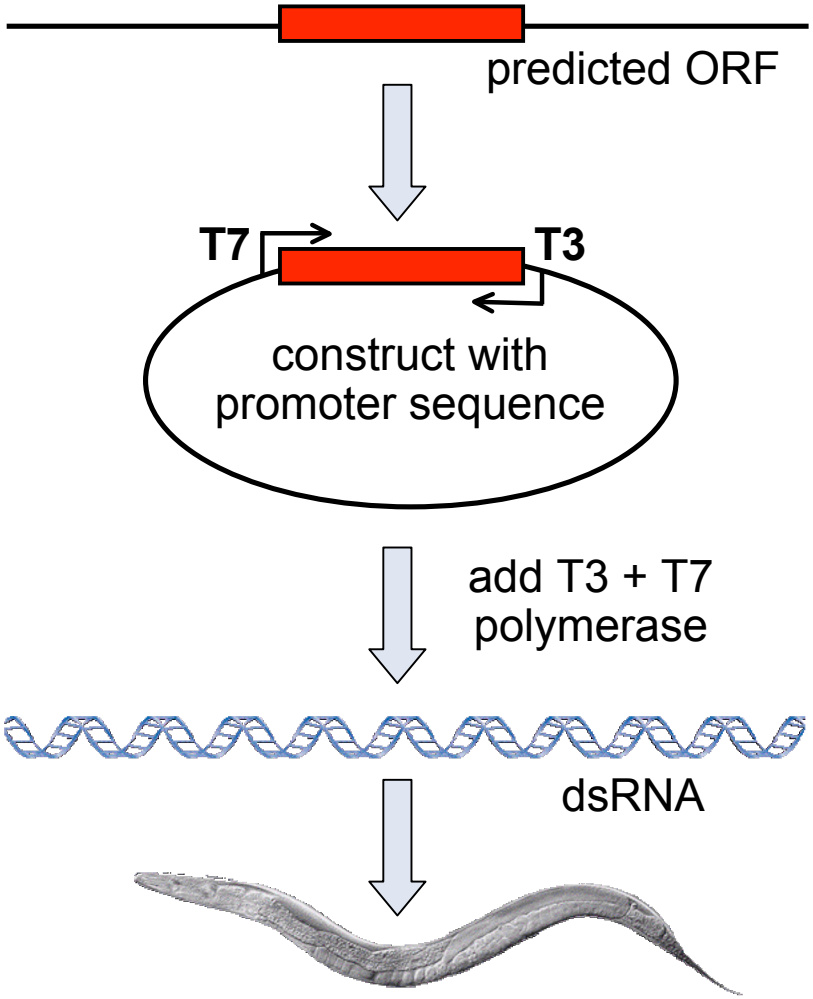
# Lecture 15: Functional Genomics II

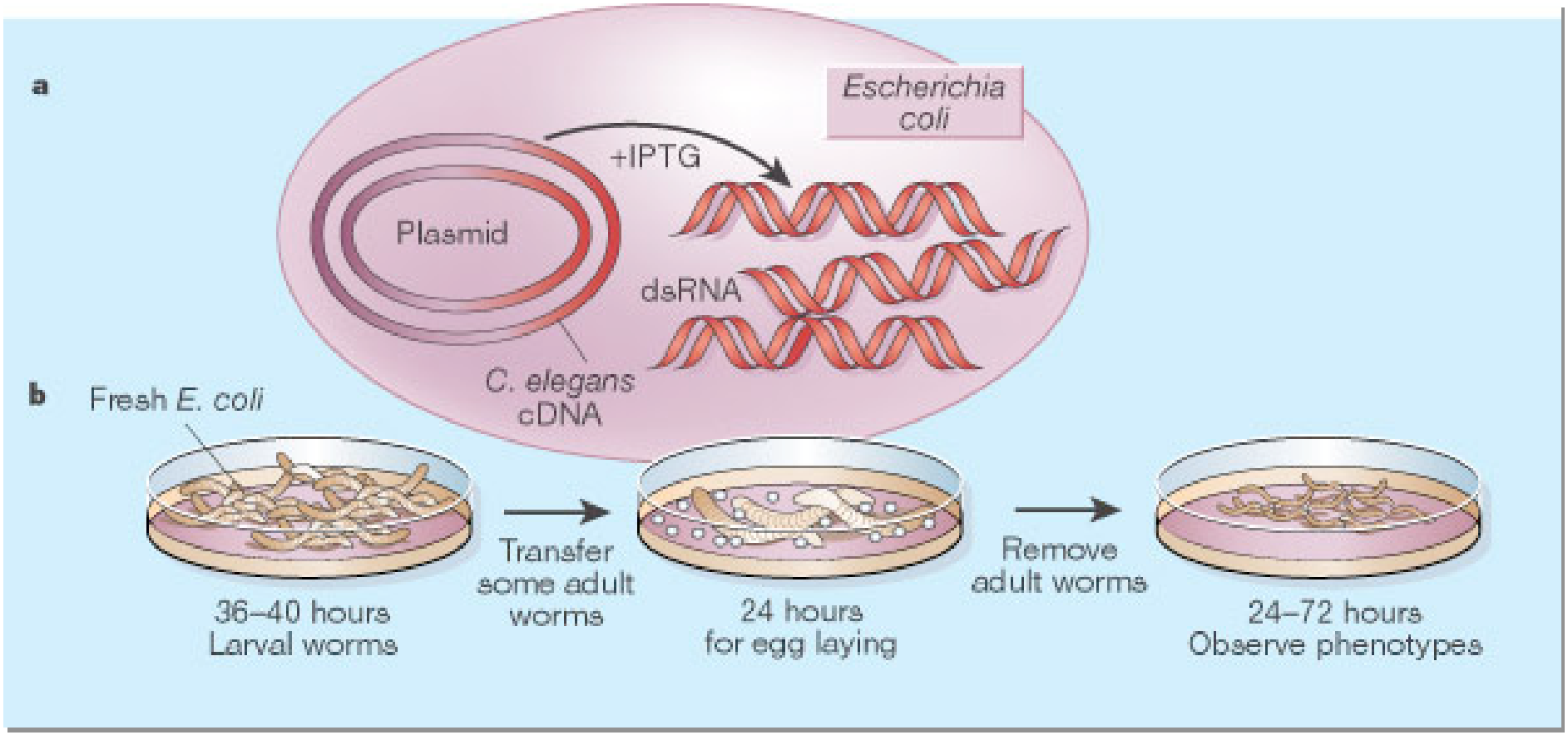
- High-throughput RNAi screens
- High-throughput insertional/chemical screens
- Homologous recombination (yeast and mouse)
- Other methods in discerning gene function
  - Activation tagging
  - Enhancer trapping
  - Modifier screens (enhancer and suppressors),
  - Yeast Two Hybrid Assay

# RNAi all genes on chromosome III -Gönczy, et al, 2000

- **Goal: In *C. elegans*, determine function of all 2,300 genes on chromosome III**
- **RNAi constructs made for each gene**
- **Worms microinjected with double-stranded RNA**
- **Videos made of embryonic phenotypes**



