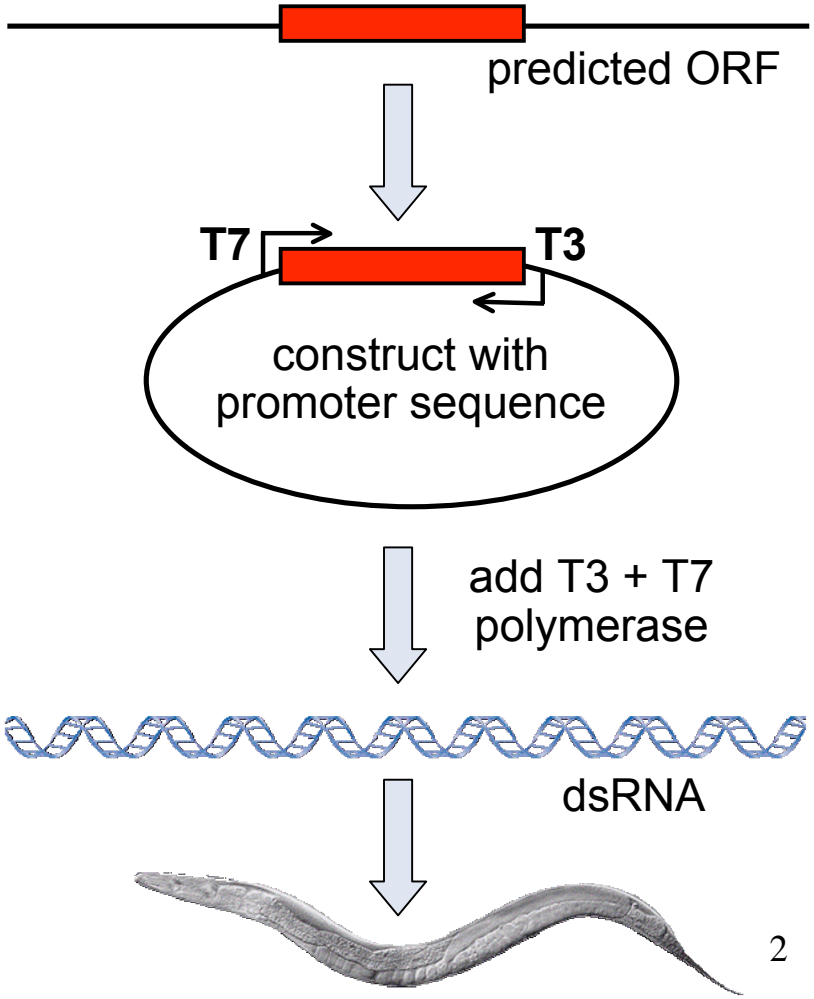
Lecture 16: Functional Genomics II

- High-throughput genetic screens
- Insertional mutagenesis
  - Activation tagging
  - Enhancer trapping
- Modifier screens: enhancer and suppressor screens, synthetic lethal
- Yeast Two Hybrid Assay

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

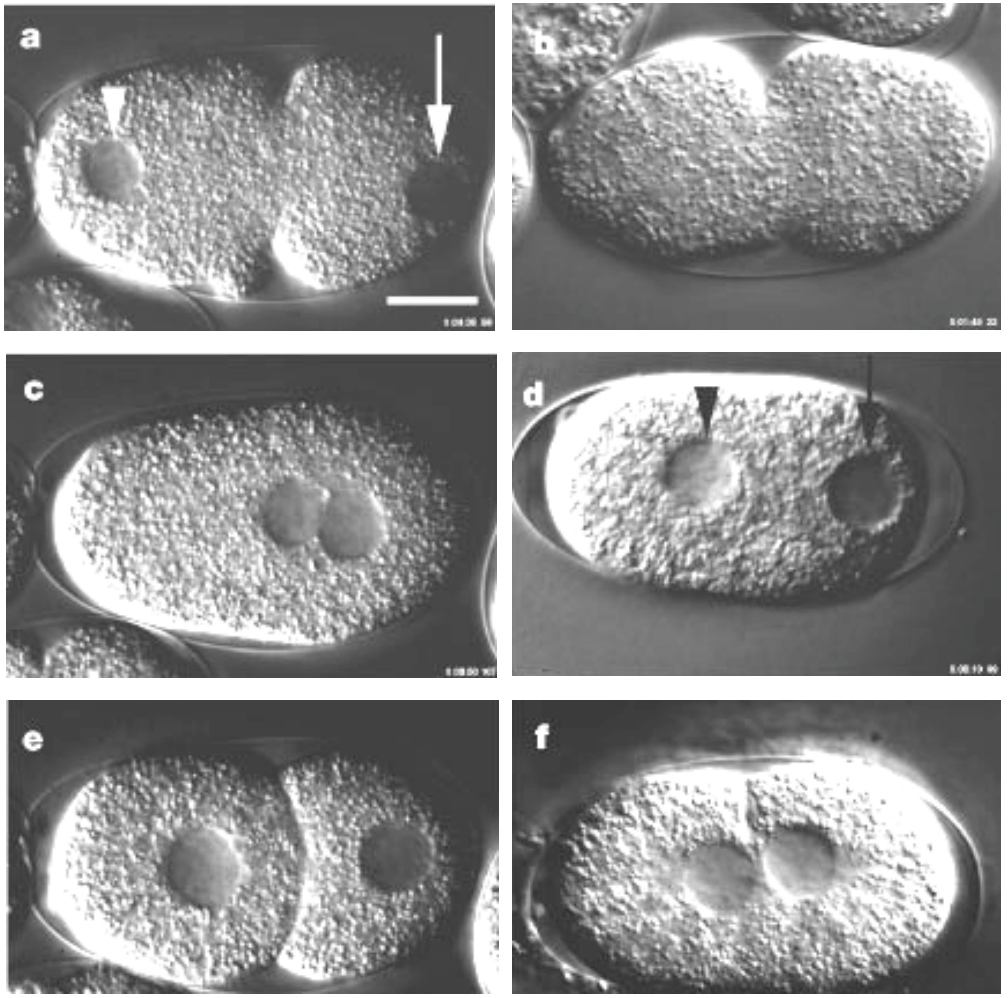
- **Goal: In *C. elegans*, determine function of all 2,300 genes on chromosome III**
- **RNAi constructs made for each gene**
- **Worms microinjected with double-stranded RNA**
- **Videos made of embryonic phenotypes**



