7.02 Fall 2001 Genetics Day 5 Recitation

ANNOUNCEMENTS: GENETICS REVIEW SESSION BEFORE THE QUIZ :
Wednesday, September 26th 7-9 pm in 4-270
(No lecture, just a question and answer period)

I. How have we generated and stabilized our *ara::lacZ* translational fusion

A. Transposon mutagenesis of *E. coli* BW140 genome with mini-Tn*10* transposon
   1. λ1205 was our delivery vehicle
   2. Select for transposon insertion into the genome and screen for Ara- mutants on MacConkey plates containing arabinose

B. Established pure clones by picking and purifying mutants **(GENETICS DAY 2.5)**

C. Further characterize arabinose and *lacZ* phenotypes of mutants **(GENETICS DAY 3)**
   1. Screen Ara- mutants phenotypes
      a. M9 Ara plates: Ara- mutants can t grow on these plates because they don t have a utilizable carbon source
      b. M9 Glucose plates: Would expect Ara- mutant to grow; no growth indicates problem with strain or patching (possible second mutation)
      c. Mac Ara Kan. White colonies should grow - a second indicator of possible problems in patching or with strain
   2. Test whether *lacZ* +/- and whether transcription is inducible by arabinose or constitutive

D. Identify and inoculate *ara::lacZ* translational fusions for P1 transduction **(GENETICS DAY 3.5)**

E. Stabilizing the mutation
   1. Why do you need to stablize the mutation? (Transposase is still present in the cell. Even though we aren t inducing transcription of the transposase gene any more with IPTG, there can still be a little bit of activity from this promoter. Don t want transposon that generated *ara::lacZ* translational fusion to hop out.)
   2. How do you stablize mutation? P1 transduction
   3. P1 bacteriophage:
      a. Lytic life cycle
         - Phage infection and replication of DNA.
         - Production of enzymes that break host DNA into pieces
         - Head and tail proteins synthesis
         - Packaging of DNA into heads
           * It will package 100 kb of DNA
           * 1/1000 phage will package bacterial DNA - the phage are called defective phage
         - Lysis
           * Lysate composition: In 10^8 phage particles, 10^5 would be defective, but the remaining approx. 10^8 phage which aren t defective (virulent)
           * Defective phage carries 2% of bacterial chromosome - library of the bacterial chromosome in your 10^5 phage particles
   4. Transduction of BW140 with P1 lysate
      a. Defective phage delivers piece of donor DNA to cell.

- What is the fate of this piece of DNA delivered by the phage?
    * The DNA will either recombine into the recipient chromosome through homologous recombination, or it will be lost
    * Select for recombination into the chromosome

b. MOI - Would a high or low MOI be beneficial for this reaction? Why?
- A low MOI is beneficial for this reaction because we want to minimize the chance that the cell that is infected with your defective phage carrying your gene of interest is also not subsequently infected with an active phage particle and lysed.
- Have we used a high MOI during any of our experiments? (Yes, the MOI was high for the generation of the P1 lysate because we were concerned only with generating the highest number of defective phage particles in the shortest amount of time.)

5. Confirm the successful stabilization of your mutation by characterizing its phenotype.

G. Mapping *ara* region within the chromosome using P1 transduction (**Day 4, 5, 6 & 7)**

1. You can also map genes that occur around the *ara* region with P1 transduction.
2. This is true because genes that are closer together, transduce together more often.
3. We know that the *leu* and *thr* genes are close to *araBADC* in the *E. coli* genome; capable of being cotransduced.
    a. *leu* locus encodes enzymes responsible for synthesis of amino acid leucine
    b. *thr* locus encodes enzymes responsible for synthesis of threonine
    c. How would these mutations effect the cell s phenotype? (Cell would not survive without addition of leucine and threonine - can t synthesize proteins.)
4. Phenotype of C600 strain: Leu-, Thr-, Ara+, Lac-, Kan^S
5. Phenotype of donor strain: Leu+, Thr+ Ara-, LacZ(?), Kan^R
6. Tuesday/Thursday section will select for Kan^R C600 transductants; Wednesday/Friday section will select for Leu+ C600 transductants.
7. Calculate how often Leu+, Kan^R, and Thr+ by characterizing the phenotype of many different transductants. Calculate co-transduction frequencies=$\frac{\text{\# Leu+Thr+}}{\text{Total Leu+}}$

