7.02/10.702 Spring 2005

Genetics Day 5 notes
(Eric Sullivan)

I. Homologous recombination
II. Generalized Transduction: stabilization and mapping
III. Choosing controls for Day 6 patching

I. Homologous Recombination:

Homologous – strands are (sequentially) identical
Recombination – exchange of DNA between two DNA strands

- Draw our donor recombining with the host (need an even number of crosses to retain circular DNA (or cells die)) and then draw a nucleotide level view

Q: Where did our donor come from?
A: P1 infected our pNK/KBS1 Ara^- mutants

II. Generalized transduction:

- Transduction: movement of DNA via viral intermediate
- Generalized Transduction: any host DNA can be transferred (non-specifically)

Q: Why are we moving the DNA?
A: Stability, separate transposon from transposase

Q: Is transposition considered homologous recombination?
A: No, there's no homology between insert and host DNA

Q: What happens during infection?
A: Draw out different Cells and infections

(If we did the) C600 mapping experiment:
clarify – still used today, if you don't know where an insert is located ahead of time

Q: What nearby genetic loci would we be mapping?
A: *thr* and *leu*

Q: Are we actually looking at the *leu* gene (Leu+ vs. Leu-)?
A: No, Cm is inserted, so we follow CmR instead

Q: If our donor is Kan^R, Ara^-, Cm^R, and Thr^+, what must the recipient be?
A: The opposite (Kan^S, Ara^+, Cm^S, and Thr^-)

- Draw crossover example
- Next time we'll use numbers to see how we can determine the actual order.

III. Choosing Controls:

Everyone will need to have chosen controls before entering lab on day 6:

LB Cm LB Kan Mac Ara Kan LB X-gal Kan LB Ara X-gal Kan

- Need positive and negative control – one that grows/shows and one that doesn't for each phenotype the plates test i.e. LB Kan, pos = Kan^R and neg = Kan^S

- Also, Patch the control onto all plates, not just the one(s) it's checking