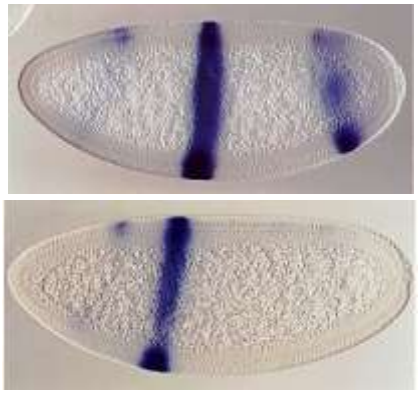




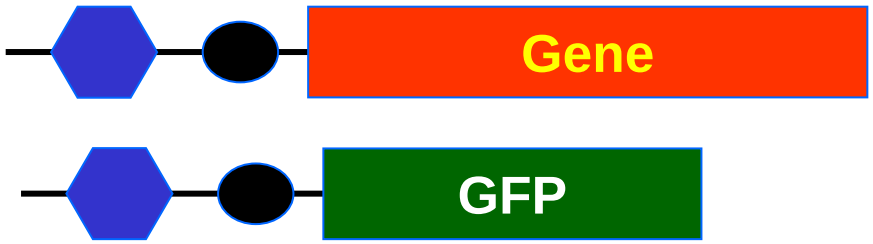


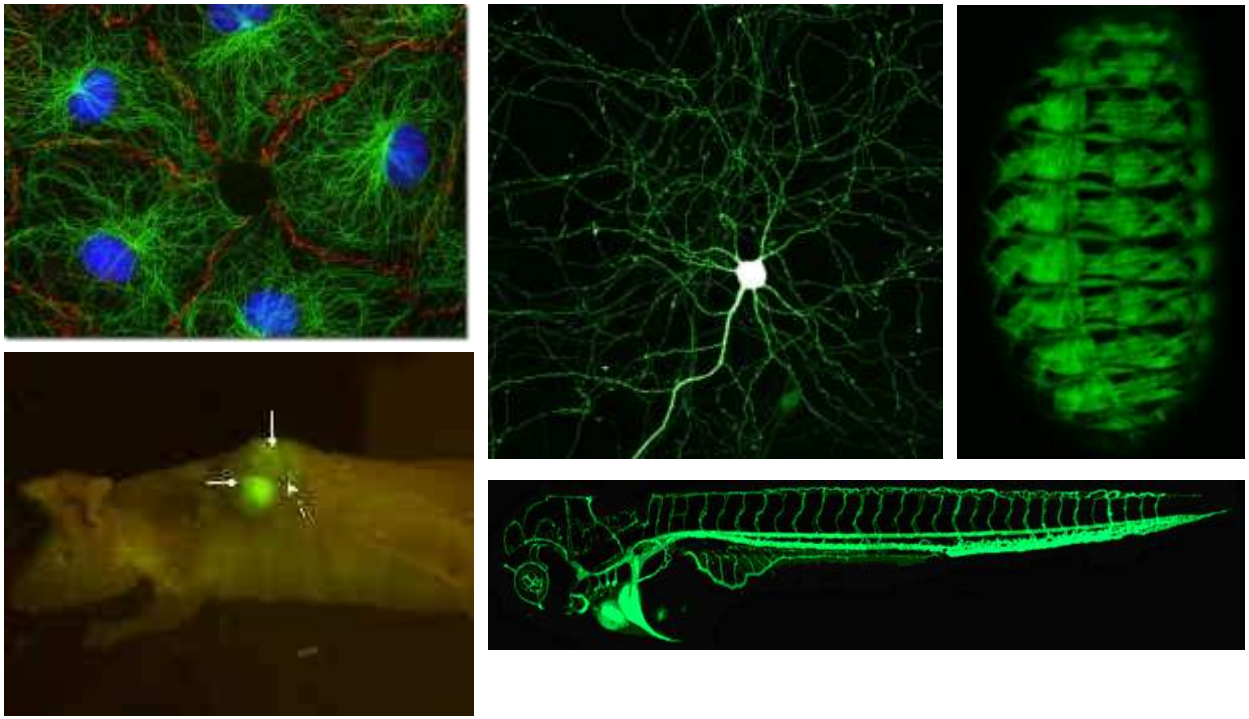






Detection of gene expression





To study the function of a gene

1. **Loss of function → Phenotype**
   - **Mutation**
   - **RNAi**

Nature, 1998

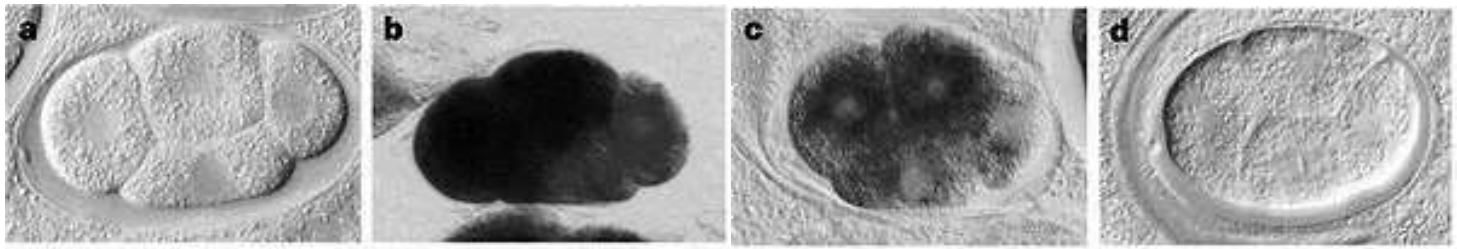
Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire[1], SiQun Xu[1], Mary K. Montgomery[1], Steven A. Kostas[1,2], Samuel E. Driver[3] and Craig C. Mello[3]