Phenotypes found in RNAi screen

Wild type

RNAi embryos

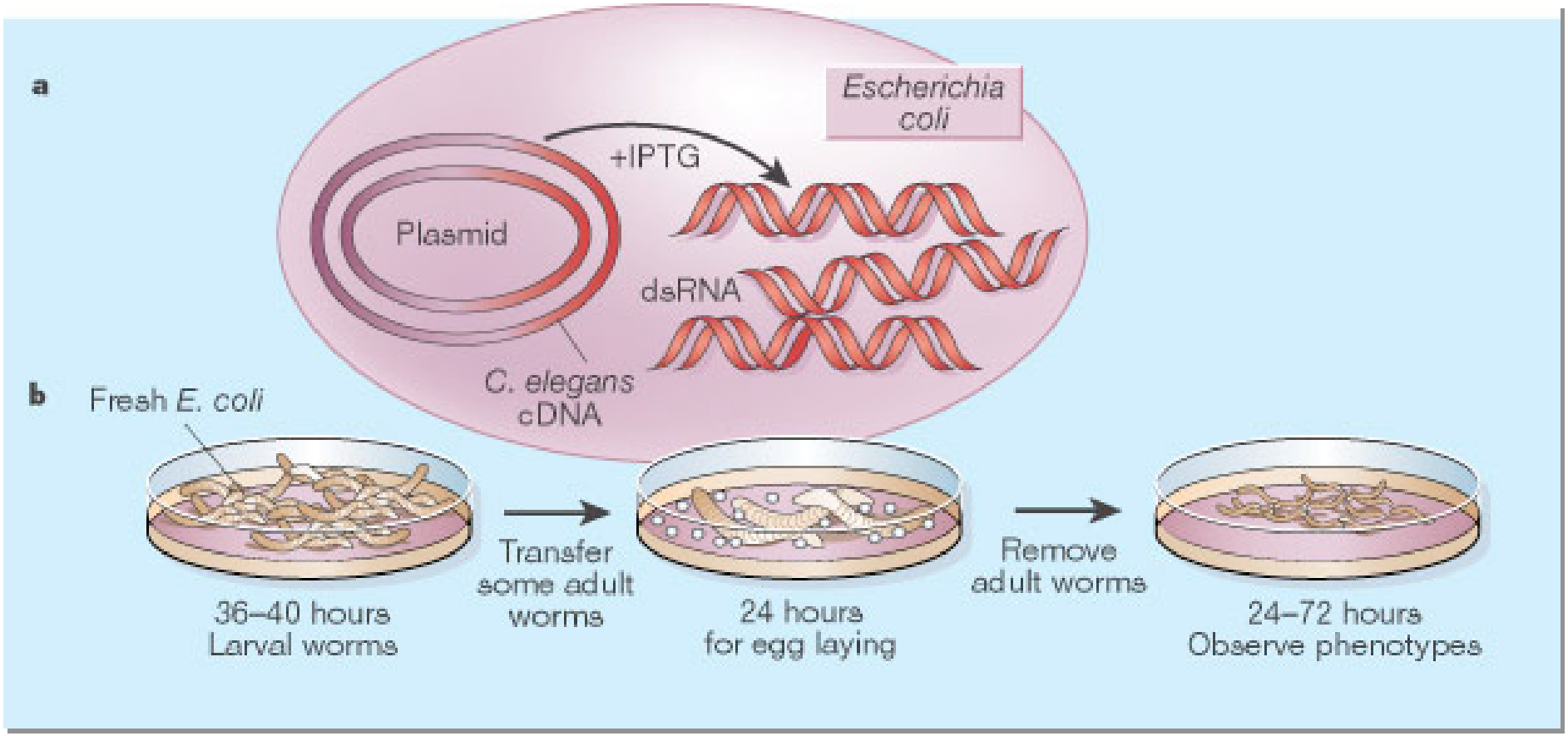


Pronuclear/nuclear appearance (phenotypic class B1)

Pronuclear migration (phenotypic class C1)

Cytokinesis (phenotypic class C6)

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



Tuschl 2003 *Nature*

Genome-wide RNAi screens in *Drosophila*
http://flyrnai.org

A functional genomic analysis of cell morphology using RNA-interference.

