2013 7.03 Exam 2
Review Sheet

**Phage Genetics**

- How does λ (Pam int–::Tn5) phage mutagenesis work?
- How does P1 phage cotransduction work?
    - For 2 factors – to determine the frequency of cotransduction (linkage).
    - For 3 factors – to determine the order of genes.

**Bacterial Genetics**

- Conjugation
    - How do bacteria exchange genetic material by conjugation?
- $F^+$ bactiera and $F^-$ bacteria
    - What is $F^+$ bacteria? What is $F^-$ bacteria?
    - How can $F^+$ bacteria transfer its F-plasmid to $F^-$ bacteria?
- Hfr bacteria
    - How does Hfr arise from $F^+$ bacteria via integration of F-plasmid into chromosome?
    - How does Hfr transfer its chromosomal DNA to $F^-$ bacteria (by DNA replication, transfer through the pilus, and integration by double crossovers)?
    - What genes are transferred early? What are transferred late?
    - Closer genes are co-transferred more frequently.
- F' bacteria
    - How does F' bacteria arise from Hfr bacteria via a looping event (single cross over) of bacterial chromosome to generate a F' plasmid?
    - How is the F' plasmid different from the F-plasmid?
    - How does a F' plasmid transfer bacterial DNA to a $F^-$ bacteria?
    - How is this transfer of bacteria DNA different from Hfr?

**Regulatory Pathways in Prokaryotes (e.g. bacteria) and Eukaryotes (e.g. yeast)**

- Lac Operon and Mal Operon (bacteria); Gal4-Gal80 pathway (yeast).
- Types of mutations in a regulatory pathway:
    - Cis-regulatory elements/ DNA sequences:
        - Promoter, operator (in bacteria);
        - Promoter, UAS, URS (in yeast).
    - Trans-regulatory elements/ diffusible factors:
        - Activator (loss-of-function, dominant-negative activator, super-activator)
        - Repressor (loss-of-function, dominant-negative repressor, super-repressor)
- How to assess the phenotype:
    - Measure the gene product (e.g. LacZ activity).
    - Insert a reporter into the operon, downstream of the gene of interest.
      (Promoter of gene A – GeneA – Reporter)
    - Place reporter downstream of the cis-regulatory sequences of gene of interest, replacing the coding sequence of the gene.
      (Promoter of gene A – Reporter)
- Regulated pathway (inducible) VS dis-regulated pathway (uninducible or constitutive).
- How to set up the test and interpret the results for
    - recessive VS dominant mutation?
    - mutation in same gene VS different genes?
    - cis VS trans acting components?
- Epistasis test - identify order of components in a linear pathway
    - Require distinct phenotypes of each single mutant A- and B-
    - If A-B- phenotype = A- phenotype, A is epistatic (works downstream) of B.