in situ hybridization of 4-cell stage embryos

Negative control

mex-3 mRNA expression



antisense RNA

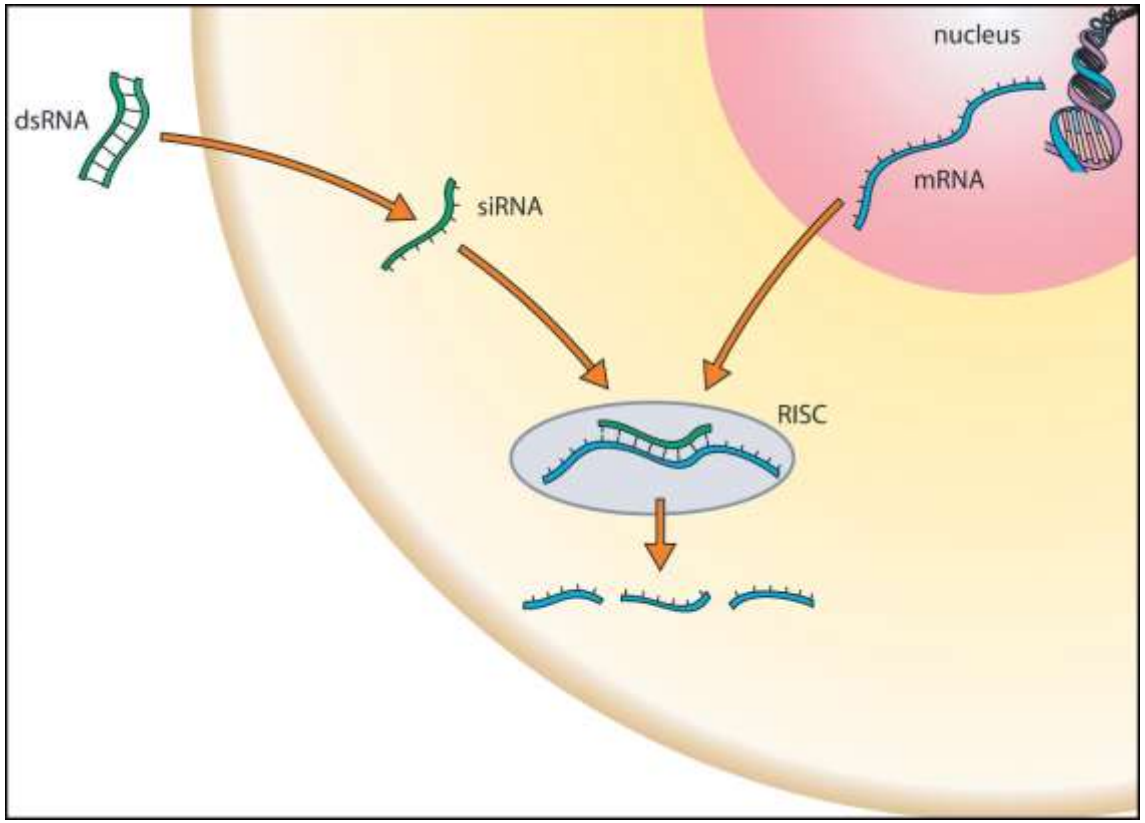
dsRNA

The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

Andrew Fire

Craig Mello



To study the function of a gene

1. **Loss of function → Phenotype**
   - **Mutation**
   - **RNAi**
   - **Dominant negative**