# Genome screen by feeding worms with dsRNA expressing *E. coli*



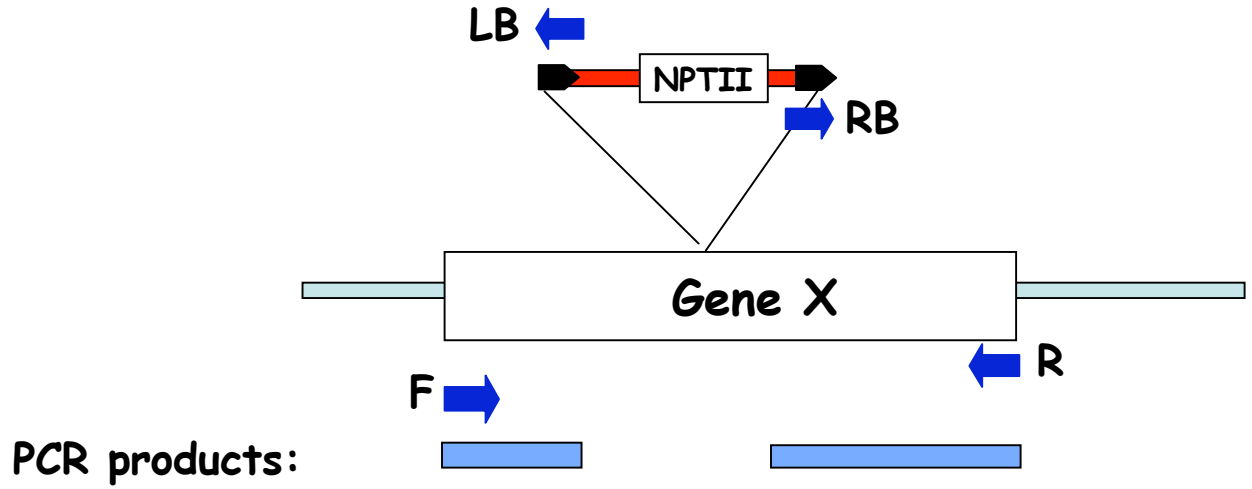

Tuschl 2003 *Nature*

# Identify gene function by insertional or chemical mutagenesis

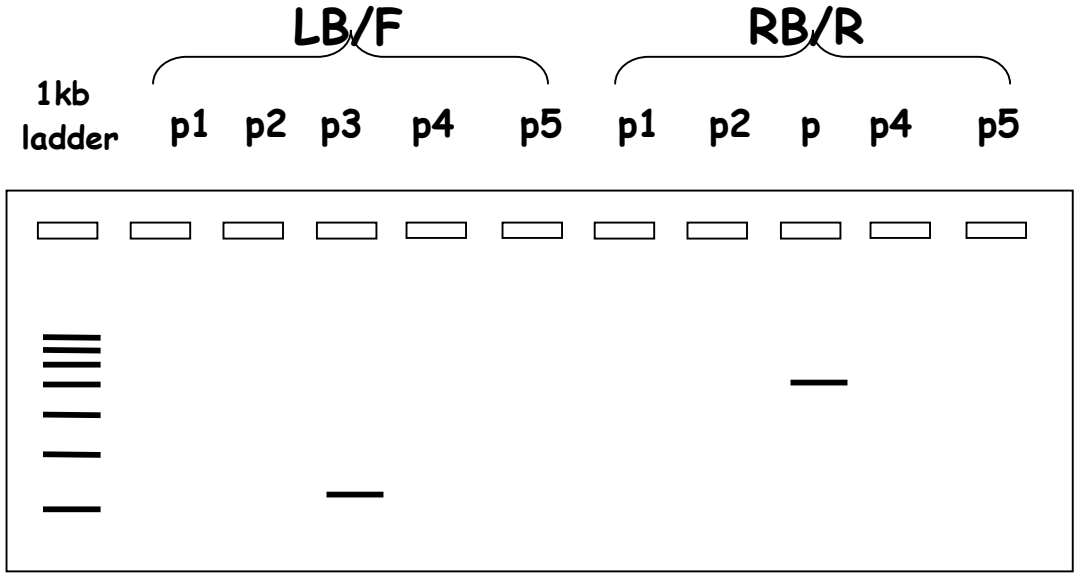
1) T-DNA or transposon insertions and PCR-based screens

