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

PCR products:

Screening pools (p1-p5)

1kb ladder  LB/F  RB/R

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Gene X

LB ← NPTII → RB

F ← R
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)

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
Yeast Two-Hybrid Assay for Interaction Between LUG and SEU

- LUFS
- LUFS+Q-rich
- Q-rich+WD
- LUFS+Q-rich+WD
- Positive Control

Constructs:
- LUFS
- LUFS+Q-rich (89-184, 449-470)
- 7 WD

SeU-GAL4-AD constructs

Comparison: LUFS-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

[Diagram showing the light deprotection process involving a mask and molecular structures with OH and T modifications]
Sporulation gene expression profile in budding yeast


Several classes of sporulation gene expression after transfer to sporulation media
Survey of 1116 genes during sporulation in budding yeast