Necessary 必要

2. **Gain of function → Phenotype**
   - **Over-expression**
   - **Constitutive active**

Sufficient 充分

Mutation – by effect on structure

Small-scale mutations:

- change one or a few nucleotides
- affecting a single gene

Large-scale mutations:

- change of chromosomal structure
- affecting multiple genes

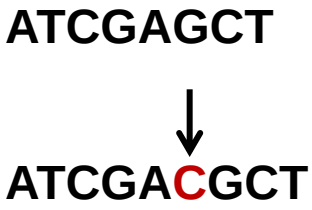
Small-scale mutations:

Point mutations

- Change of a single nucleotide in the DNA

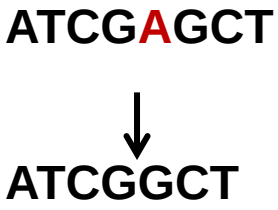
Insertions

- Add one or more extra nucleotides into the DNA.



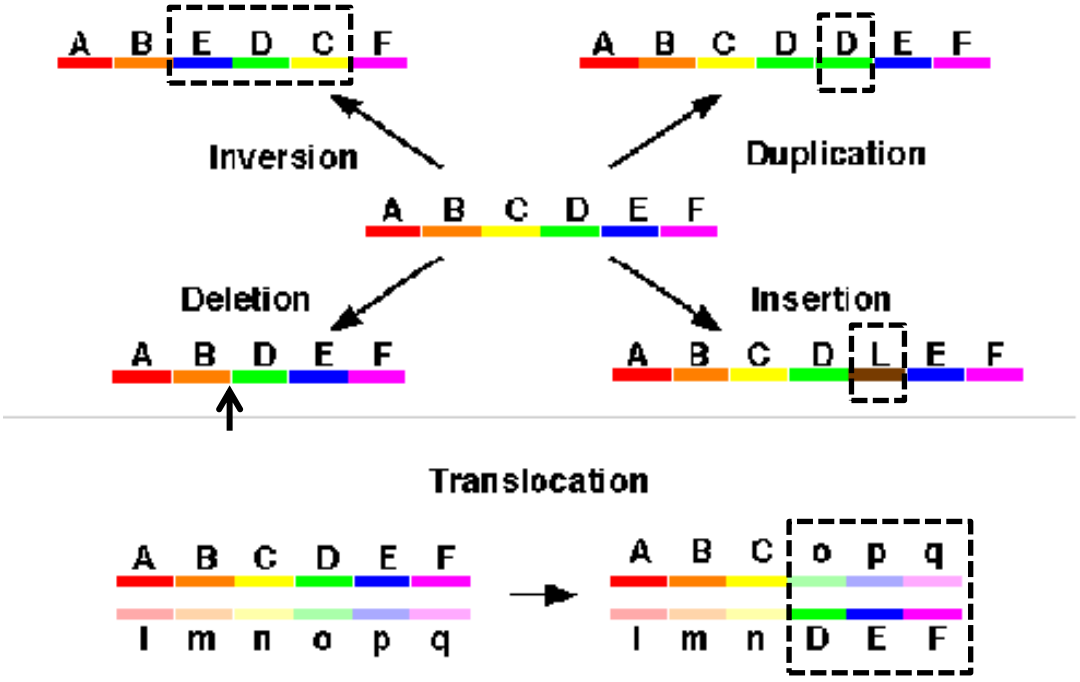
Deletions

- Remove one or more nucleotides from the DNA.



Large-scale mutations:

- Inversions: reversing the orientation of a chromosomal segment
- Duplication: leading to multiple copies of a chromosomal region
- Insertion: A chromosome region is inserted into another chromosome
- Deletions: leading to loss of the genes within those regions
- Translocations: interchange of genetic parts between chromosomes



Mutation – by effect on function

Loss-of-function mutations

the gene product having less or no function.