J Biol. 2003;2(4):27. Epub 2003 Oct 01

Kiger AA, Baum B, Jones S, Jones MR, Coulson A, Echeverri C, Perrimon N

Genome-Wide RNAi Analysis of Growth and Viability in Drosophila Cells

Science. 2004 Feb 6;303(5659):832-5

Michael Boutros, Amy Kiger, Susan Armknecht, Kim Kerr, Marc Hild, Britta Koch, Stefan A. Haas, Heidelberg Fly Array Consortium, Renato Paro, Norbert Perrimon

Parallel Chemical Genetic and Genome-Wide RNAi Screens Identify Cytokinesis Inhibitors and Targets

PLoS Biol 2(12): e379.

Ulrike S. Eggert, Amy Kiger, Constance Richter, Zachary E. Perlman, Norbert Perrimon, TimothyJ. Mitchison, Christine M. Field

Functional genomic analysis of the Wnt-Wingless Signaling Pathway

Science. 2005 Apr 7

DasGupta R Kaykas A, Moon RT, Perrimon N

Terminal cytokinesis events uncovered after an RNAi screen.

Curr Biol. 2004 Sep 21;14(18):1685-93.

Echard A, Hickson GR, Foley E, O'Farrell PH.

Genome-wide RNAi screens in mammalian cells

A large-scale RNAi screen in human cells identifies new components of the p53 pathway.

Berns K, Hijmans EM, Mullenders J, Brummelkamp TR, Velds A, Heimerikx M, Kerkhoven RM, Madiredjo M, Nijkamp W, Weigelt B, Agami R, Ge W, Cavet G, Linsley PS, Beijersbergen RL, Bernards R. Nature. 2004 Mar 25;428(6981):431-7.

An approach to genomewide screens of expressed small interfering RNAs in mammalian cells.

Zheng L, Liu J, Batalov S, Zhou D, Orth A, Ding S, Schultz PG.

Proc Natl Acad Sci U S A. 2004 Jan 6;101(1):135-40.

Genome-wide screens in plants, planaria, …

RNA Interference Identifies a Calcium-Dependent Protein Kinase Involved in Medicago truncatula Root Development.

Ivashuta S, Liu J, Liu J, Lohar DP, Haridas S, Bucciarelli B, Vandenbosch KA, Vance CP, Harrison MJ, Gantt JS. Plant Cell. 2005 Nov;17(11):2911-21. Epub 2005 Sep 30.

RNA silencing in plants.

Baulcombe D. Nature. 2004 Sep 16;431(7006):356-63. Review.

Identification of genes needed for regeneration, stem cell function, and tissue homeostasis by systematic gene perturbation in planaria.

Reddien PW, Bermange AL, Murfitt KJ, Jennings JR, Sanchez Alvarado A.

Dev Cell. 2005 May;8(5):635-49.

Opening a new can of worms: a large-scale RNAi screen in planarians.

Newmark PA. Dev Cell. 2005 May;8(5):623-4.

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**

Insertional mutagenesis for both forward and reverse genetics

- **Alternative to chromosome walking**
  - To reduce time and effort required to identify mutant gene
  - Use inserted DNA to identify mutated gene
- **Inserts randomly in chromosomes**
- **Conventional: DNA inserts disrupt genes**
- **Variations: activation tagging and enhancer trap**

Insertional mutagens

- **Transposable elements**
  - Mobile elements jump from introduced DNA
    - ***P* elements** in *Drosophila*

- **Single-insertion elements**
  - **T-DNA** in plants
    - Once insert, can't move again

