Lecture 9-10. Plant genomics II

Functional genomics: Identify the function of each and every gene in the genome. Since the characterization of the function of a protein domain in one organism generally provides hint to its function in another organism, the first goal of functional genomics is to identify as many genes as possible in major model organisms

Basic Approaches

A. Forward genetics: Random mutagenesis, screen for traits of interests

Chromosome walking or transposon-tagging

B. Reverse genetics: disrupt a particular gene or set of genes with known seq.

C. Fine structure genetics

D. Gene expression profile (analyses of transcriptome)

B. High-throughput reverse genetics

1. PCR-based screen for T-DNA or transposon insertion mutations in specific genes---Wisconsin knockout facility

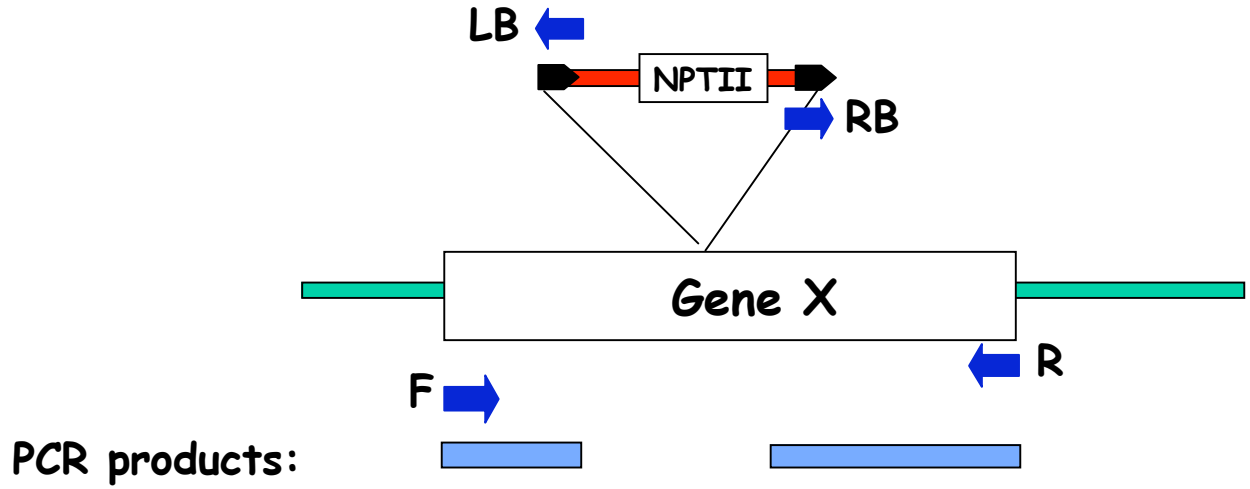
2. Database searches--salk institute lines

3. TILLING

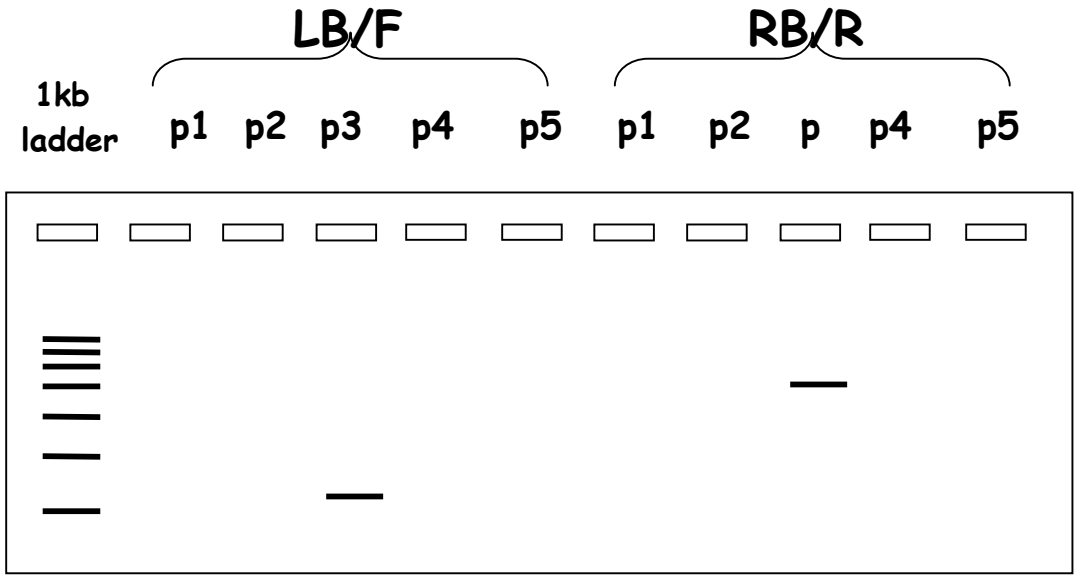
4. RNAi (RNA interference)