- Amorphic mutation: complete loss of function
- Hypomorphic mutation: partial loss of function

Phenotypes associated with such mutations are most often recessive.

Gain-of-function mutations

the gene product gains a new and abnormal function.

these mutations usually have dominant phenotypes.

Dominant negative mutations

have an altered gene product that interfere with the wild-type gene product.

Mutation – by effect on fitness

Harmful mutation:

decreases the fitness of the organism.

Beneficial mutation:

increases the fitness of the organism.

Neutral mutation:

has no harmful or beneficial effect on the organism.

Mutation – by effect on protein sequence

Silent mutation:

- a point mutation that does not result in a change to the amino acid.

Neutral mutation:

- a point mutation that results in a different, but chemically similar amino acid.

Missense mutation:

- a point mutation that cause substitution of a different amino acid, which renders the resulting protein less functional.

Nonsense mutation:

- a point mutation that results in a premature stop codon, and a truncated protein.

Frameshift mutation:

- insertion or deletion of a number of nucleotides that is not evenly divisible by three, disrupt the reading frame and results in different translation of amino acids.

Mutation – by effect on protein sequence

Silent mutation:

UUA - UUG

Neutral mutation:

CUU - AUU

Missense mutation:

CUU - GUU

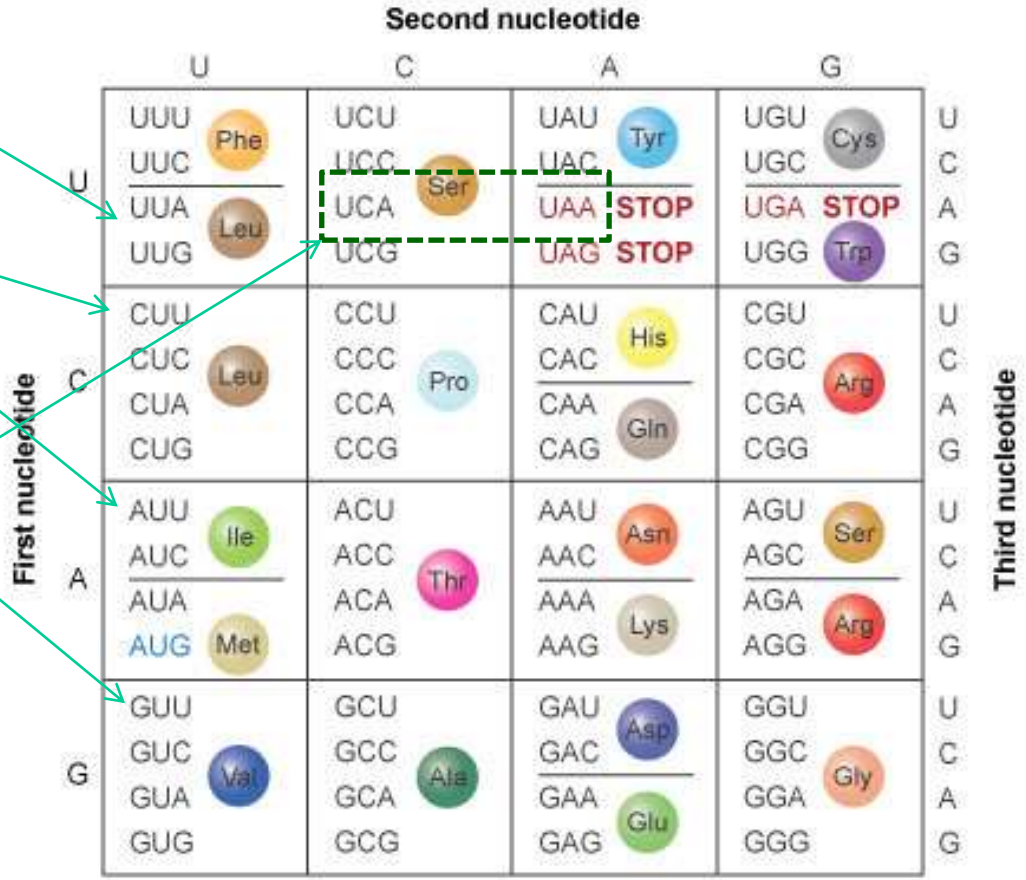
Nonsense mutation:

UCA - UAA

Frameshift mutation:

AUG UUA UCA

AUG UA UCA



Mutagenesis

Radiation:

X-ray

The Nobel Prize in Physiology or Medicine 1933

"for his discoveries concerning the role played by the chromosome in heredity "

Thomas Hunt Morgan

(1866 –1945)

1906, began his work on D. melanogaster at Columbia University

1910, reported the white eyed mutant – the first gene (white) identified

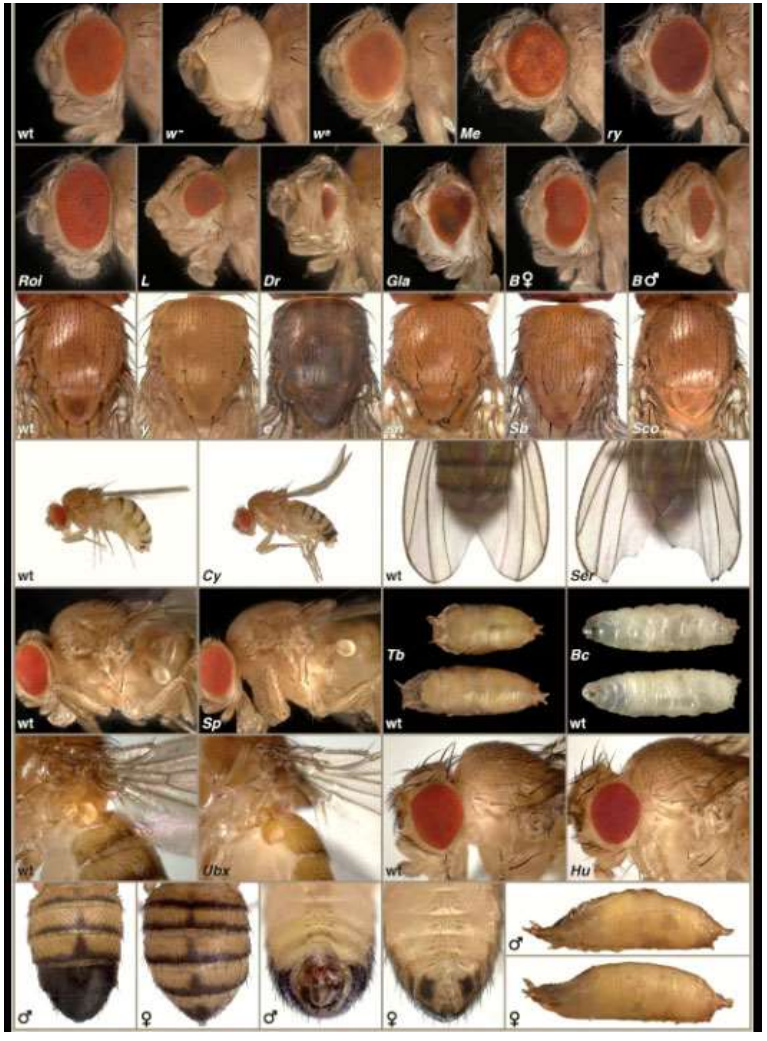
1933, rewarded Nobel Prize in Medicine

The Nobel Prize in Physiology or Medicine 1946

"for the discovery of the production of mutations by means of X-ray irradiation "

Hermann Joseph Muller

(1890 –1967)



Mutagenesis

Radiation:

X-ray

UV

Discovery: 1890s, in Bordeaux, France.
- wine workers showed an high incidence of **skin cancer** on the back of the neck.

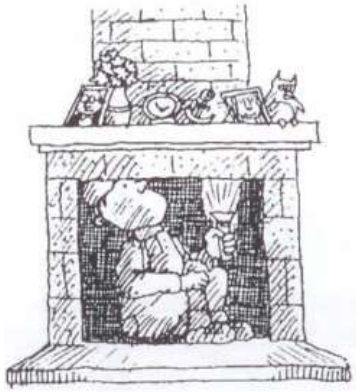
Explanation: They exposed the back of their necks to the **sun** while bending over in the fields picking grapes.

Conclusion: The ultraviolet (**UV**) radiation in natural sunlight was later identified as a **mutagen**.

Mutagenesis

Radiation:

Chemical:



Discovery: 1775 in England, Dr. Pott noticed a high incidence of **cancer** in chimney sweeps.