2) Arabidopsis Tilling project

# 1. Screen for T-DNA (or Ds) insertion in specific genes

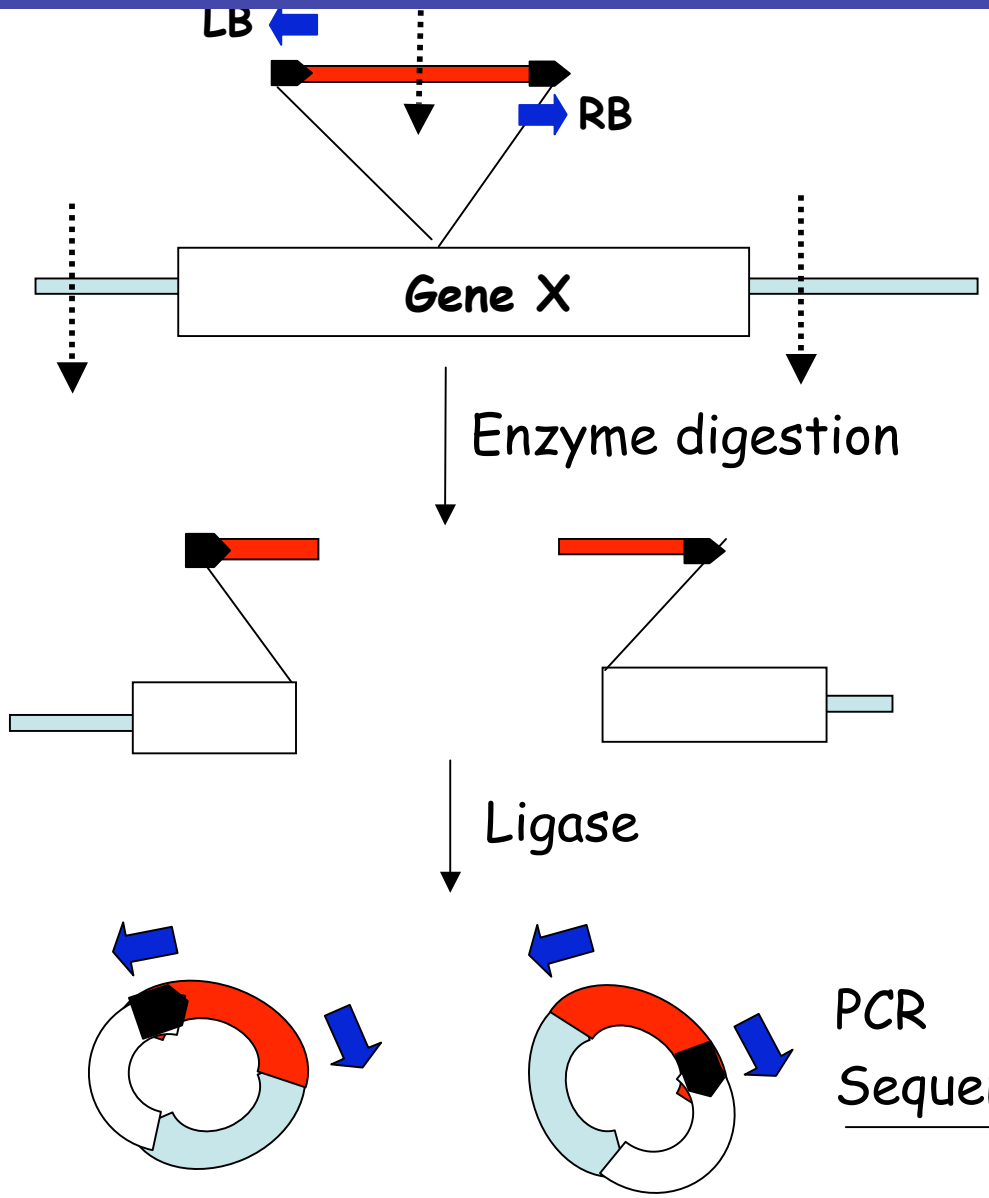



## Screening pools (p1-p5)



# Data-base searches for T-DNA insertions in the genes of interests

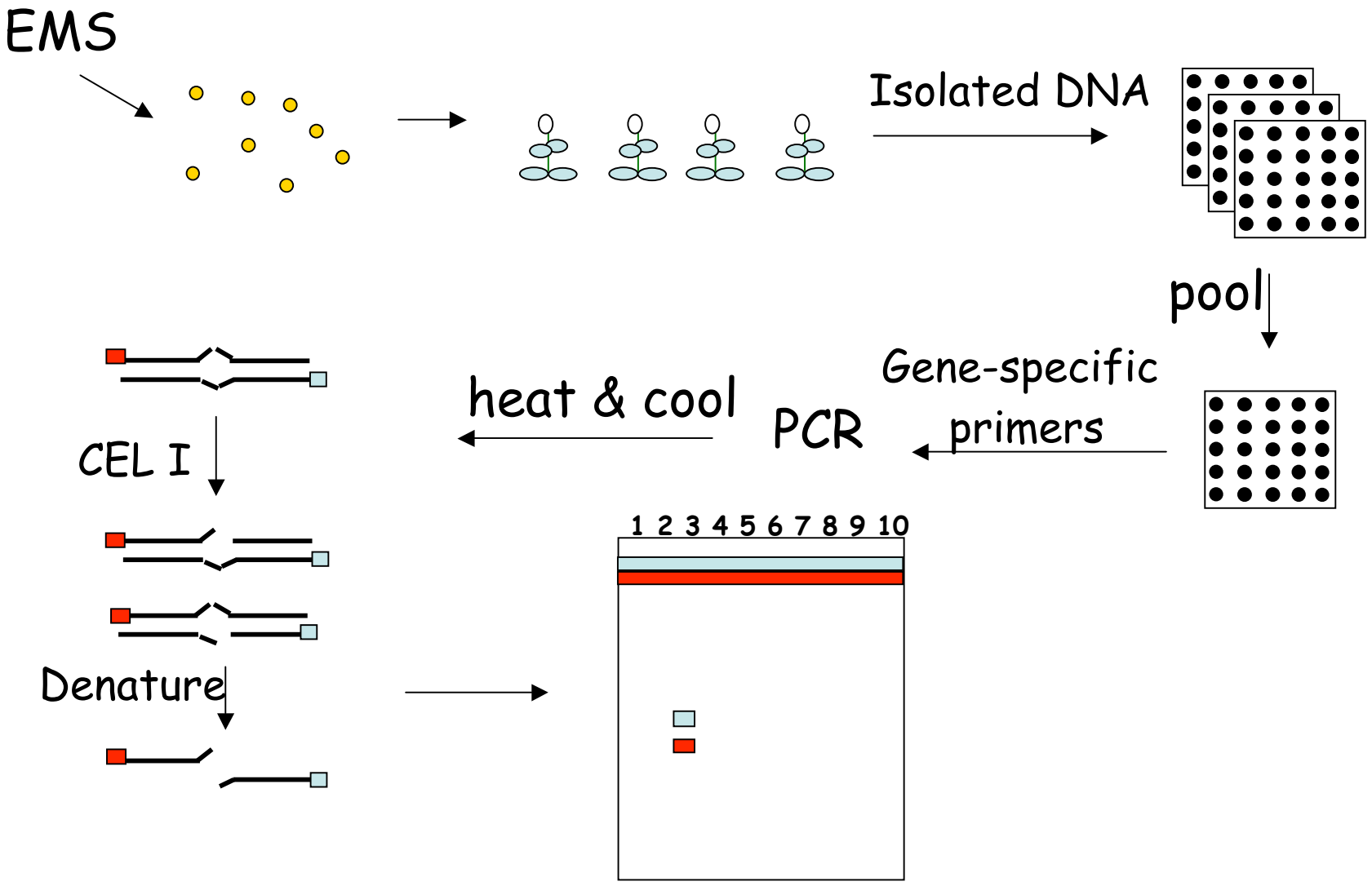
**Salk Institute Genomic Group** (http://signal.salk.edu/cgi-bin/tdnaexpress)




**LEUNIG="At4g32550**
SEU=At1g43850

# 2.TILLING (Targeting Induced Local Lesions IN Genomes)

Arabidopsis Tilling website:http://tilling.fhcrc.org:9366/



# Classical mutagenesis vs. RNAi

- **Diversity of mutations**
  - **(point mutations, deletions, inversions, etc.)**
- **Heritable, stable, and quantitative**
- **Saturating the genome requires hitting multiple genes repeatedly**

- **Give each gene equal attention**
- **High throughput**
- **Reasonably equivalent disruption of each locus**
- **“Mutations” automatically mapped**
- **Not heritable**
- **Doesn’t generate full depletion of target RNA**