5. Gain-of-function (activation-tagging) mutagenesis

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

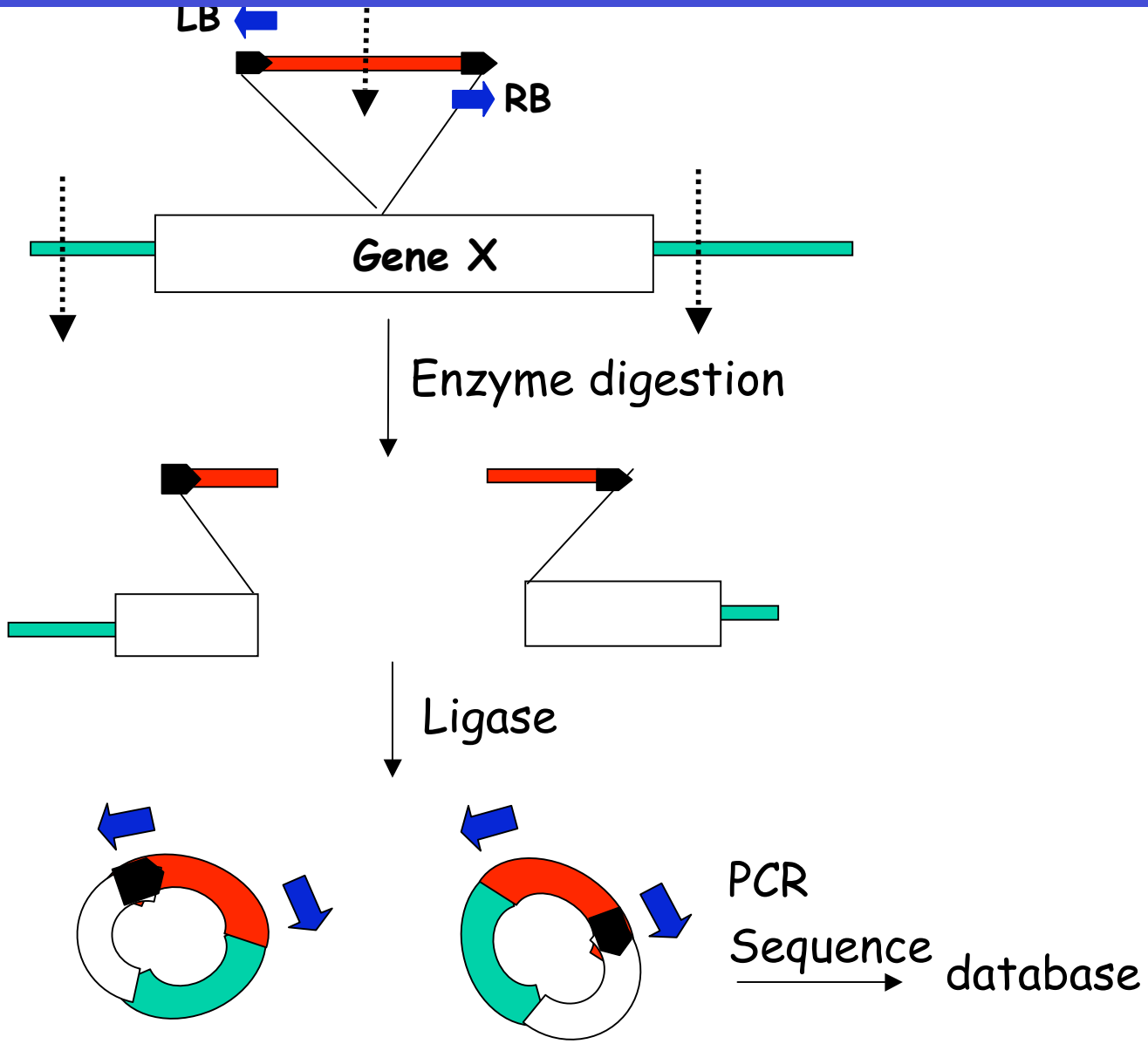


Screening pools (p1-p5)



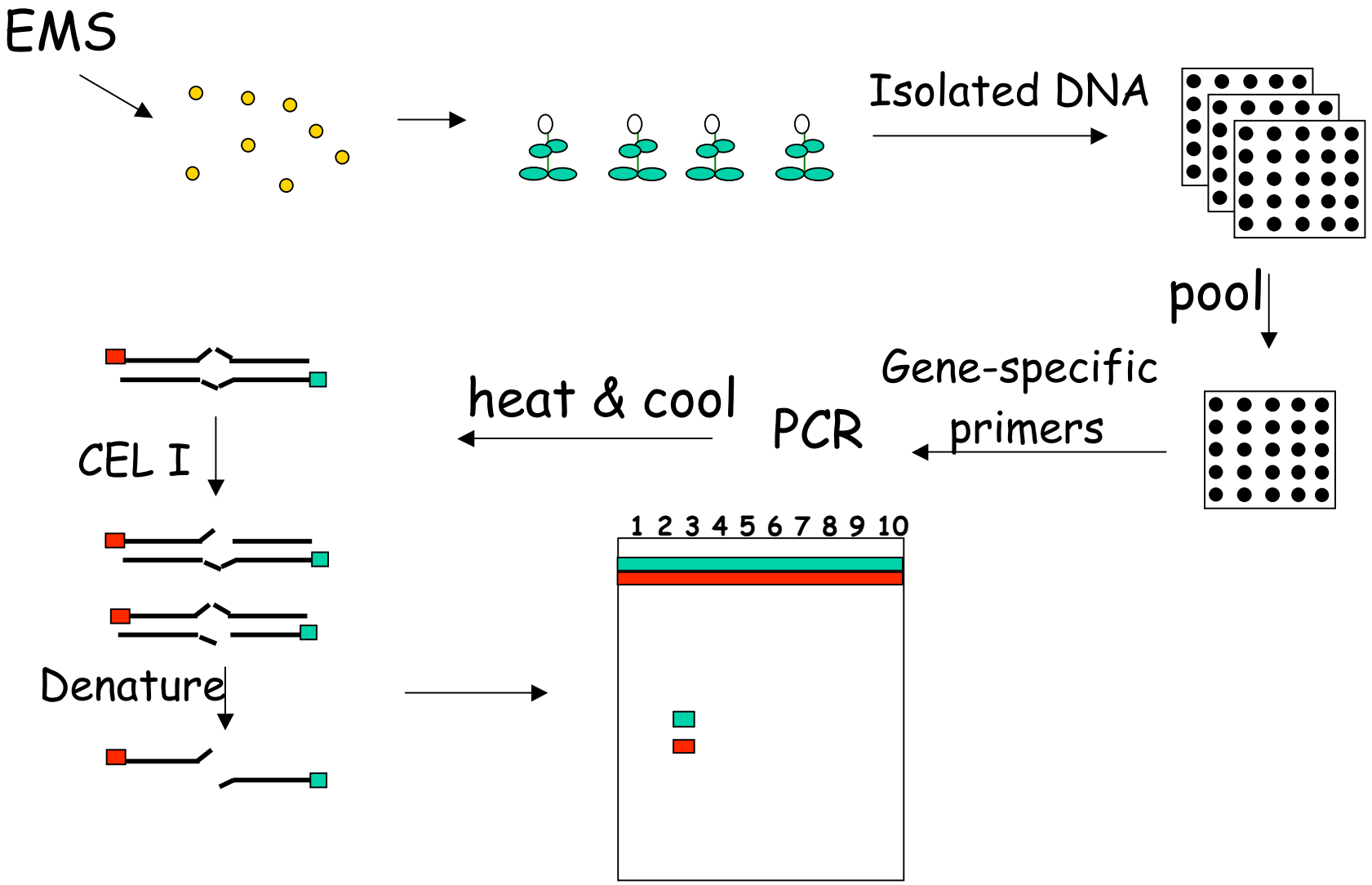
2. Data-base searches for T-DNA insertions in the genes of interests