Explanation: chimney soot contained **carcinogens** that could cause cancer.

Conclusion: Over 150 years later, chimney soot was found to contain **hydrocarbons** (烃) capable of **mutating DNA**.

Mutagenesis

Radiation:

Chemical:

Transposons: transposable element

Barbara McClintock
(1802 –1992)

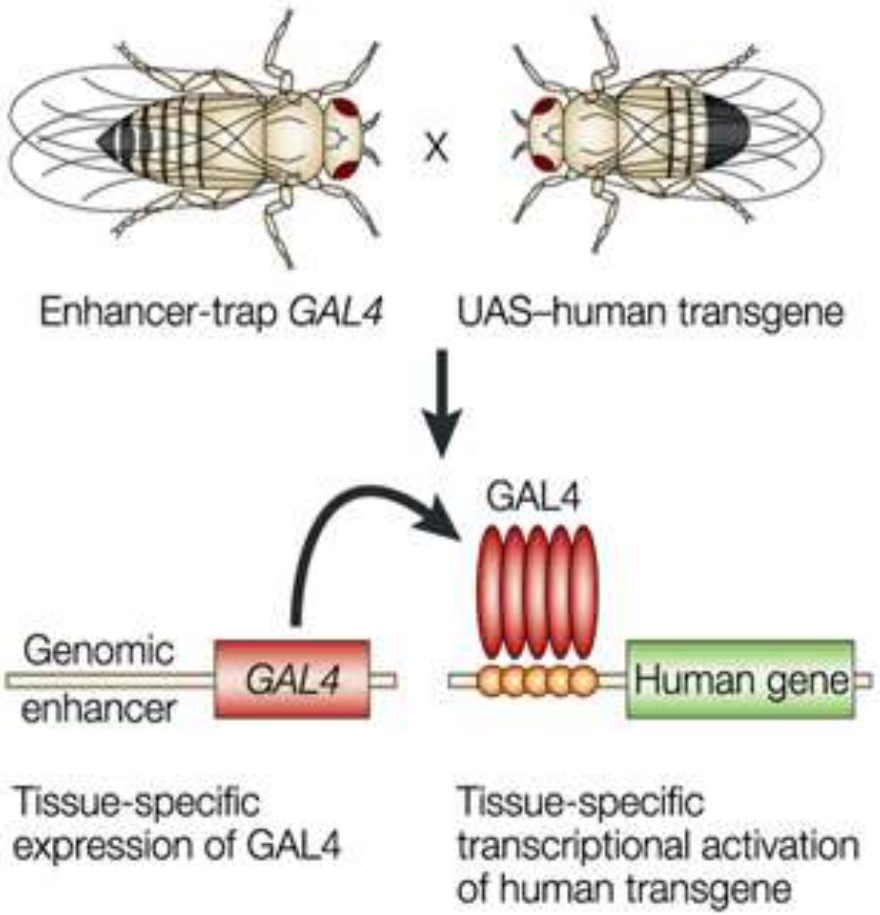
jumping genes

The Nobel Prize in Physiology or Medicine 1983

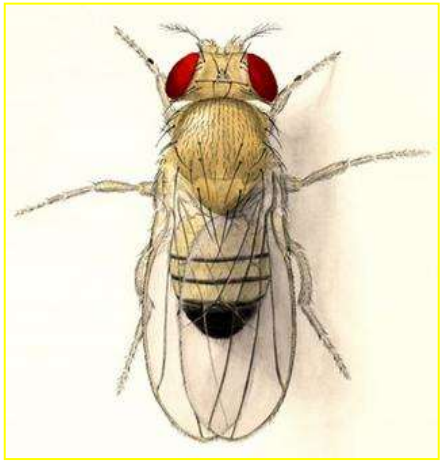
"*for her discovery of mobile genetic elements*"

Gain of function phenotype

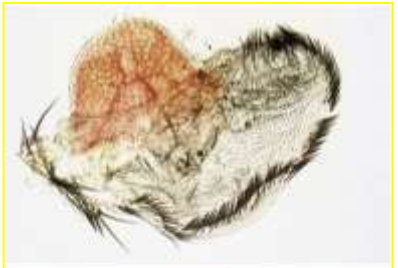
Over-expression



Induction of ectopic eyes by targeted expression of the eyeless gene



Ectopic Eyeless expression



Expression of apoptotic genes induce cell death