

Functional Genomics

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1 Methodology

1.1 Goal of functional genomics

- defining the function of all genes. function may be defined at many levels (biochemical, . . . , behavioral)
- Accelerate the process of genetic analysis. you always need the same basic functional information: sequence, mutants, expression, protein interactions
- Strategy is automation!

1.2 Forward genetics (phenotype \rightarrow DNA)

Given a phenotype, use random mutagenesis, screen progeny to identify genes related to it, positionally clone affected locus

1. saturation screening - mutate genome or chromosome to guarantee at least one mutation per progeny (EMS, x-ray, gamma ray). Want unbiased hit distribution over genome
2. insertional mutagenesis using transposable element linked to a marker
 - easy to revert to wild type
 - easy cloning of a site
 - but entry sites are biased
 - controlled transposition using transposase
3. mutant testing to identify which mice contain a mutation yielding phenotype
 - F1 (dominant) and F3 (recessive) genetic screens
 - (a) Having mutagenized a slew of male mice with say ENU in F0
 - (b) cross these males to homozygous recessive females (test cross). If a mutation is dominant, you will recover mutant heterozygotes in the F1 generation. Keep the males, which are founder males. Do a second 'confirmation' cross to the F0 female.
 - (c) If nothing seen in F1, take the F1 founder males and cross again to the F0 females. Then backcross the heterozygous females (all females) from F2 to the F1 males or intercross with F2 males. The F3 generation should yield recessive mutants, however a fourth cross is needed to confirm, in which you cross the F3 mutant to someone from F1 (grandfather or grandmother) of opposite sex.
 - complementation grouping: given a set of affected mice, cross homozygous mutants against homozygous dominants, and determine the complementation sets.
 - lethal recessive mutations - balancer chromosomes for fly and mouse
4. 'pre'-positional cloning to find out what is already known about the phenotype/genotype
 - additional markers, eg functional SNPs
 - find all genes in a candidate region, expression patterns of these genes, any existing mutants?
5. positional cloning to identify the mutated locus. The idea is to gradually narrow down upon the locus:
 - (a) Genetic map: Determine linkage with other loci using a genetic map. Continue to backcross to homozygote recessives, looking for linkage with other traits. Once you can determine chromosome, you can mate with a balancer mouse, to make it easy to breed the mutant.

- (b) Physical map: Use these associated loci to search for physical markers, simple repeats, STSs, and SNPs. You might be able to cut the set of possible loci in half, eg.
 - (c) Genomic resources: Now that you have found two relatively close markers, check out the genome sequence between these markers and identify all known genes. Test each gene until you find the affected locus. Depending on the resolution desired, you can sequence your mutant to see the point mutation, et al.
6. Determining function more precisely: Pathway, system, disease
- Expression: Microarrays, fluorescent or whole mount in situ hybridization, tissue sections, EST mining, reporter assay using transgenic mice
 - Gene Ontology annotations
 - promoter regulation, protein binding interactions (place in a pathway)
 - Subsequent mutant characterization: loss of function allele, etc

1.3 Reverse genetics (DNA → phenotype)

- given a set of genes, identify phenotypes caused by their disruption using directed mutagenesis
- methods
 - homologous recombination - insertional cassettes with reporters
 - knock-ins - fully replace target sequence, or alter transcriptional control of a gene
 - * position-effect - expression determined by genomic location
 - transposon mutagenesis
 - RNAi + screening
 - * transient loss of gene function
 - gain of function
 - * inducible promoters, multiple copies of gene
 - phenocopy - environmental situation mimics mutational phenotype
- mutant testing
 - complementation grouping
 - inverse PCR sequencing (following large scale mutagenesis)

1.4 Fine-structure genetics

- manipulate topology of genes to generate novel functions and interactions
- balancer chromosome
 - contain recessive lethal to prevent appearance of homozygous balancer
 - inversions to inhibit chromosomal recombination
 - dominant visible marker to identify organism with balancer
- modifier screen (pathway based screen)
 - identify genes that interact with a mutant gene that affects phenotype
 - synthetic lethal - two independently viable mutants are inviable together
 - * uncover redundant functions and interacting genes
- enhancer trapping
 - insert reporter gene transposable element adjacent to target gene both of which loci are controlled by the same enhancer region
 - * indicates when a particular gene is expressed
 - ectopic expression - use GAL4 reporter in TE to drive expression of UAS enhanced gene at another locus
 - * allows tissue specific expression
- floxing - specific gene disruption in time and/or space
 - homologous recombination to insert *lox* site flanking an exon - target sequences of *cre* recombinase
 - mate mouse with *lox* site to mouse with *cre* gene, and progeny have missing exon
 - drive expression of *cre* using inducible or tissue/time specific promoters