Lecture 18 Reverse Genetics

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. Gene expression profile (analyses of transcriptome)

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

D. Fine structure genetics
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**

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2. 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

Gene X

Enzyme digestion

Ligase

PCR Sequence database
3. TILLING (Targeting Induced Local Lesions IN Genomes)

4. RNA interference (RNAi)

loss of gene activity
(a) Synthesis of dsRNA

(b) A result of par-1 dsRNA treatment
5. Gain-of-function (Activation tagging) mutagenesis

Over-express Gene X
Leading to gain-of-function phenotype