# Homologous recombination and gene knock-out in yeast and mouse

Fig. A.8

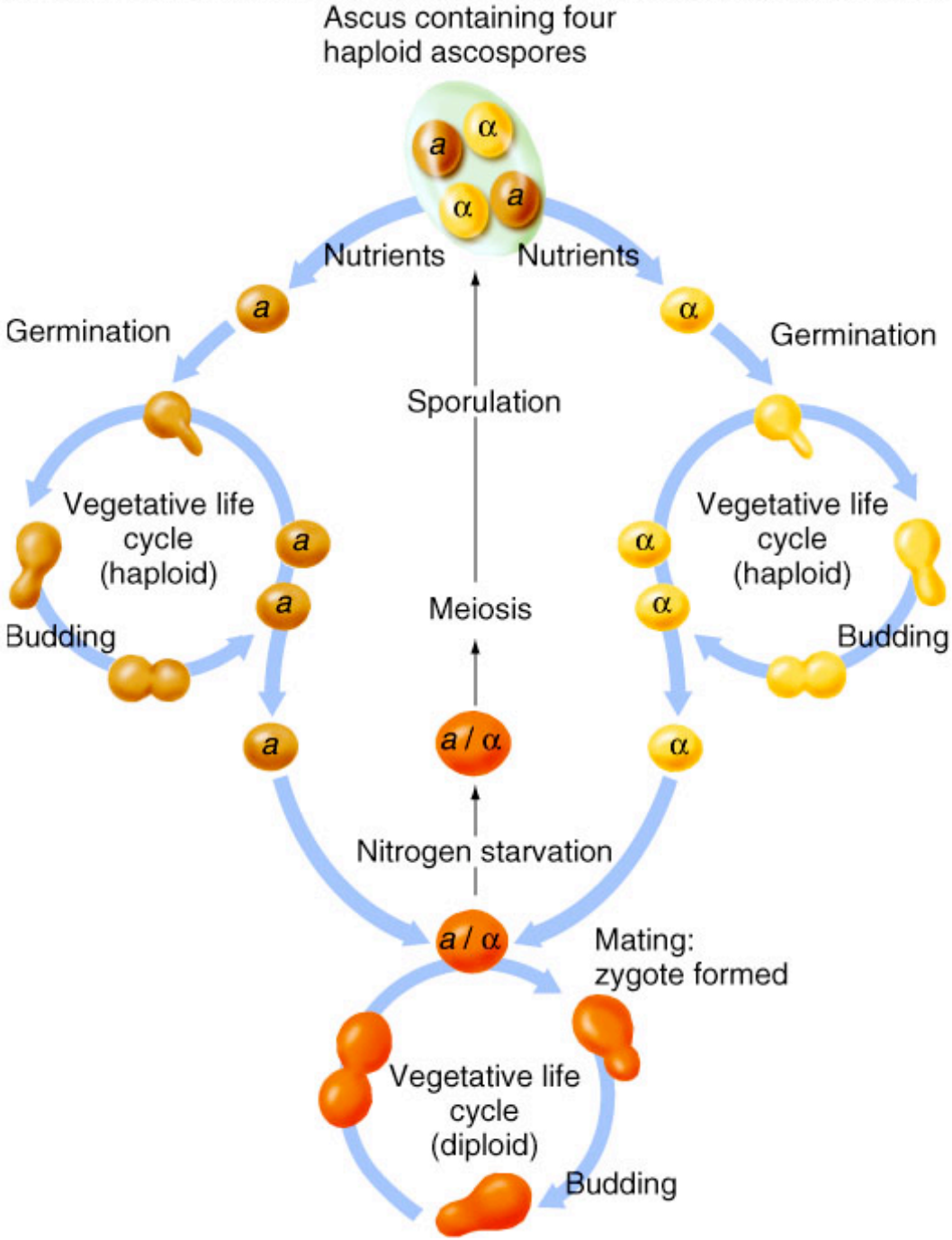
