7.02 Fall 2001 Genetics Day 4 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. Generation and stabilization of *ara::lacZ* translational fusion
- A. Used λ1205 to deliver the mini-Tn10 transposon to the *E. coli* strain pNK/BW140
  1. Transposon gene inserted randomly into the genome
- B. Select for transposon insertion into the genome
- C. Screen for Ara- mutants on MacConkey plates containing arabinose
  1. Enzymes responsible for converting L-arabinose to D-xylulose-phosphate encoded by *araBAD*
  2. Expresion from *araBAD* is regulated by *araC*
  3. *araC* expressed in the presence and absence of arabinose
- D. Established pure clones by picking and purifying mutants **(GENETICS DAY 2.5)**
- E. Further characterize arabinose and *lacZ* phenotypes of mutants (**GENETICS DAY 3)**
  1. Screen Ara- mutants phenotypes
     a. Patch on M9 Ara plates. Ara- mutants can not grow on these plates because they don t have a utilizable carbon source
     b. Also streak on M9 Glucose plates. Allows detection of a second, auxotrophic mutation if it exists
     c. Streak on Mac Ara Kan. (Colonies should grow - serves to indicate any possible problems in patching.)
     d. Patch from least rich (M9 Ara) to most rich (Mac Ara Kan)
     e. Patched appropriate control strains
  2. Test whether *lacZ* is transcribed and translated and whether transcription is inducible by arabinose or constitutive
     a. Used two plates to identify constitutive and arabinose inducible insertions. What are the phenotypes of inducible and constitutive mutations on the two plates?

	Inducible	Constitutive
- LB Ara X-gal Kan:	Blue	Blue
- LB X-gal Kan:	White	Blue

     b. Controls are important - inducible mutants may look a little blue on LB X-gal, not nearly as blue as constitutive mutation.

- E. Identify and inoculate *ara::lacZ* translational fusions for P1 transduction (**GENETICS DAY 3.5)**
- F. 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. Phage that infects gram negative bacteria (E. coli is gram negative -

reflection of cell wall/outer cell envelope composition.)

b. After it infects, phage replicates its DNA. It also produces enzymes that break host DNA into pieces

c. Also makes head and tail proteins for packaging

d. Packaging of DNA into heads

- P1 phage is unlike some other phage in that it doesn t have as stringent of a sequence specificity for what it packages into its heads
- It will package 100 kb of DNA that carry a packaging site - site similar to this occurs in host DNA
- 1/1000 phage will package bacterial DNA - the phage are called defective phage

e. Phage lyse bacterial cells and phage are released into medium and are capable of infecting more bacteria

- If a phage lysate contained 10^8 phage particles, how many of these would be defective? (10^5)
- Defective phage carries 2% of bacterial chromosome - library of the bacterial chromosome in your 10^5 phage particles

f. Phage that carry bacterial DNA infect new bacterial cells. They aren t able to replicate and lyse the infected cell because they don t contain any phage genetic material to direct this process.

g. 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 degraded.
- If the piece of DNA that recombines into the chromosome contains a marker, such as our kanamycin-resistant transposon, then we can select for it

4. Can then test the phenotype of your newly generated mutant to make sure that you transduced your *ara::lacZ* fusion.

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. You will use an *E. coli* strain that has mutations in two genes that are known to be in close proximity of the arabinose operon within the *E. coli* genome to determine how often these two genes are transduced with the arabinose operon.

II. Mechanics of P1 phage transduction

A. You obtained your overnight cultures that had been growing in LB + $CaCl_2$ (Ca++ required for adsorption of P1 phage to bacterial surface.) (Why don t you need maltose? P1 has different surface receptor than λ.)

B. You took the OD of these cultures and could proceed only if the OD was above a certain level. Why did you do this? (You wanted to make sure there were enough cells present to produce a sufficiently large phage titer. This increases your possibility of transducing the mutation you want.)

C. You added 50 μL of P1 lysate to your donor cultures. There was a sixth tube containing BK1 that you didn t add P1 lysate to. What is this important for? (Uninfected control - allows you to compare turbidity of your lysed cells to

something that hasn t lysed.)

D. Now you re incubating for 2.5 hrs. Why is the incubation time so long? (You are allowing for multiple rounds of infection and lysis until most bacterial cells have been infected and lysed.)

E. After you ve determined lysis has occurred. You ll add chloroform to your tubes.
   1. What is the function of chloroform? (Lyses any remaining unlysed cells)
   2. Is chloroform a volatile or non-volatile liquid? (Volatile)
   3. Do you remember from your training lab how that will affect the way it behaves when pipeted? (Often dribbles from pipet tip.)
   4. How can you avoid dribbling any chloroform on yourself?
      - Pay attention to what you are doing
      - Wear gloves
      - Work under the hood

F. You centrifuge your lysates in Epppendorf tubes.
   1. Separating P1 lysates from cellular debris and the bulk of the chloroform

G. Transfer supernatent to new Eppendorf and add 10 μL chloroform. After vortexing, make sure you label this tube and give it to your TA to keep in their ice bucket.
   1. What have you just generated? (A P1 lysate from you donor strain containing an *ara::lacZ* insertion.)
   2. What are you going to do with this? (Use it to infect a recipient strain of bacteria and introduce your mutation into the chromosome of this strain.)

III. Determining phage titer and calculating moi

1. You are also going to count the plaques from your phage titration that you did on T/W. This is very similar to what we did on day 2 with the results of our viable counts - Use electronic counting pens to save time and don t count plates that have more than 300 plaques. We are also going to be generating a weighted average for the number of plaques we count.)
   a. Remember that with a weighted average, we give the most confidence (1) to the plate that we have the most confidence in, our lowest dilution.
   b. Sample calculation - Average the numbers from your two plates

10^{-5} - TNTC
10^{-6} - 50 plaques
10^{-7} - 4 plaques
10^{-8} - 1 plaques

$$\frac{50 + 4 + 1}{1.11} = 50$$

$$\textbf{pfu/ml} = \frac{50 \text{ plaques}}{(0.4 \text{ ml})(0.3\text{ml}/1.2\text{ml}) * 10^{-6}} = \frac{\#\text{ of plaques}}{(\text{vol. plated})(\text{vol. phage/total vol.})(\text{dil. factor})}$$

pfu/ml = $5*10^{8}$ pfu/ml

2. You should calculate your cfu/ml by converting the OD_{550} that you took of your pNK/BW140 (Remember it was a 1/50 dilution) during the mutagenesis on Day 3 with the OD_{550}/CFU/ml correlation factor you calculated from Day 2 s results. (Should you use the correlation factor for minimal media or LB? Why? (LB, because that was the type of media we used for the transposon mutagenesis.)

3. You can use these two numbers to calculate your moi.

$$\text{MOI} = \frac{\#\ \text{phage (pfu/ml} * \text{vol. added to mutagenesis reaction/total volume)}}{\#\ \text{cells (cfu/ml} * \text{vol. added to mutagenesis reaction/total volume)}}$$