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

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

eyeless gene

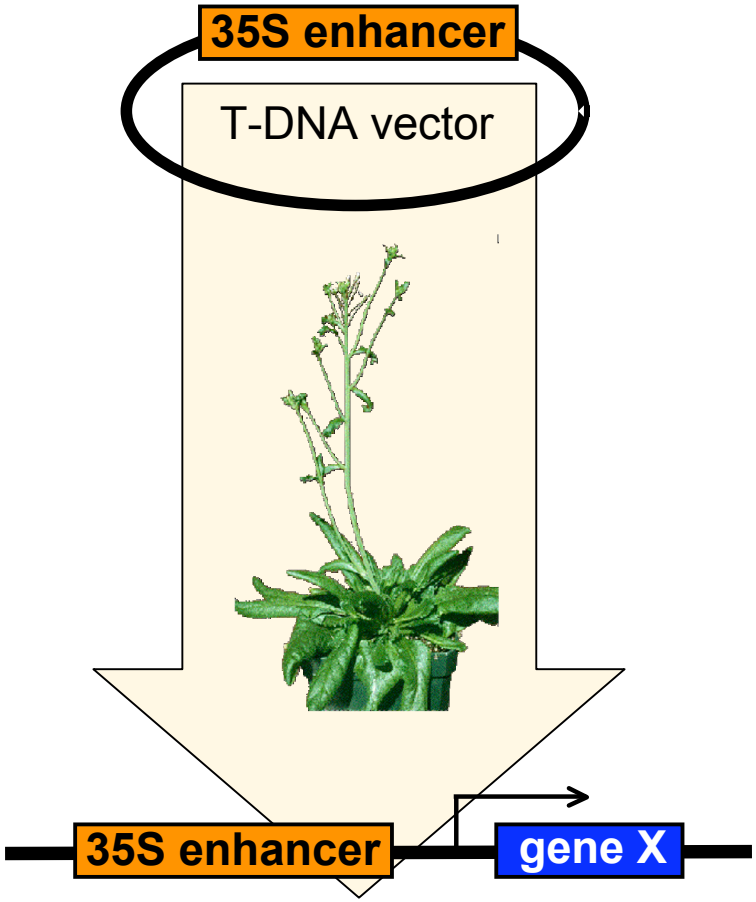
Wild type

eyeless

Ectopic expression of *eyeless*

Activation tagging in *Arabidopsis*

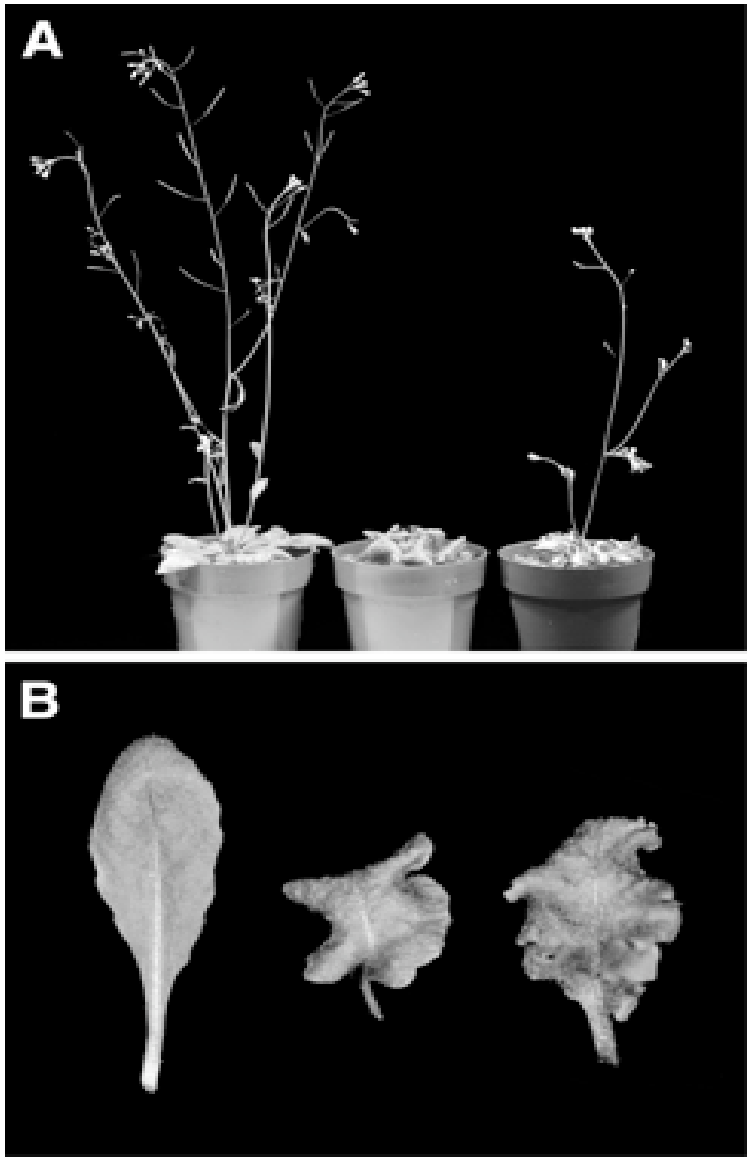
- **Strong constitutive viral promoter**
  - **CaMV 35S**
- **Inserted randomly in genome**
  - **With T-DNA**
- **When inserts are near a gene promoter, the following results occur:**
  - **Activation**
  - **Constitutive expression**
- **Because many genes are expressed in specific cells or tissues,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