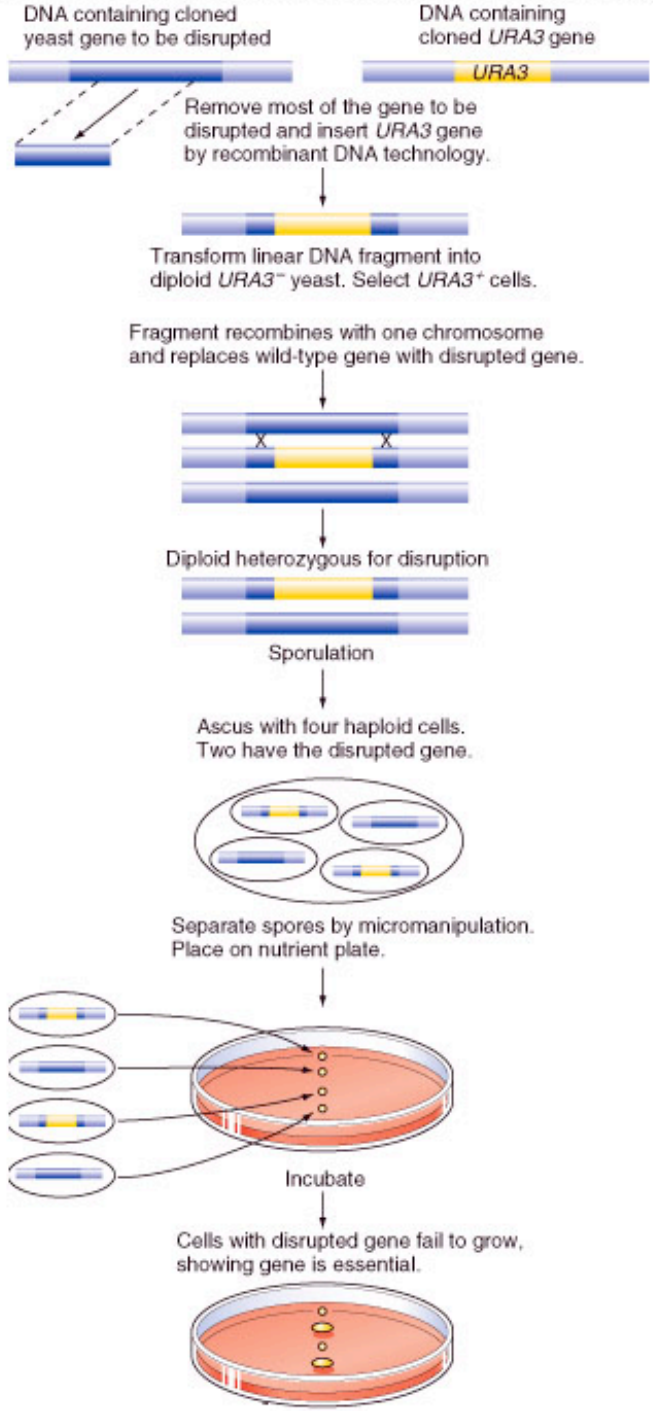
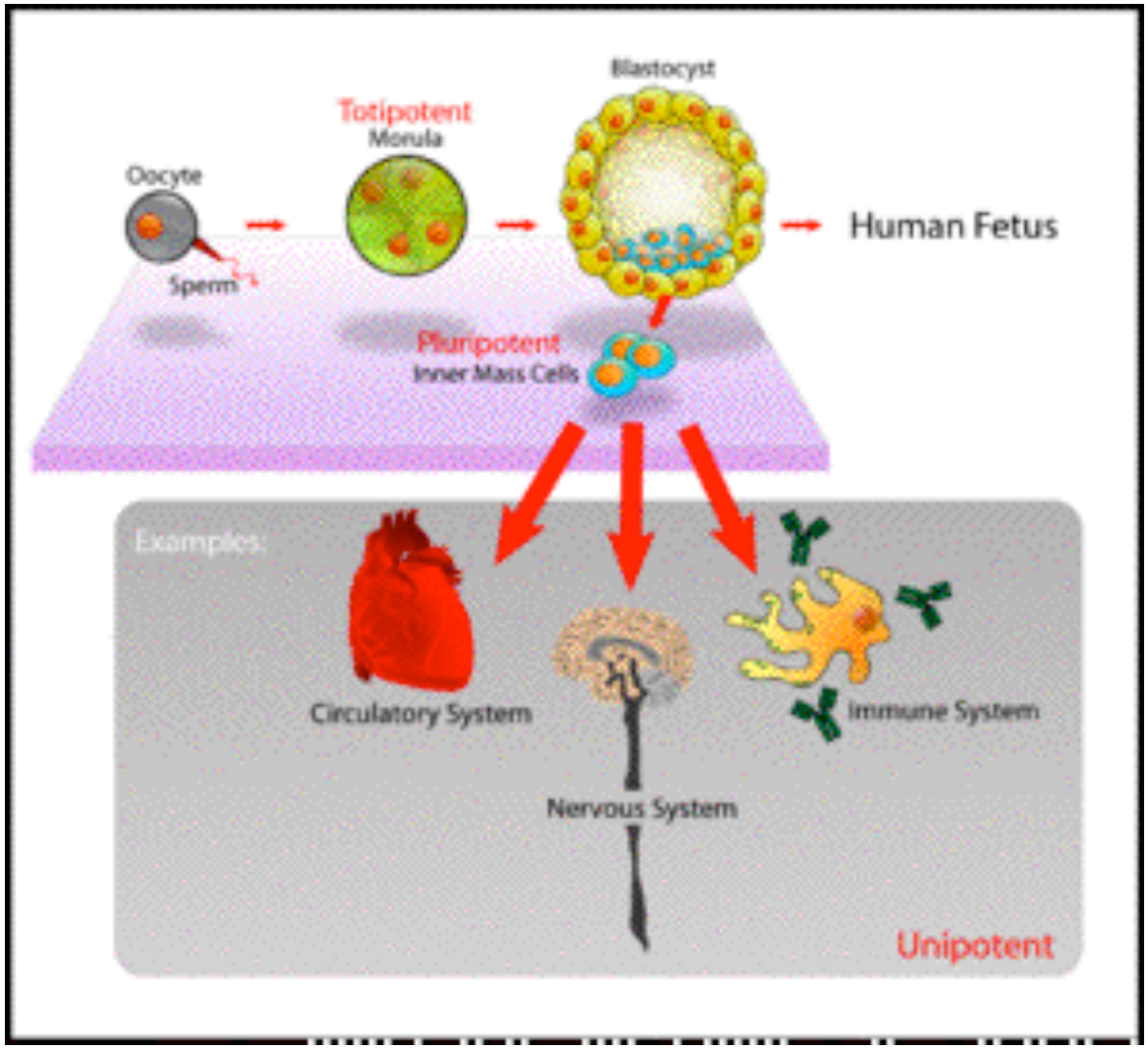