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

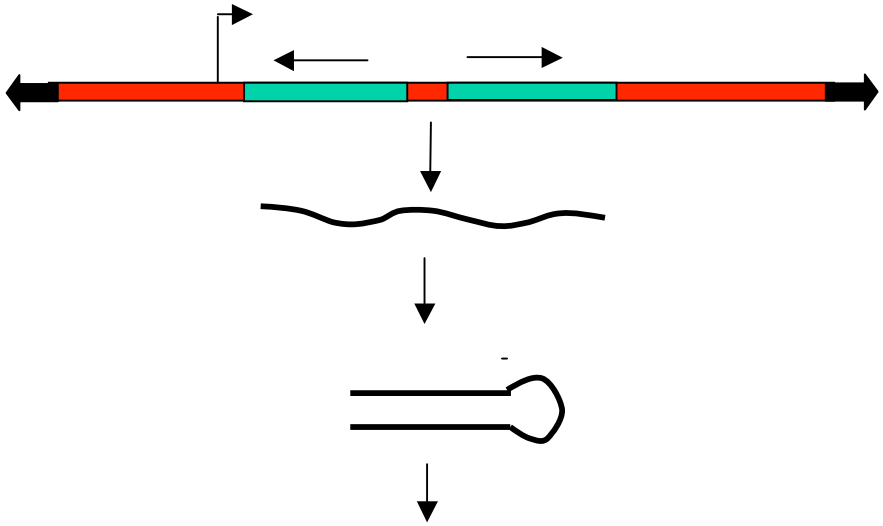


3. TILLING (Targeting Induced Local Lesions IN Genomes)

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

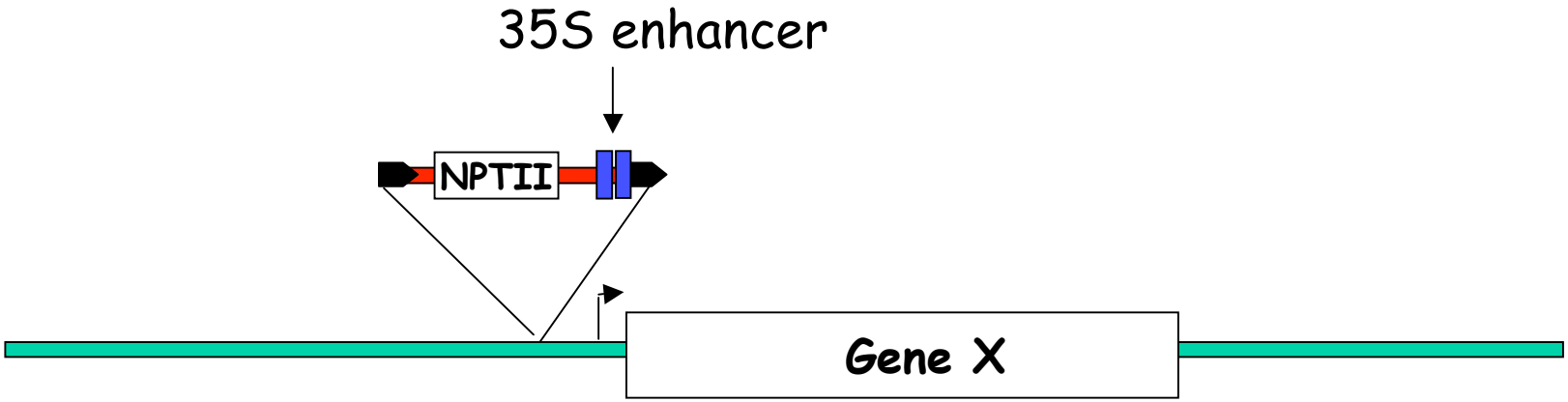


4. RNA interference (RNAi)



loss of gene activity

5. Gain-of-function (Activation tagging) mutagenesis



Over-express Gene X

Leading to gain-of-function phenotype

C. Fine Structure Genetics

1. Modifier screens: enhancer and suppressor screens, synthetic lethal

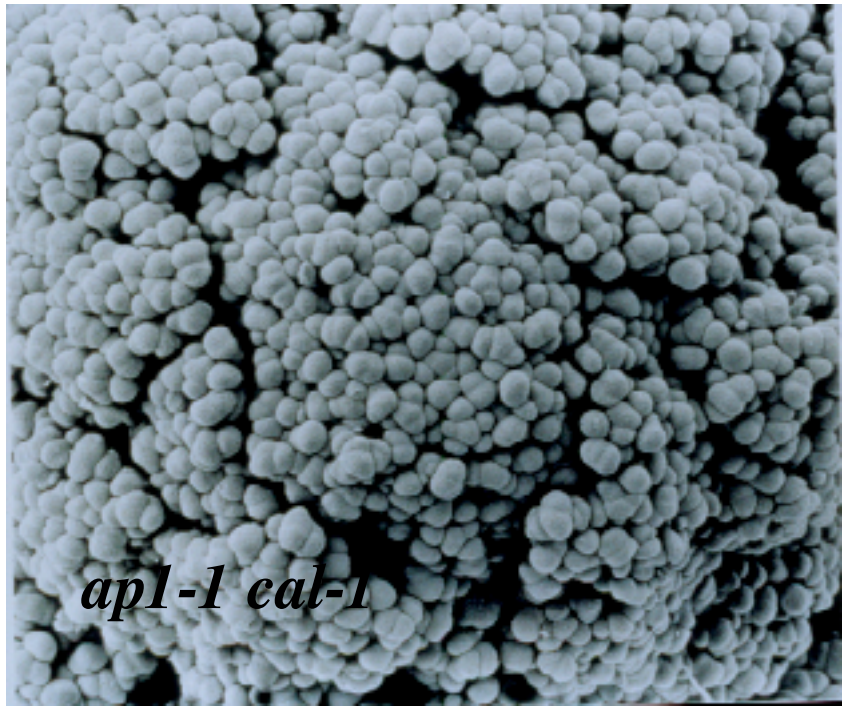
2. Enhancer-trap (using GFP or GUS)