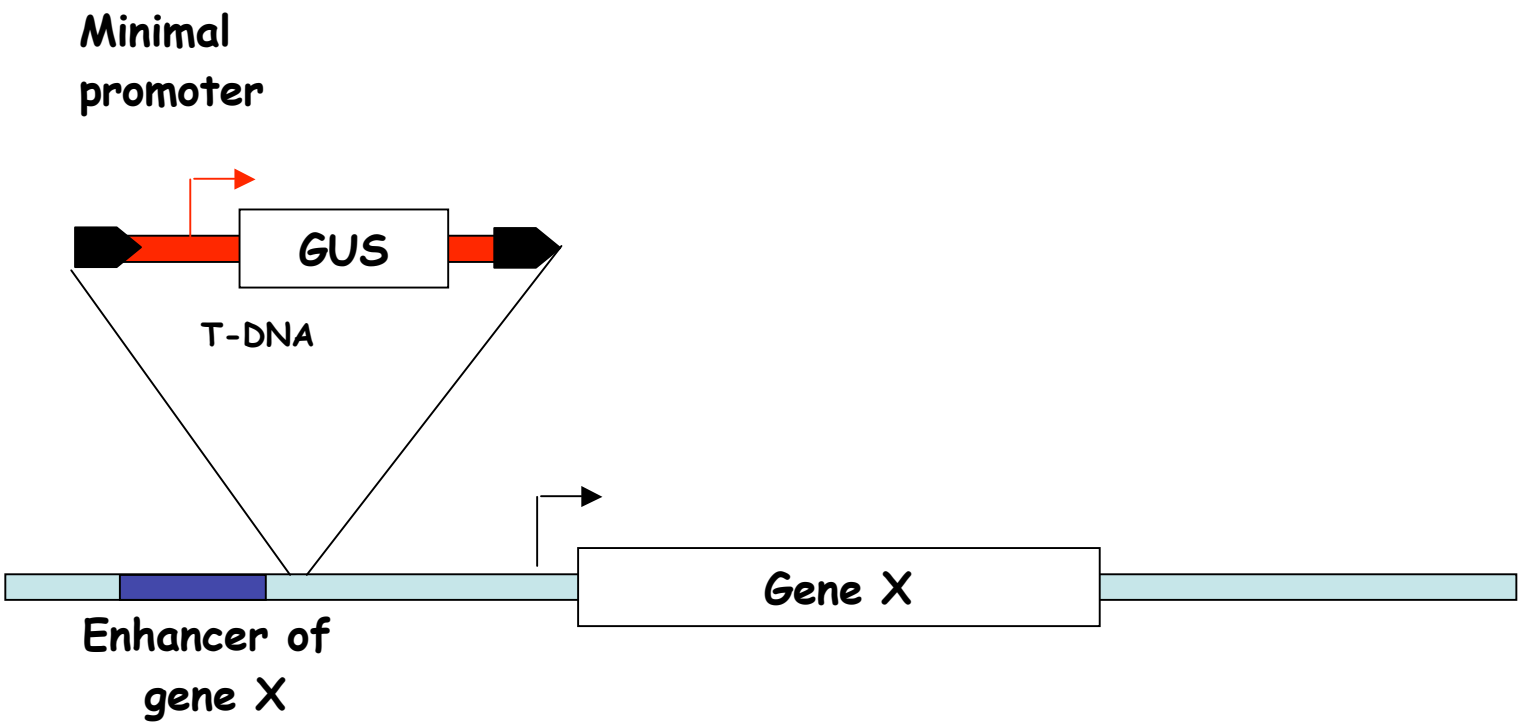
Activation tagging examples in *Arabidopsis*

Identification of genes required for flowering time (A) and plant leaf and shape (B)