Fig. A.7

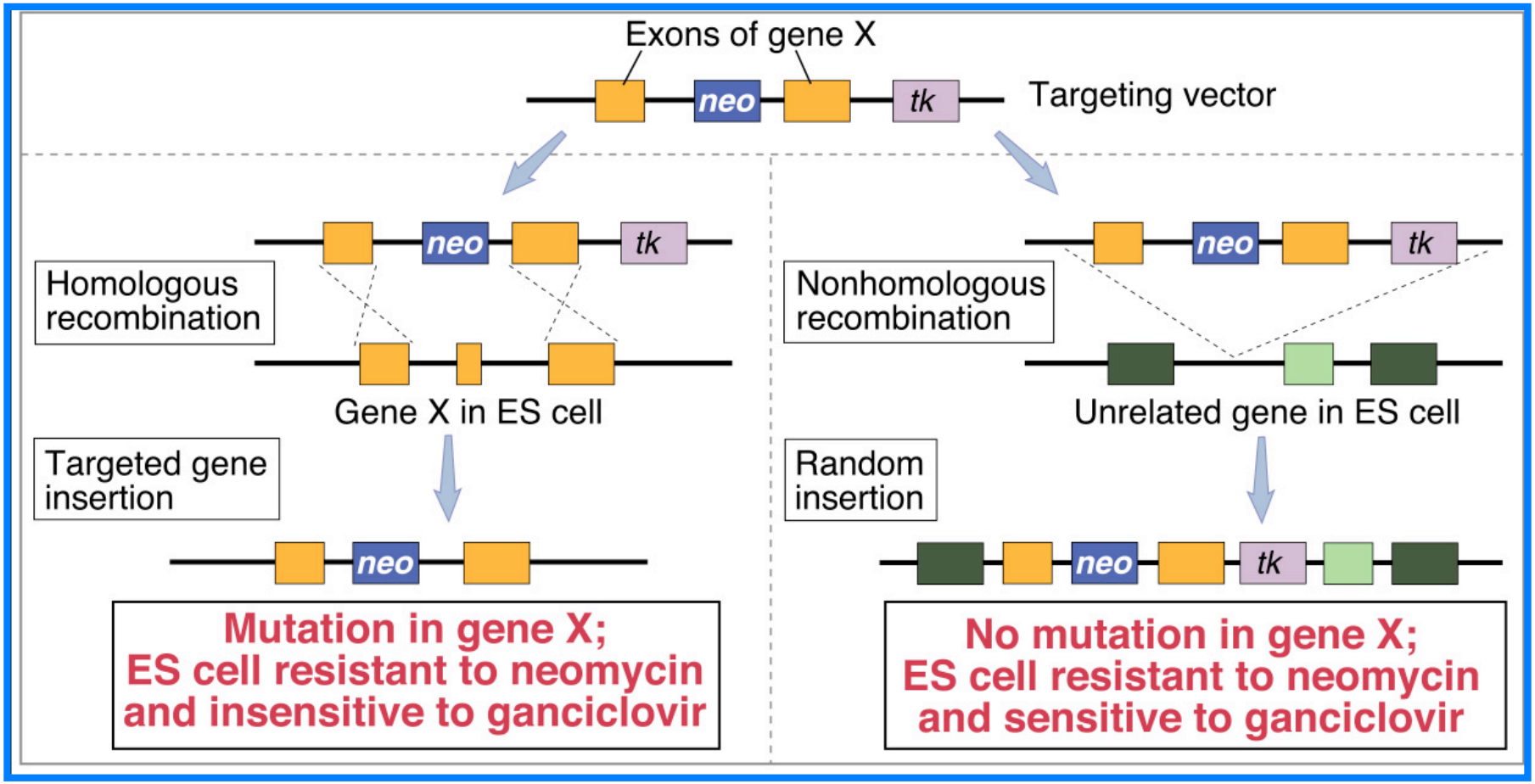


# Transgenic mouse depends on ES (embryonic stem) cells

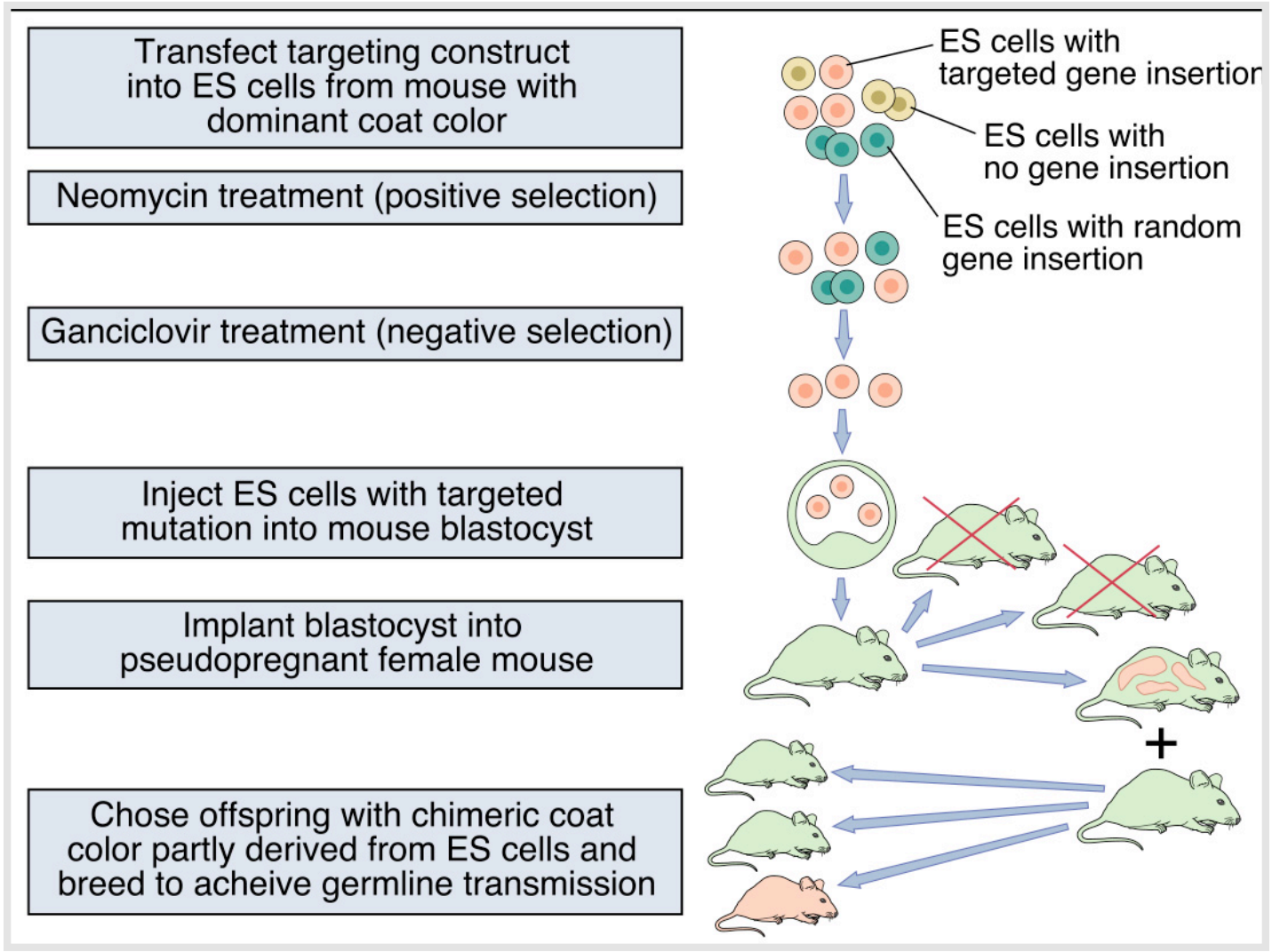



**Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta.**

# Knockout Mice Targeting Constructs for Homologous Recombination

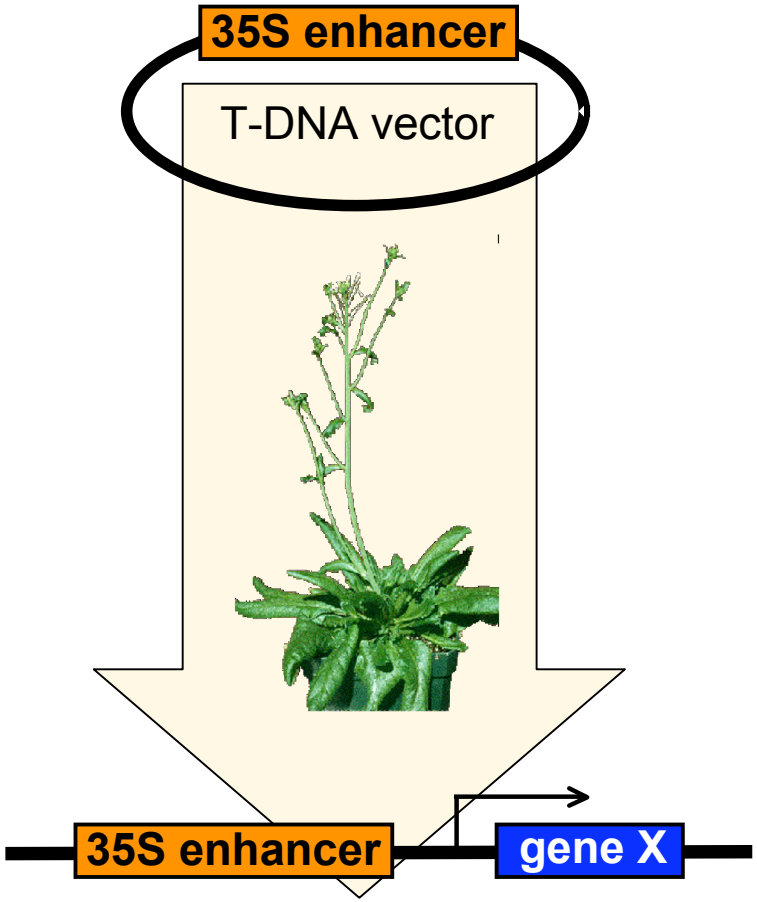


# Transgenic Mice
# ~Generation of Knockouts~



# Activation tagging in *Arabidopsis*

- **Strong constitutive viral promoter**
  - **CaMV 35S**
- **Inserted randomly in genome**
  - **With T-DNA**
- **When inserts are near a gene promoter, the following results occur:**
  - **Activation**
  - **Constitutive expression**
- **Because many genes are expressed in specific cells or tissues, activation in all tissues can result in abnormal phenotypes**