3. GAL4-mediated over-expression

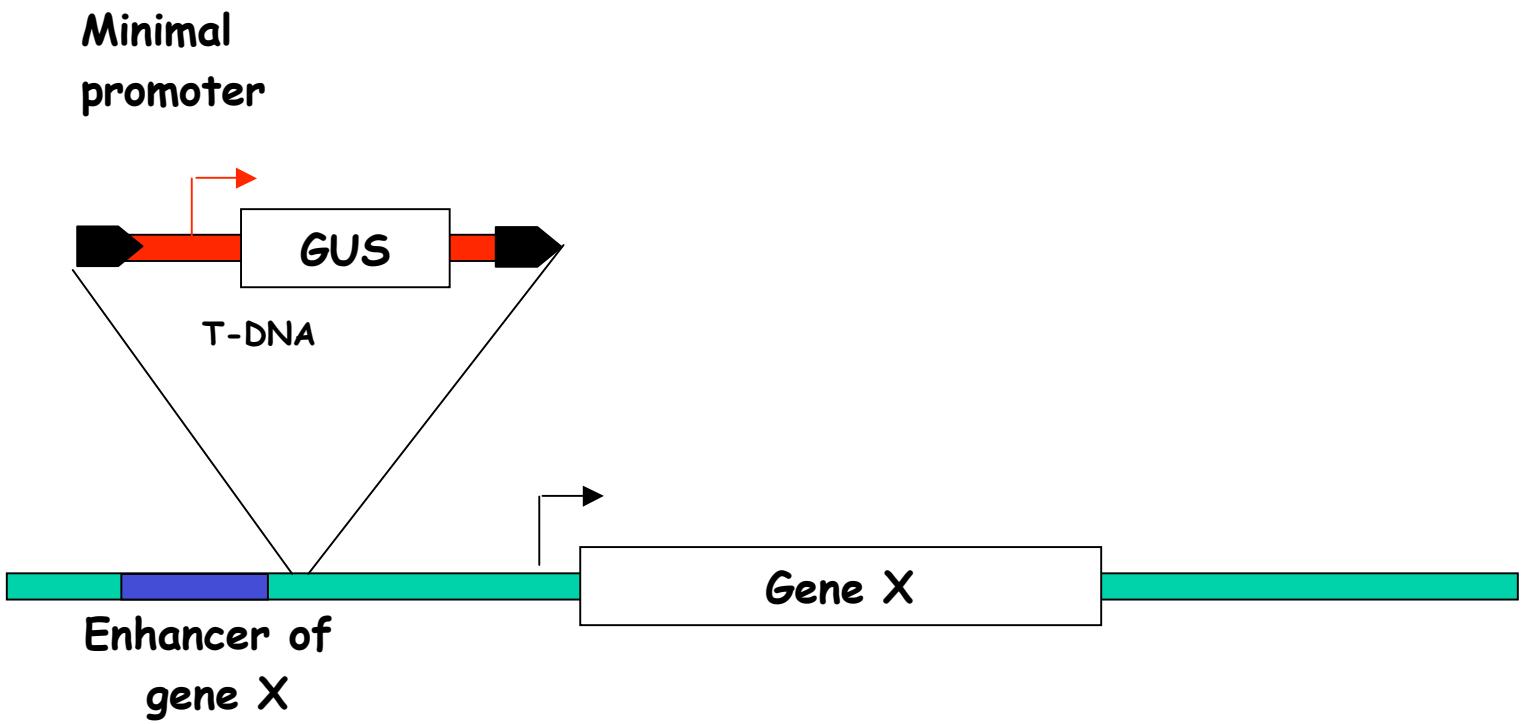
4. Yeast Two-hybrid screen (Y2H)

