BIS101 F2014: lecture 16 Forward and Reverse Genetics

Forward vs. reverse genetics

- draw phenotype & genotype w/ arrows
- forward: no prior genetic knowledge required
    - forward: requires lots of mutagenesis and screening
- reverse: need to "know" locus
    - only gets @ effect of 1 gene

Mutagenesis important for both approaches

- chemical -- EMS, high rate, usually hypomorph/hypermorph
    - alylates G's, leading to G||C A||T mutation (transition?)
- physical -- radiation, medium, usually loss of fxn, rearrangement
- biological -- TEs, low, can be loss of fxn in gene or hypomorph in reg. how could you make mutants with TEs ? Ac/Ds

Often make mutant "libraries" of lines of mouse or rice or whatever with known mutations. Broad use to community.

Forward: "phenotype forward"

Identify strange phenotype. From nature or often from mutant libraries.

Identify mode of action. How? crosses (either with Natural mutant or artificial one)

- why are dominant mutations rare? (most will be loss of function or hypomorphic)
- advantage of haploid organisms? (all mutations seen)
- if multiple mutants, do mutations complement? same vs. different.
- under what conditions does mutation have effect? gets at function?

Map mutant

- QTL mapping & fine mapping
- what needed ? genetic map, physical map (reference genome sequence) to put it in context with other genes

Test candidate loci:

- do they have differences from WT (nonsyn etc.). ? PCR and sequence
- look for size differences (indels etc.) ? PCR
- Show expression diffs? (how measure? RNAseq or qPCR or Northern)

Reverse

Start with locus of interest. Want to know what it does.

What does sequence tell us ? Important domains, shared promotoers, etc.

e.g. we can see it has a zinc-finger-domain, so we know it binds DNA.

Mutagenize and look for fxn.

- knockout or knockdown (how do latter? RNAi)
- CRIPS/CAS editing

Where/when is it expressed?

- RNAseq in different tissues.
- Transform promoter/enhancer w/ reporter gene (example? tb1 transient assay)
- in situ hybridization: label DNA/RNA and see in what tissues it hybridizes

Levels of protein:

- Western blots

If protein interacts with DNA? Chip-seq (redraw?)

If it's DNA sequence how can we tell methylation status?

- bisulfite sequencing (methlyated C's protected)
- methylation sensitive restriction enzymes