D. Weigel's lab



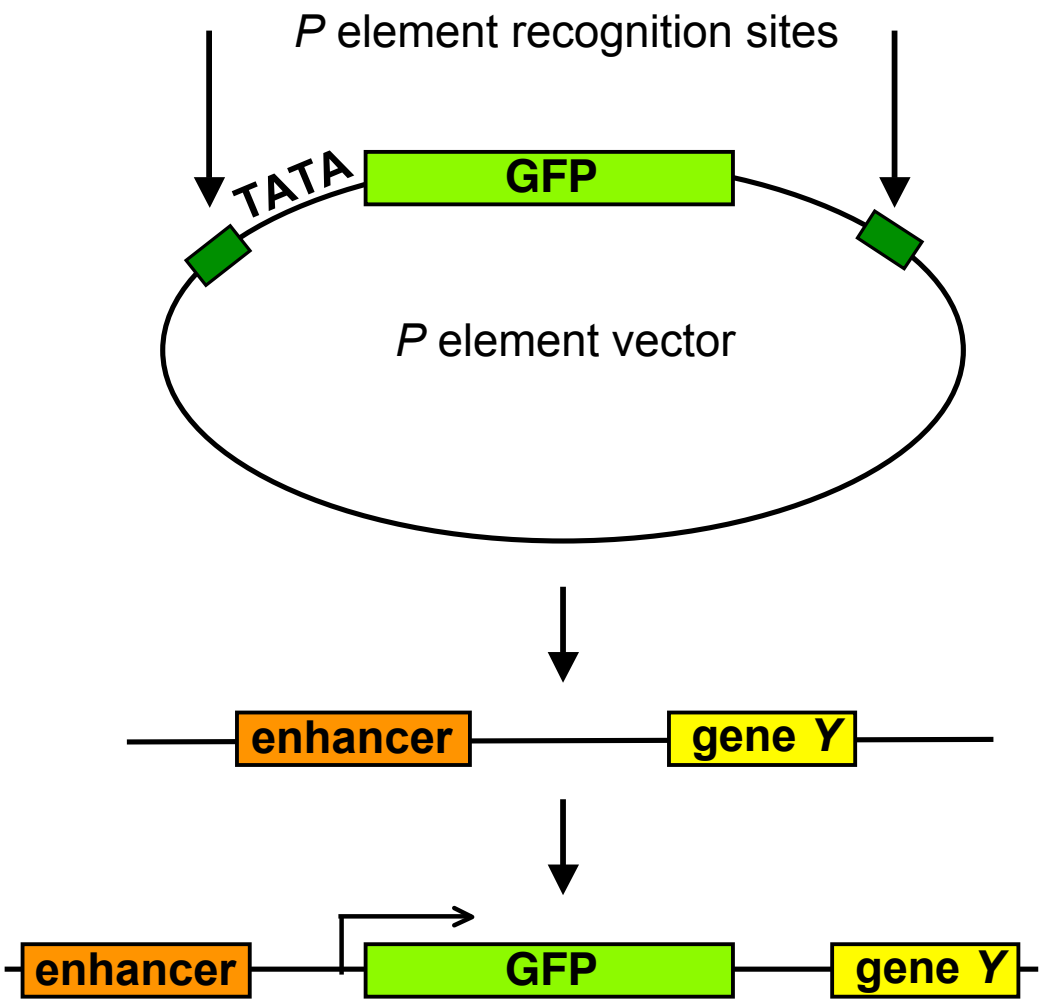
Enhancer trapping in Arabidopsis



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

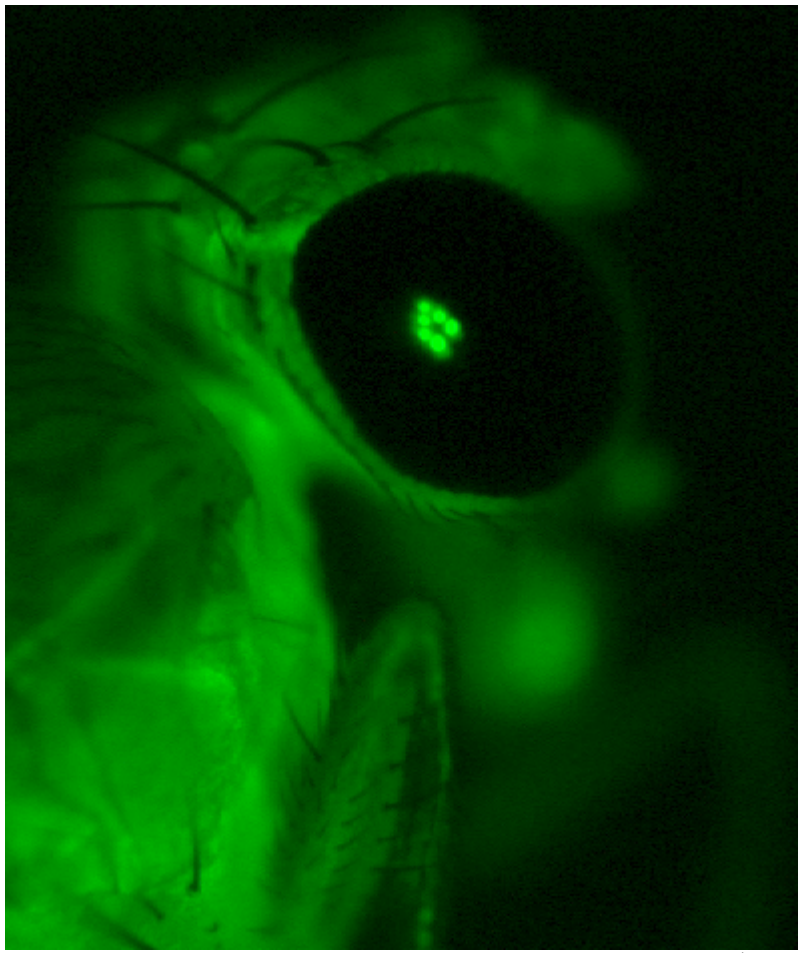
Enhancer trapping in *Drosophila*

- **Use transposon *P* element**
- **Carries reporter gene**
  –GFP
- **Hops into genome**
- **When lands near enhancer, activates gene expression**
- **Expression similar to that of neighboring gene**