1. Enhancer or suppressor mutations

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



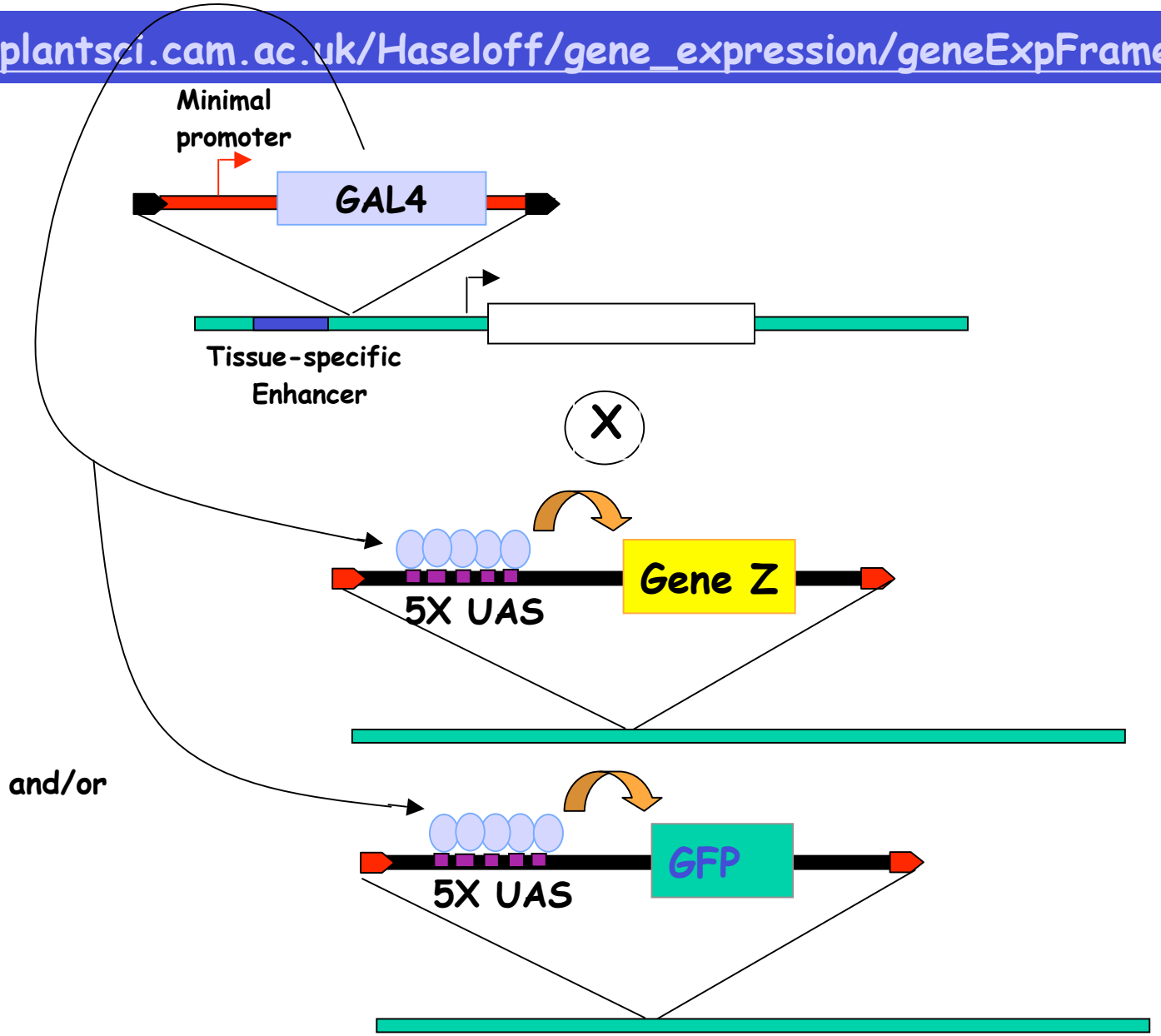
2. Enhancer-trap



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

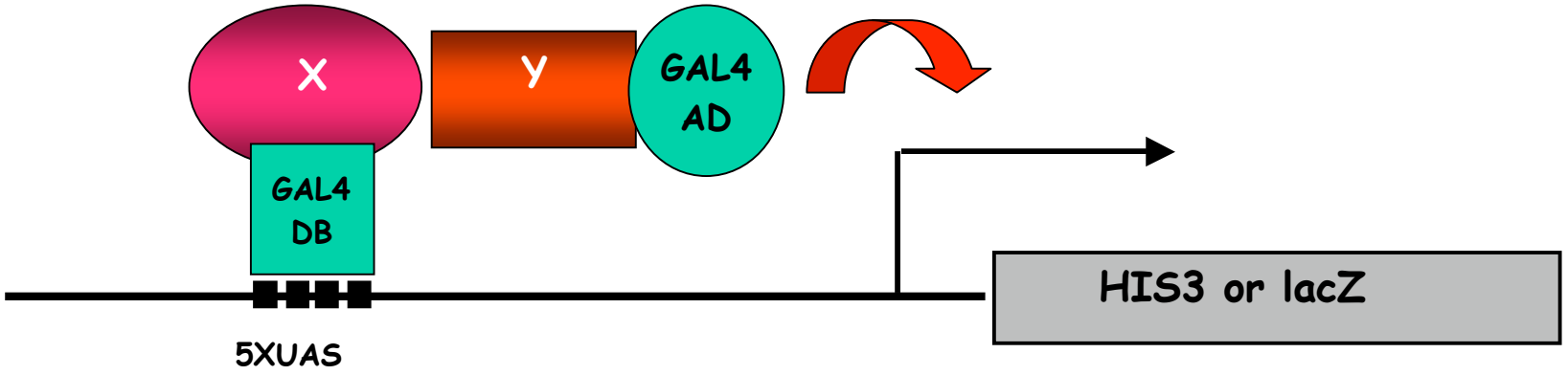
3. GAL4-mediated over expression

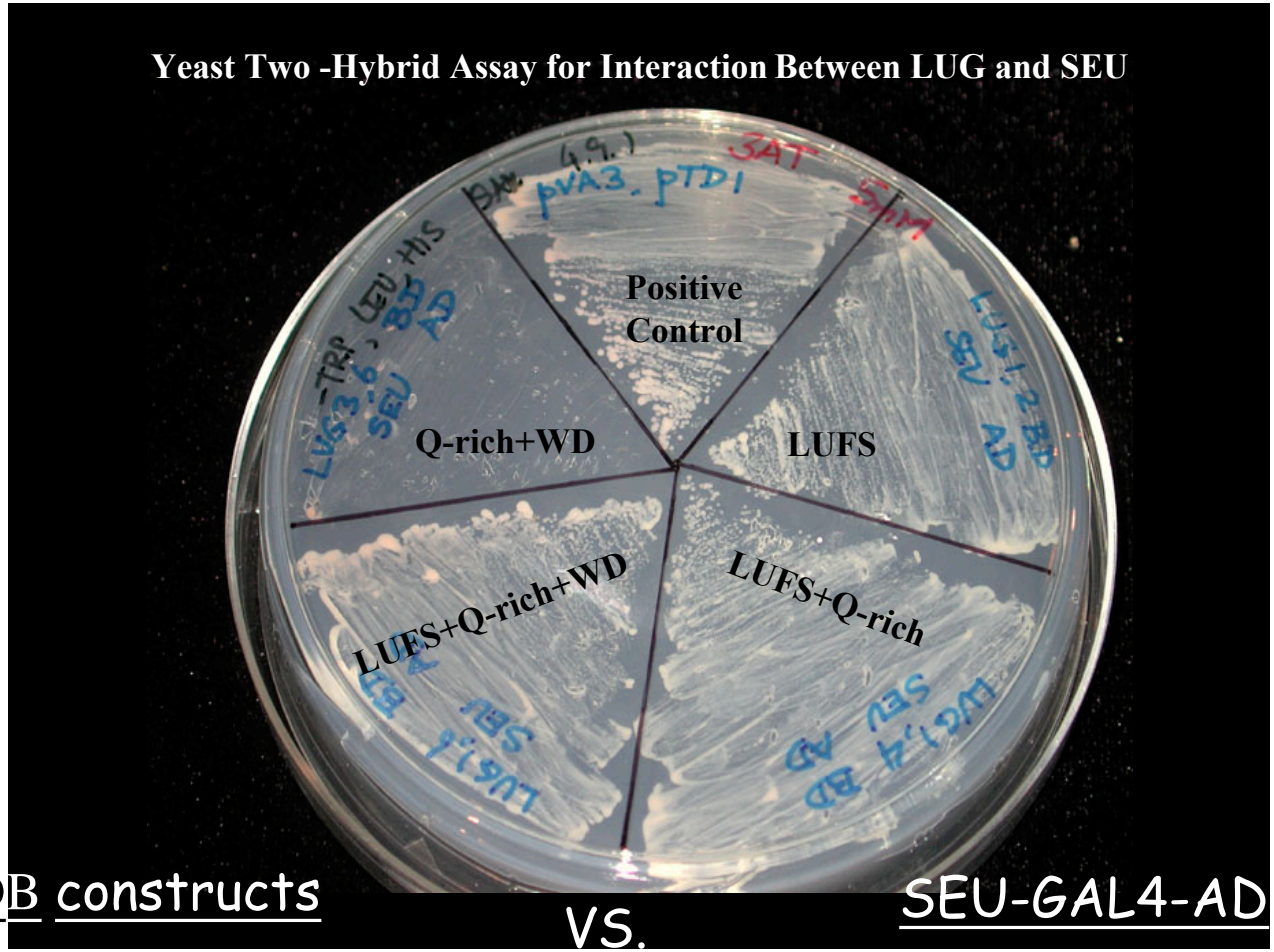
www.plantsci.cam.ac.uk/Haseloff/gene_expression/geneExpFrameset.html