# Loss-of-function vs. gain-of-function usually give opposite phenotypes

## *eyeless* gene

Wild type

*eyeless*

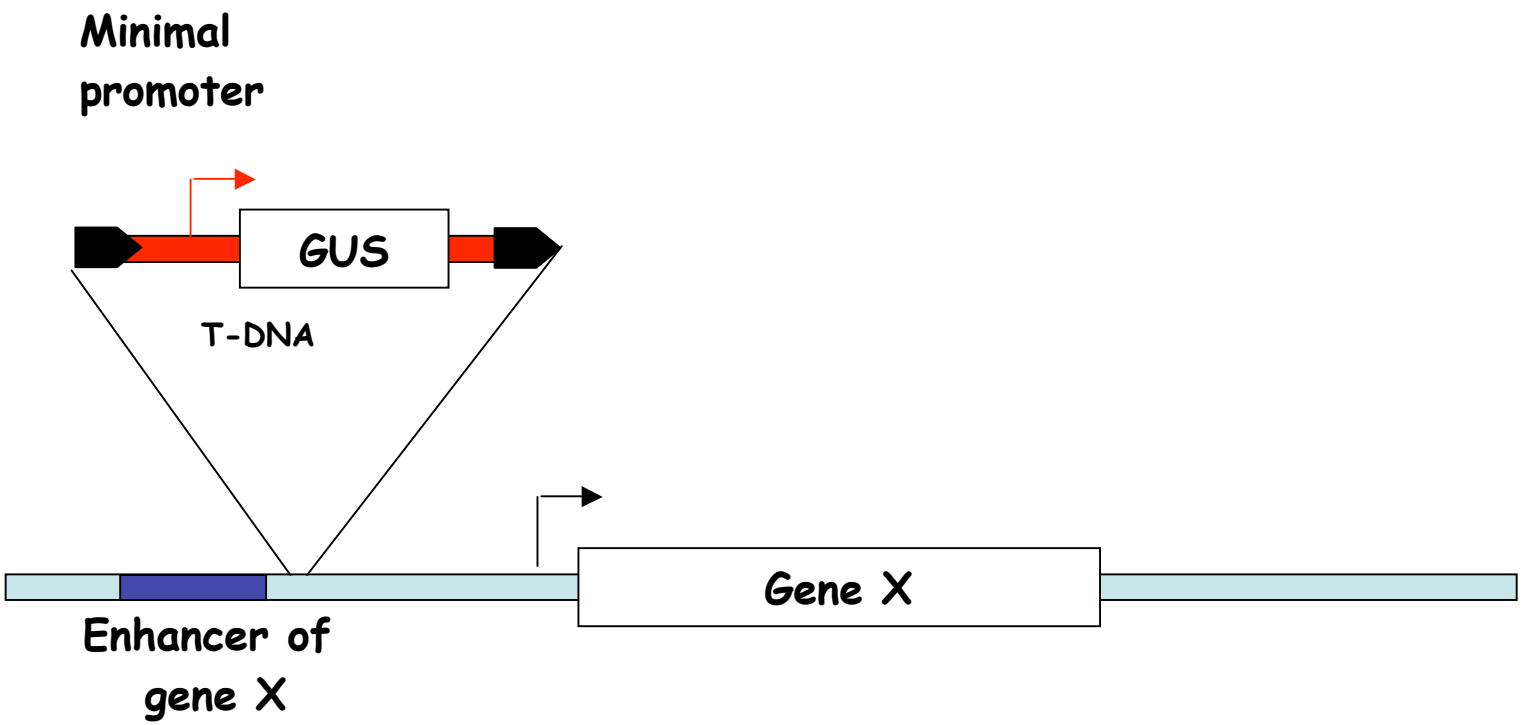

**Ectopic expression of *eyeless***

# Activation tagging

- **A variation on insertional mutagenesis**
  - Makes **gain-of-function mutations** instead of **loss-of-function** mutations
  - An insertion that carries a strong constitutive promoter or enhancer

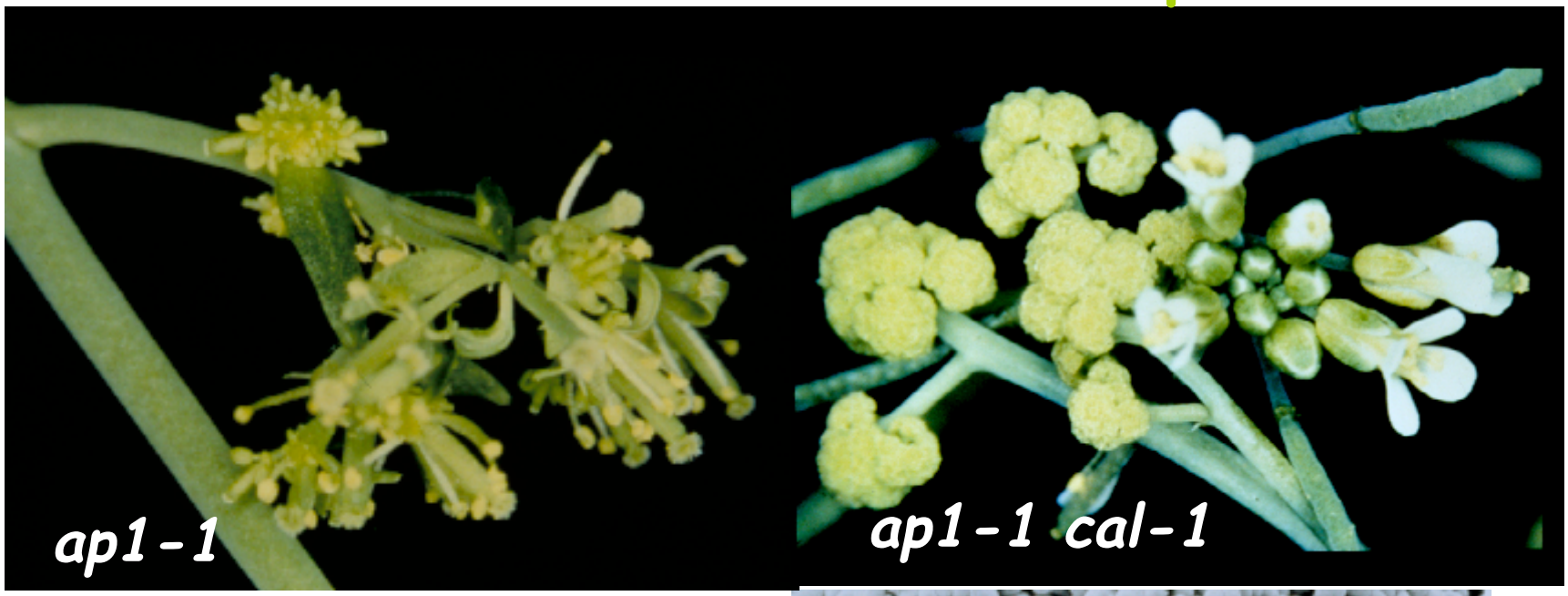
- **Potential to identify gene function not detectable through loss-of-function screens**
  - Useful for the following cases:
    - Functionally redundant genes
    - Genes required for viability