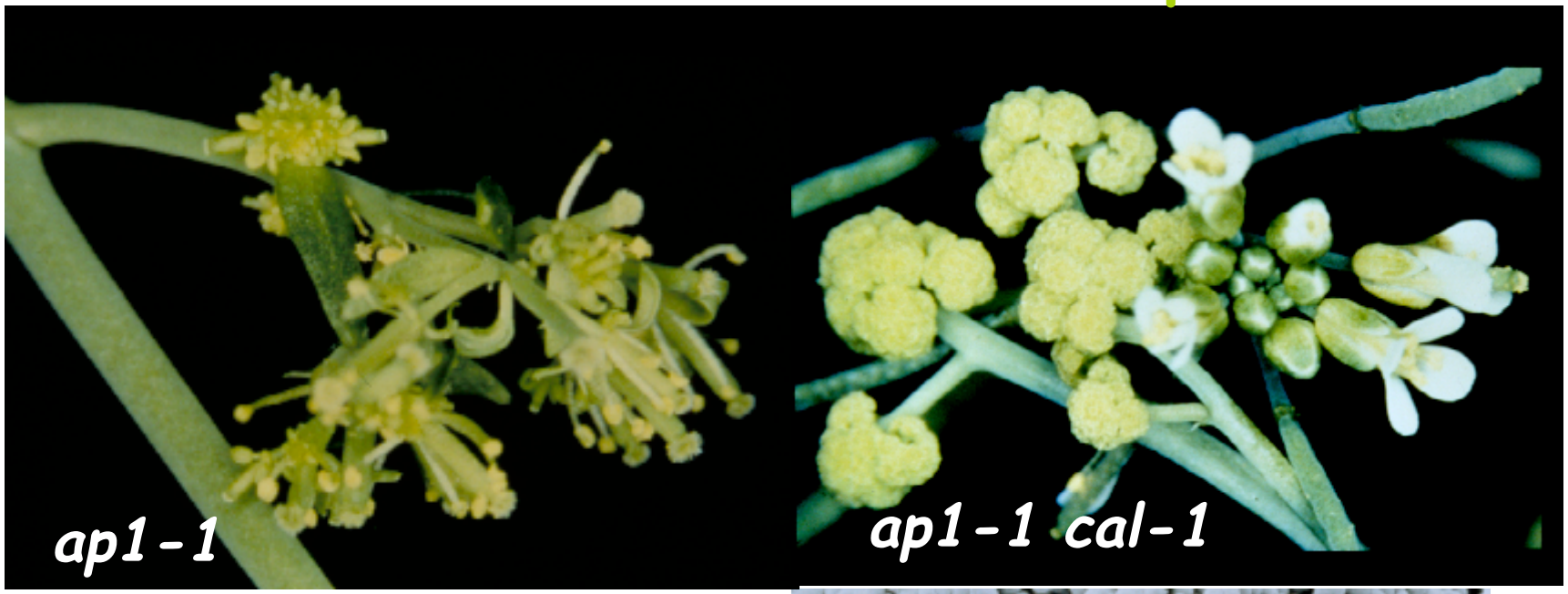


Enhancer trapping in *Drosophila*

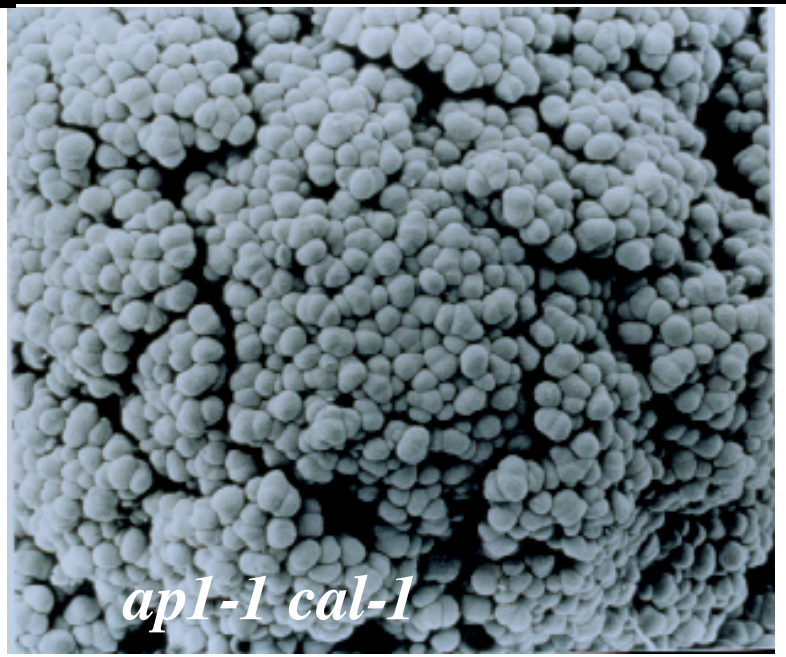
- **Reporter gene: Green Fluorescent Protein (GFP)**
- **The enhancer trap has inserted into a gene expressed in part of the fly eye**