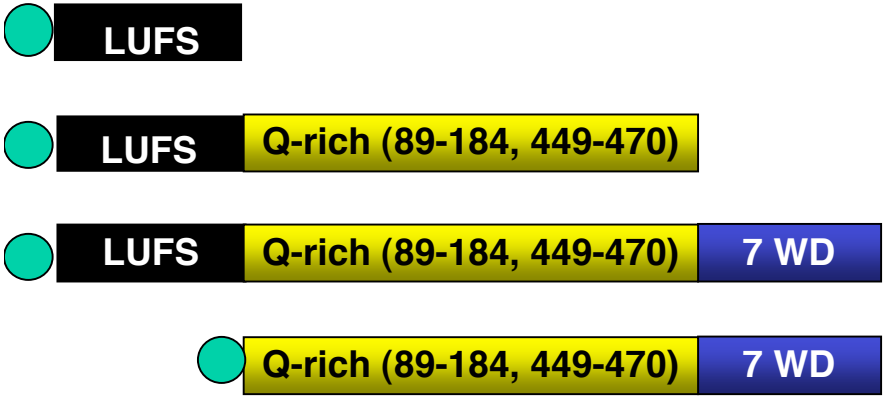
4.Yeast Two Hybrid (Y2H) Assay

to test interaction between two proteins





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



D. Analyses of the transcriptome

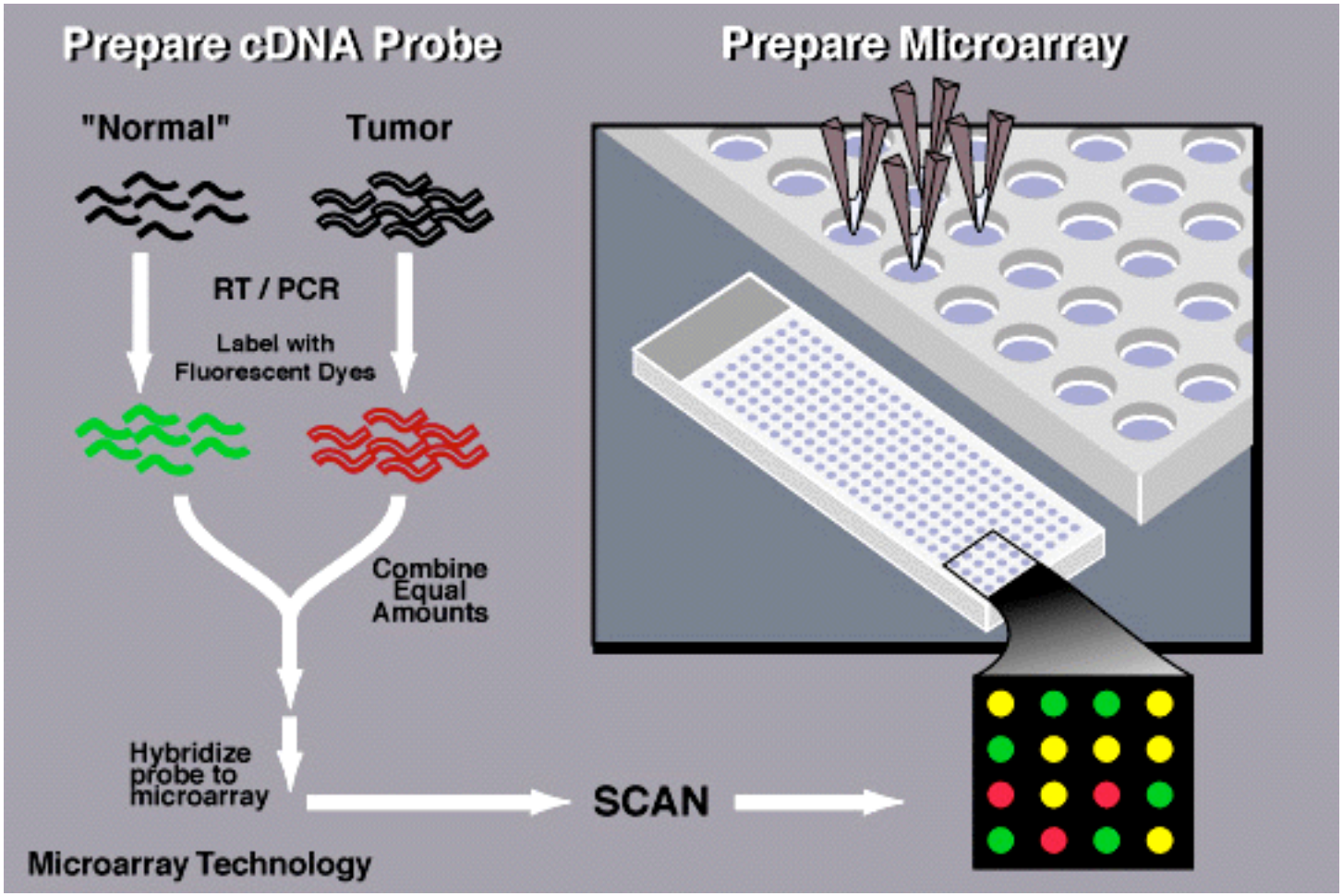
Documenting gene expression on a genome wide scale

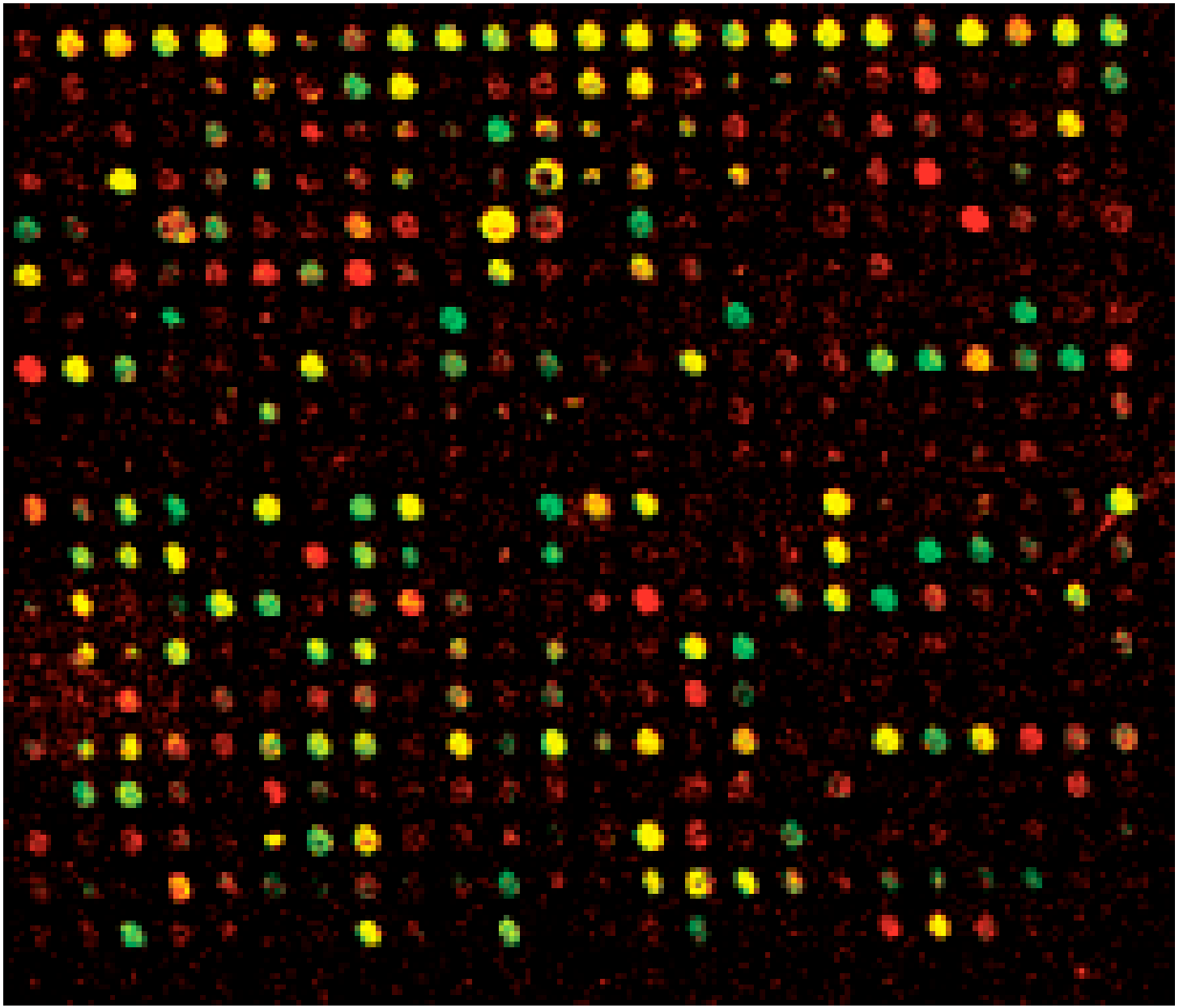
Transcriptome: complete set of transcripts and their relative expression levels in a particular cell or tissue under defined conditions