# Enhancer trapping in Arabidopsis

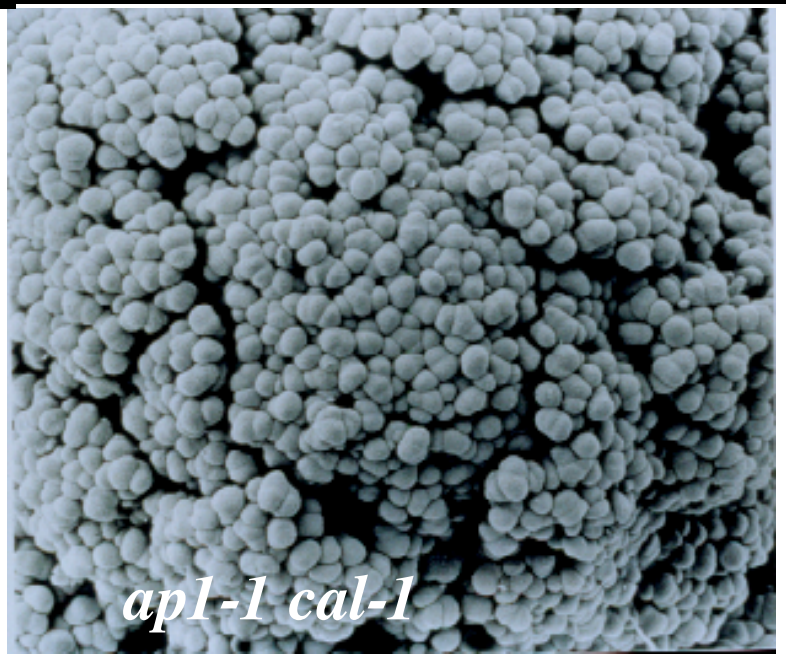



Dr. Tom Jack's website
http://www.dartmouth.edu/~tjack/

# Modifier screen example

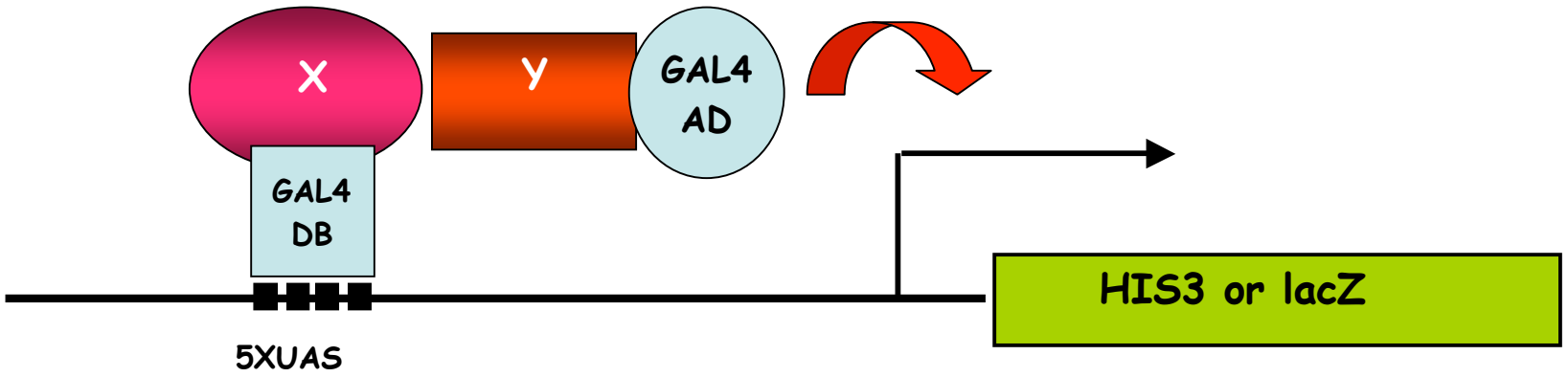


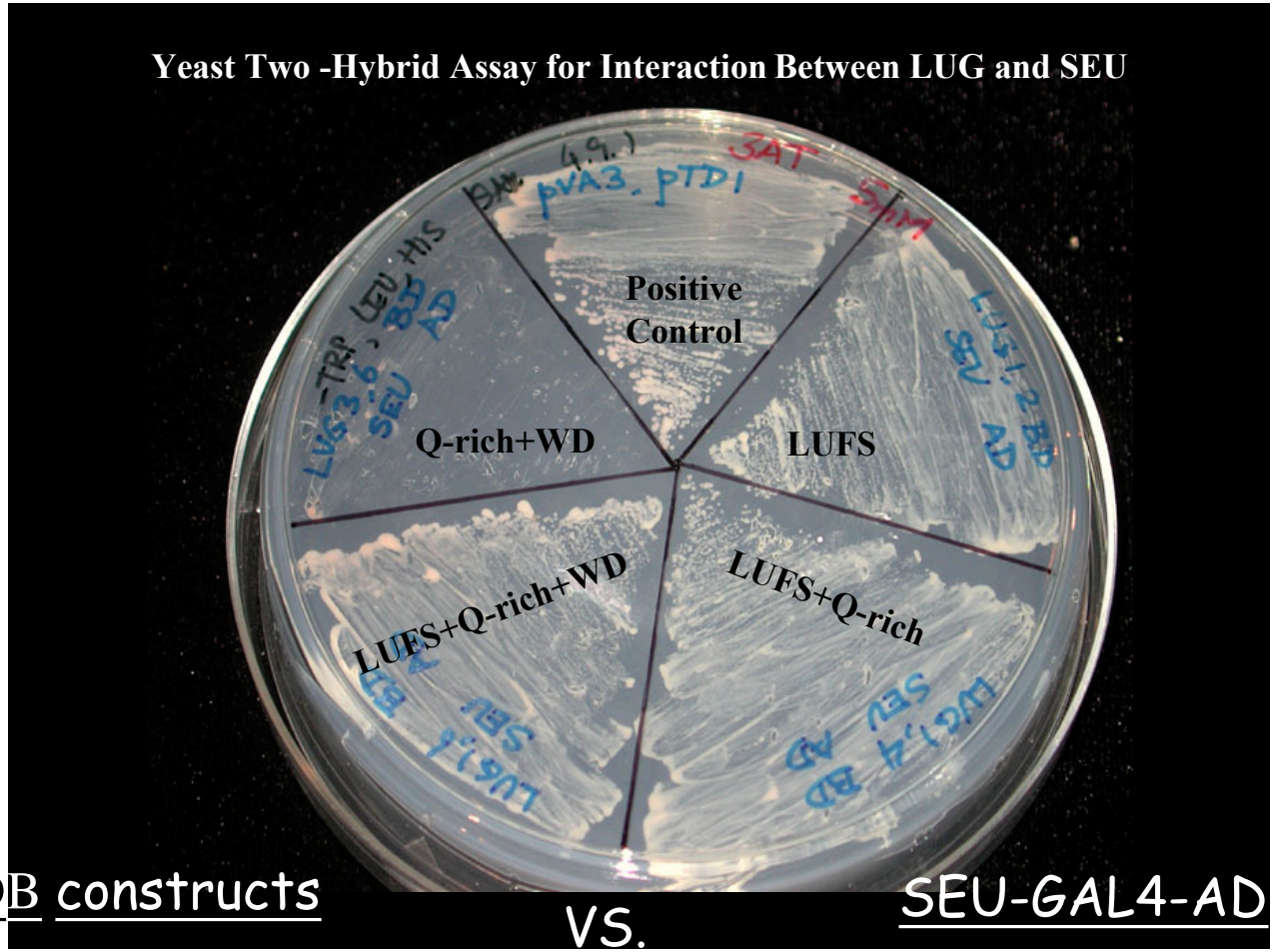

*cal-1*: wild-type looking
*ap1-1*: flower mutant
*ap1-1 cal-1*: cauliflower



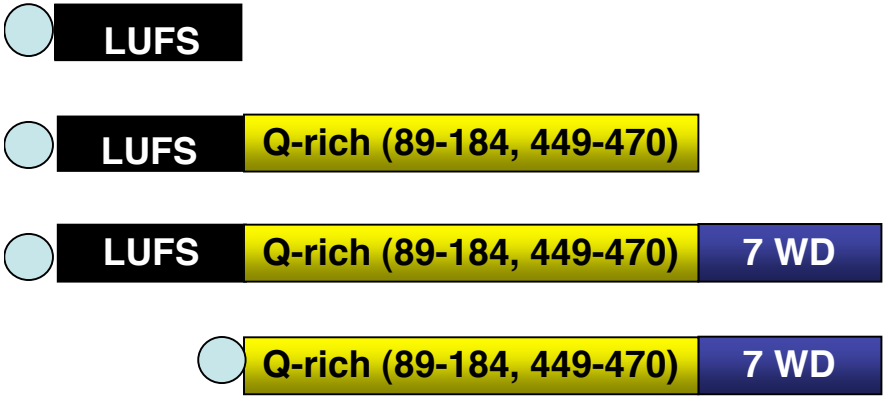
# Yeast Two Hybrid (Y2H) Assay

## to test interaction between two proteins






LUG-GAL4-DB constructs VS. SEU-GAL4-AD




Use NCBI's Books to search for:

Yeast two hybrid assay
Activation tagging
Enhancer trap
Etc.