H. Hypothetical Example of co-transduction mapping

1. Three markers A, B & C
2. Donor strain = A+ B+ C-
3. Recipient strain = A- B- C+
4. We transduced the recipient strain with lysate from the donor strain, selected for transfer of the A+ marker. We screened for transfer of the two other markers and observed 4 transductant classes at different frequencies.

A+ B- C+ : 56.9%
A+ B+ C+ : 37.9%
A+ B+ C-: 4.7%
A+ B- C- : 0.5%

This suggests that the gene order is

A----B----------C

or

C-----------------A-----B

because the cotransduction frequency between A&B(42.6%) is much higher than A&C(5.2%)

5. How do we determine the correct genome order? Two ways
    a. We know that rare transductant classes occur from very rare events. A quadruple cross over with the genome between a 100 Kb region of transducing DNA that carries all three markers is a very rare event.
        - Why is this so rare? Packaging of all 3 markers doesn t happen all the time.
        - 2 recombination events occurring simultaneously is rarer than a single recombination event
        - 2 Recombination events occurring simultaneously within a span of 100 Kb is extremely rare. - Which transductant class does this represent? A+ B- C-
        - Would you be able to see this rare quadruple crossover if you selected for receipt of the middle marker? (No, B- cells won t grow. The rarest class of transductants generated when you select for B+ is transfer of all three markers from your donor - A+ B+ C-)
    b. A second strategy for determining gene order is to transduce and select for two different markers. (i.e. What we are doing in class - Tuesday/Thursday is selecting for Kan^R; Wednesday/Friday are selecting for Leu+) The co-transduction frequency between B & C will allow you to rule out one of the two models.

II. Mechanics of P1 phage transduction

A. You obtain C600 and BW140 cultures that grew overnight in LB + $CaCl_2$.
    - Ca++ necessary for phage adsorption

B. Centrifuge for 2 min. Pour off the supernatent and resuspend in MC medium.
    1. Make certain that cells are thoroughly resuspended - no cell clumps
    2. Calcium in MC medium is good for phage infection

C. Add 10 μL of P1 lysate to each set of recipient tubes. Protocol mentions that this is expected to be 10^8 phage/ml. Why is this important? (Trying to establish an MOI of 0.1 - dense O/N culture has cell count approx. 10^9 cells/ml) Why do we want a low MOI? (Low MOI prevents multiple infections.)

D. Mix by shaking gently and incubate on bench for 30 min. (Time of phage adsorption) You have an extra tube of each recipient, what are they for? (Uninfected controls)

E. Add sodium citrate. Why? (Chelates divalent cations in media - stops phage adsorption)

F. Add 1 ml LB and shake at 37 °C for one hour. What s going on during this hour? (Recombination of DNA into the chromosome; synthesis of protein encoding kanamycin resistance; synthesis of protein involved in leucine synthesis; cell replication)

G. Transfer cultures to eppendorf tubes, centrifuge 2 min., decant supernatent and resuspend cells in 50 μL LB + sodium citrate (Wed/Fri resuspended in M9 + sodium citrate). Why have sodium citrate? (Chelating any remaining free Ca++ ions) Why did Wed/Fri use M9 instead of LB? (Didn t want to add any nutrients to their M9 Thr plates.)

   Spread cells on appropriate plates
    1. Everyone spreads BW140 on LB Kan. Tuesday/Thursday spread C600 on LB Kan, Wednesday/Friday spread C600 on M9 Thr

H. Also spot P1 lysate on LB Kan plates