I. cDNA microarrays

II. Oligonucleotide arrays

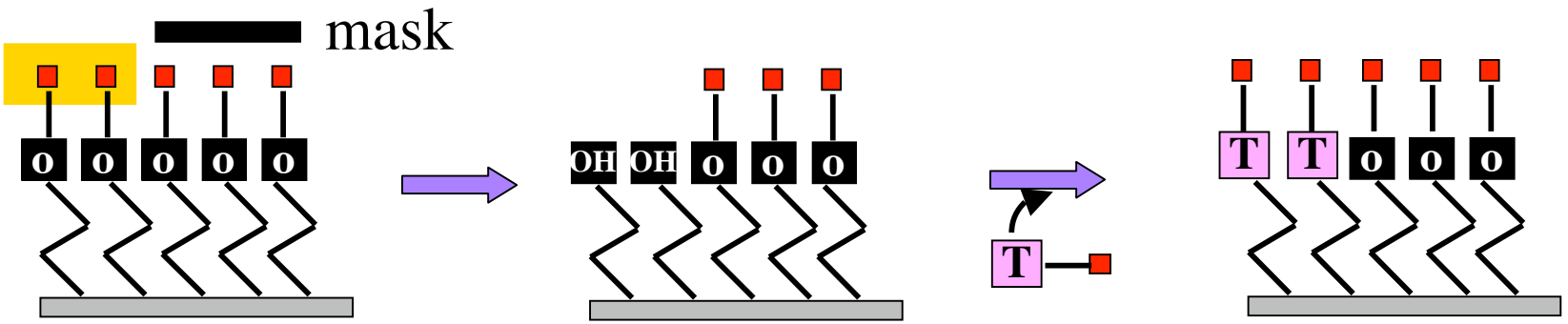
I. cDNA microarrays

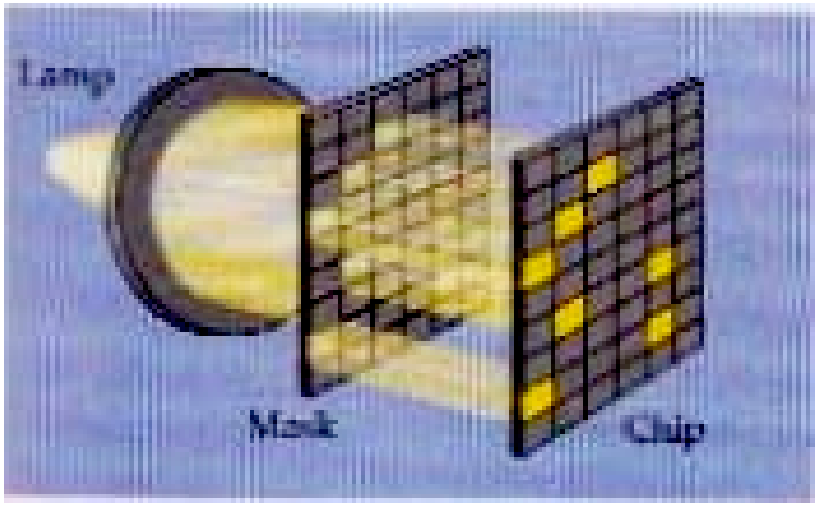




II. Oligonucleotide microarrays (Affymetrix GeneChip)

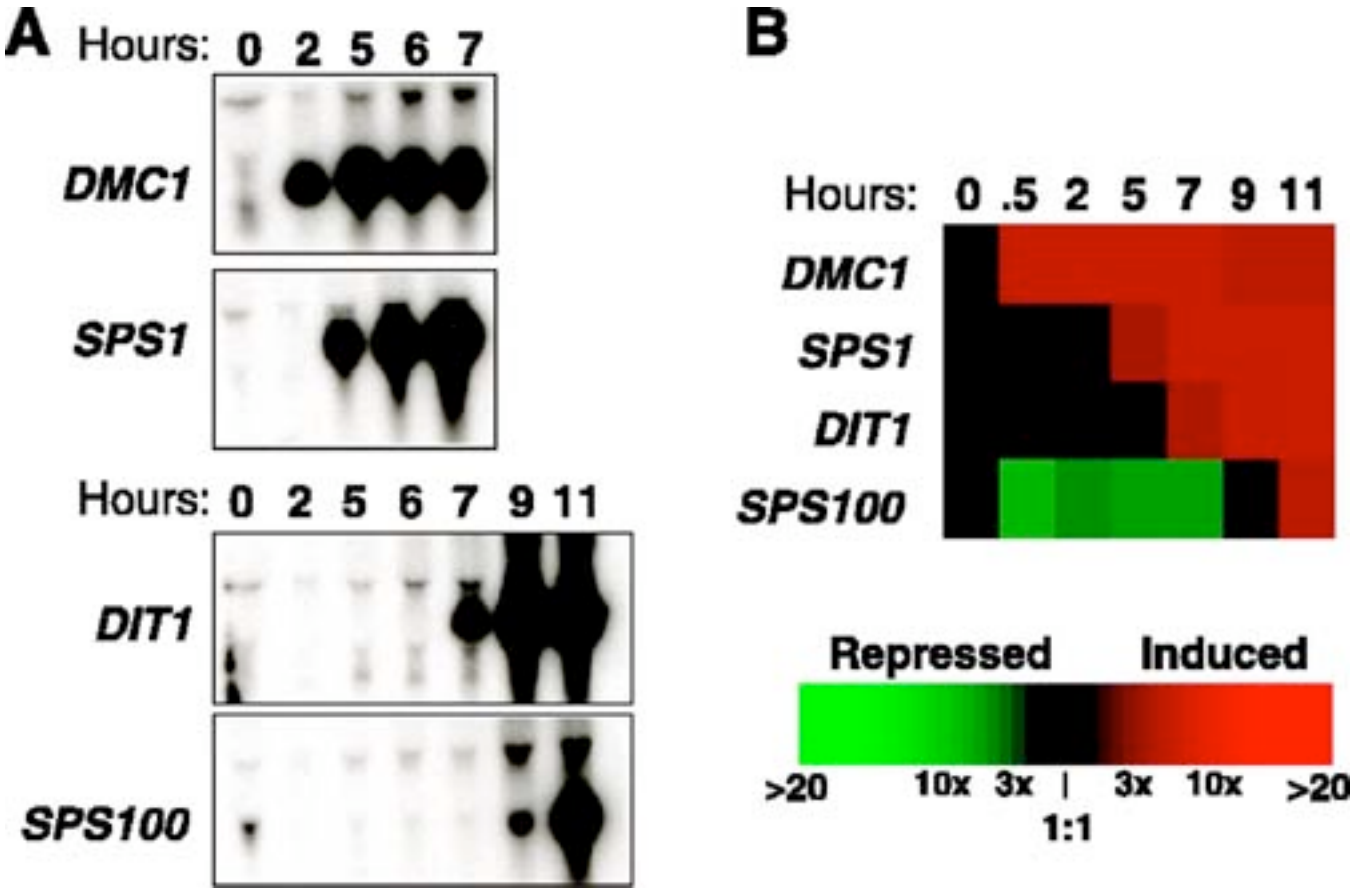
Light deprotection



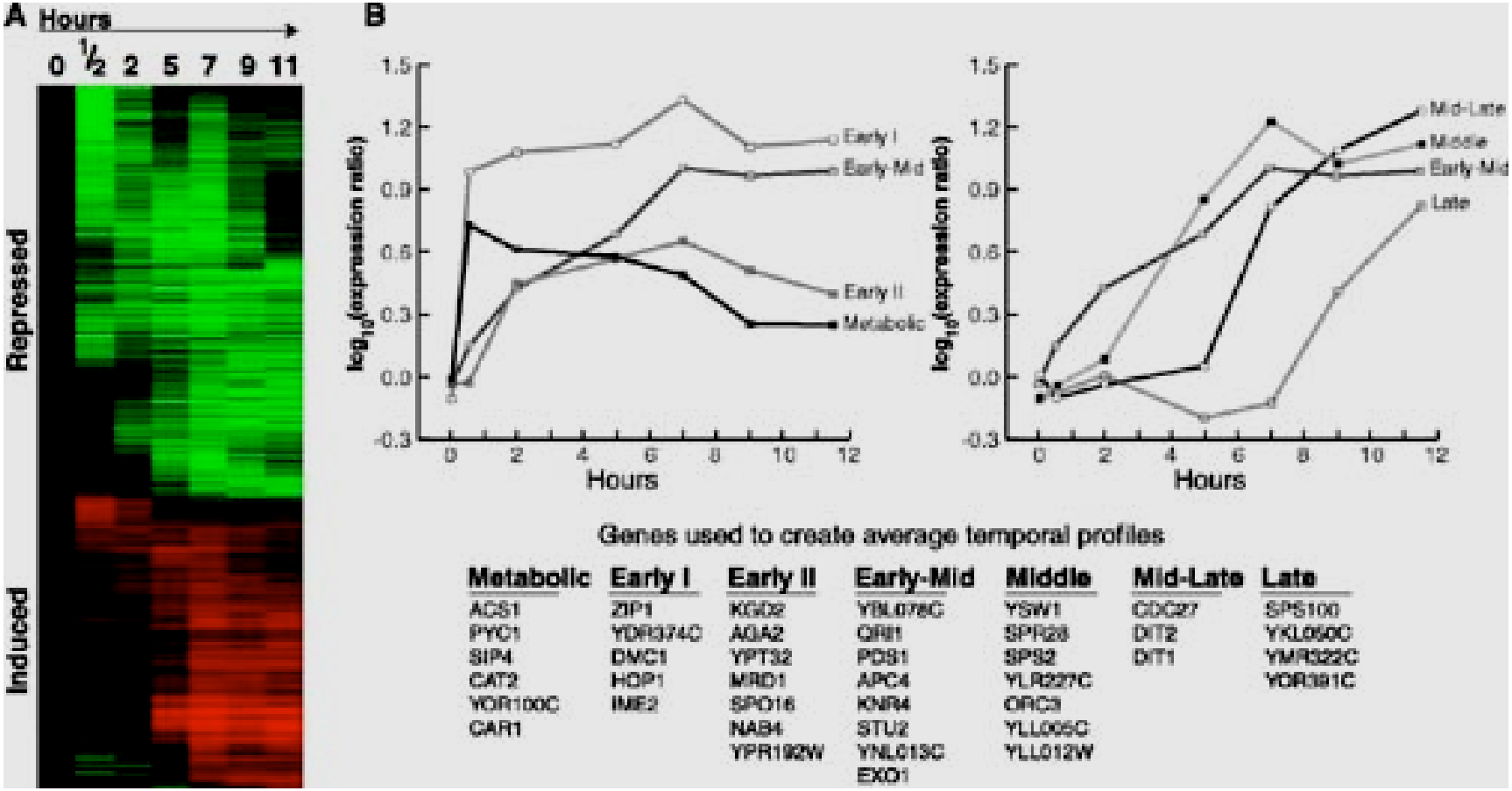


Sporulation gene expression profile in budding yeast

Chu et al., (1998) Science 282, 699-705



Several classes of sporulation gene expression after transfer to sporulation media



Survey of 1116 genes during sporulation in budding yeast

Chu et al., (1998) Science 282, 699-705