Modifier screen example

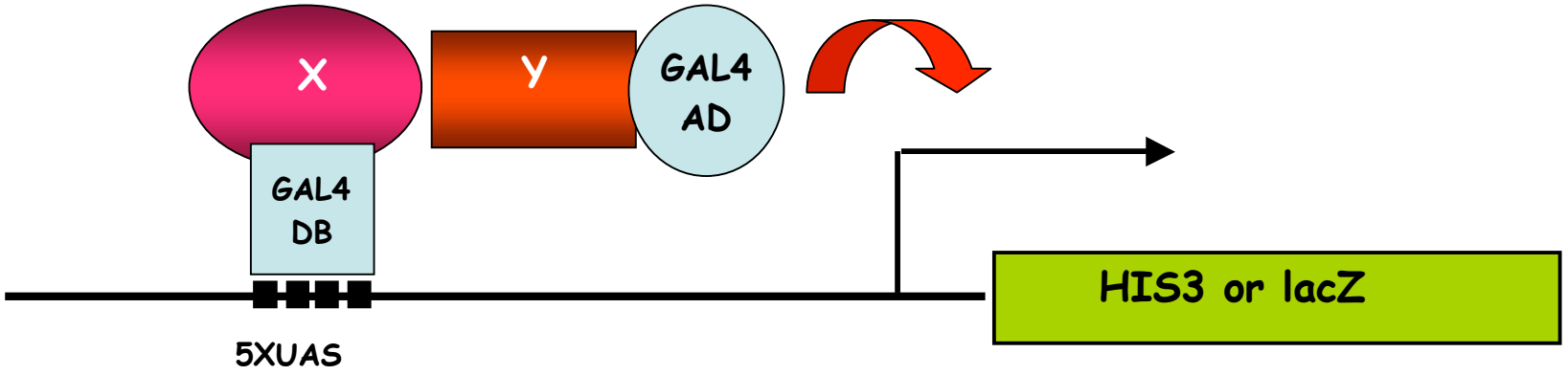


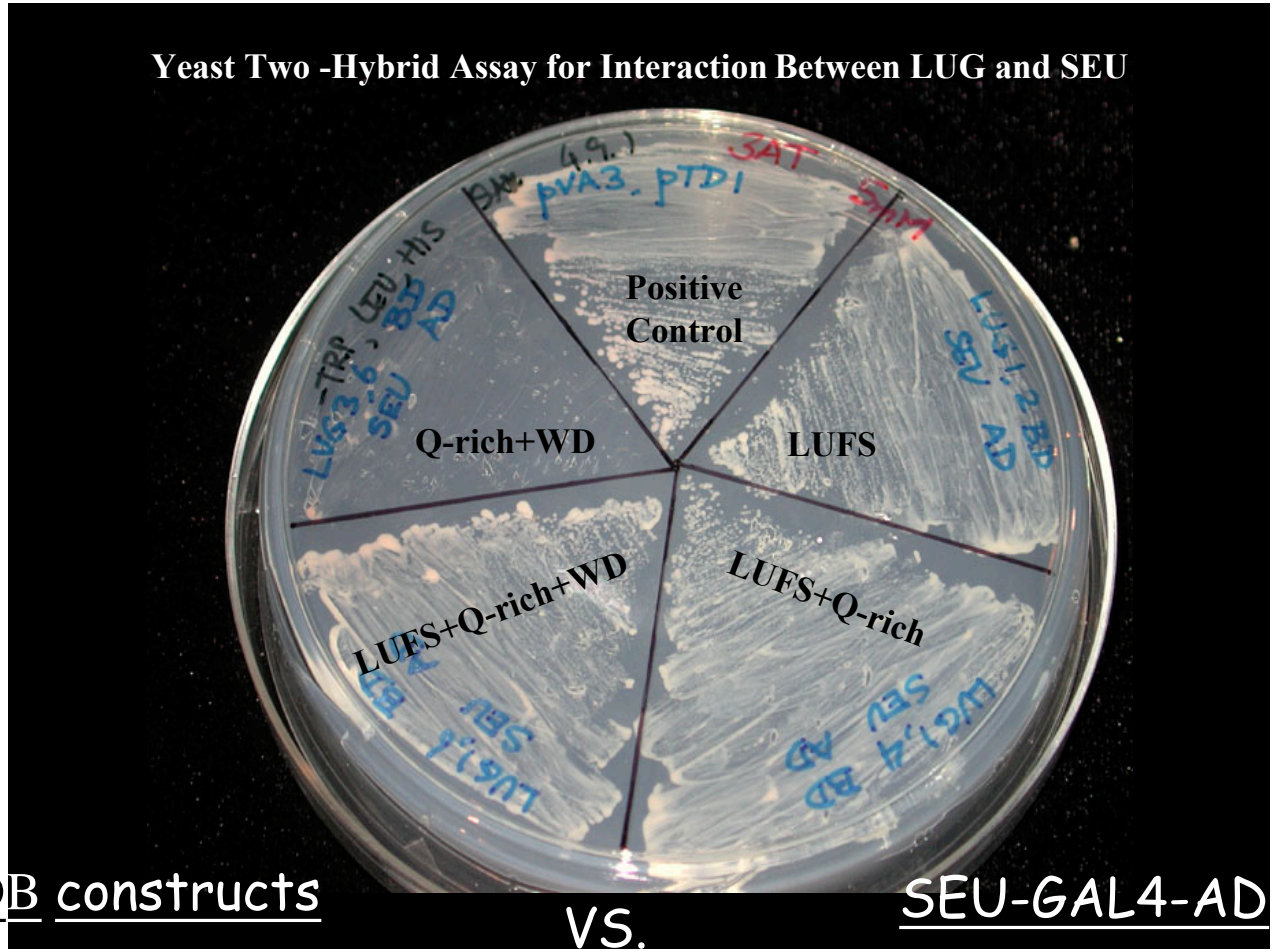
cal-1: wild-type looking
ap1-1: flower mutant
ap1-1 cal-1: cauliflower



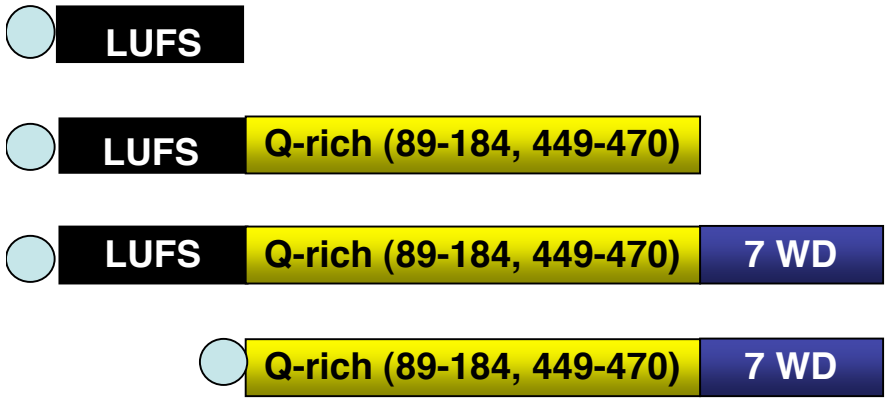
Yeast Two Hybrid (Y2H) Assay

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.