7.02 Fall 2001 Genetics Day 3 Recitation

I. Generation and stabilization of *ara::lacZ* translational fusion

A. Infected *E. coli* strain pNK/BW140 with bacteriophage λ1205 carrying the mini-Tn*10* transposon

1. λ1205 delivered the transposon into the cell.
2. Transposon gene inserted randomly into the genome when acted upon by transposase encoded by pNK

B. Select for transposon insertion into the genome — acquisition of kanamycin resistance

C. Screen for Ara- mutants on MacConkey plates containing arabinose

1. Enzymes responsible for converting L-arabinose to D-xylulose-phosphate encoded by *araBAD*
   a. Takes arabinose through a series of intermediates to D-xylulose-phosphate, which can enter central metabolism and be fermented
   b. Mutation in *araD* is lethal because toxic intermediate builds up
   c. Expresion from *araBAD* is regulated by *araC*
      i. Transcription is induced by *araC* in the presence of arabinose
      ii. *araC* represses expression from *araBAD* in the absence of arabinose
   d. *araC* expressed in the presence and absence of arabinose

   e. How are Ara- mutants generated? Into what genes must the Tn insert?
      i. Insertion into *araB, araA,* or *araC; araD* insertion = lethal

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. Will the Ara- mutants grow? (No, minimal media doesn t contain a carbon source other than arabinose for the bacteria to use.) Why do we patch on M9 Ara plates? Can you think of an instance where an Ara+ strain produced a white colony?
   b. Also streak on M9 Glucose plates. Will the Ara- mutants grow? (Yes)Can you think of an instance where Ara- mutants wouldn t grow? (If they also have an auxotrophic mutation.)
   c. Streak on Mac Ara Kan. What do you expect to see on this plate? (White colonies)
   d. The order in which we patch our plates is important — you want to patch from most restrictive (M9 Ara) to least restrictive (Mac Ara Kan) - Don t want to transfer nutrients and confound our results.
   e. Patch your four colonies from each mutant strain on the same line in the grid.
   f. Make sure to patch the appropriate controls. Why do you need to patch controls? (Able to reference what you observe with previously characterized phenotypes)

2. Test whether *lacZ* is transcribed and translated and whether transcription is inducible by arabinose or constitutive
   a. *lacZ* will only be transcribed and translated if it has inserted into a

gene in the correct reading frame and orientation
- Reading frame review

ATG TAC TAC TAG CCA   ATG TAC TAT AGC CA   ATG TAC TTA GCC A

b. You will use two plates today to identify constitutive and arabinose inducible insertions? What are the phenotypes of inducible and constitutive mutations?

	Inducible	Constitutive
- LB Ara X-gal Kan:	(Blue colonies)	(Blue colonies)
- LB X-gal Kan:	(White Colonies)	(Blue colonies)

How would you expect the four control strains to behave on these plates?

	BK1	EJ1	H24	JET2
Phenotype	Ara-, LacZ+(I) Kan^R	Ara-, Lac-, Kan^R	Ara-, LacZ+(C), Kan^R	Ara+,Lac-, Kan^R
M9 Ara	(No growth)	(No growth)	(No growth)	(Growth)
M9 Glu	(Growth)	(Growth)	(Growth)	(Growth)
Mac Ara Kan	(White colonies)	(White colonies)	(White colonies)	(Red colonies)
LB X-gal Kan	(White colonies)	(White colonies)	(Blue colonies)	(White colonies)
LB Ara X-gal Kan	(Blue colonies)	(White colonies)	(Blue colonies)	(White colonies)

E. Identify *ara::lacZ* translational fusions (**GENETICS DAY 3.5) –** W/F class will do this for T/Th class on Wednesday; T/Th class will do this for W/F class on Th - you should fill in the chart as to what you patch and tape it to your plates so that the other group can complete your experiment for you. Strains that are characterized as having *ara::lacZ* translational fusions will be inoculated by your classmates into media for overnight growth before P1 transduction.

F. Stabilizing the mutation
1. Move transposon and gene in which it has inserted away from transposase.
2. Use P1 phage transduction to do this(**GENETICS DAY 4, 5 & 6)**
   a. P1 is another bacteriophage.

G. Mapping Mutation using P1 transduction (**Day 4, 5, 6 & 7)**

II. Titration of λ1205

A. λ1205 = delivery vehicle we used for transposon mutagenesis

B. Wild type λ capable of two different lifestyles — lytic and lysogenic

Lysogenic = after phage infects, it stably integrates into the host chromosome - called a prophage; replicates as part of host genome; can be induced to excise under certain conditions

Lytic = phage infects, replicates, synthesizes phage head/tail, packages DNA into phage particles and lyses cells

C. λ1205 has a mutation in the phage attachment site so it is not capable of lysogeny

D. λ1205 also has an amber mutation in a gene encoding for a protein involved in DNA replication so it is not capable of lysis, either.

E. Phage titering - determining how many infectious phage particles are in the stock
1. Plate low concentrations of phage with bacteria - each infectious phage will

create its own zone of lysis called a plaque

2. Counting plaques allows us to determine phage titer
3. Phage titering depends on the ability of our λ1205 to infect and lyse cells. How is this possible, since they contain a mutation that normally prevents DNA replication and subsequent lysis?
   a. The E. coli strain LE392 is a suppressor host that contains tRNA that are capable of reading the amber stop codon and inserting an amino acid in its place
   b. When grown on LE392, phage replication is uncrippled and lysis can occur
4. Protocol
   a. Make serial dilutions of 10^{-2}-10^{-8} of the phage stock in total volume of 1 ml in buffer TMG (How would you do this?)
   b. Label and infect four tubes containing 0.9 mL LE392 culture with 0.3 of your diluted phage stock at 10^{-5},10^{-6},10^{-7},10^{-8}
   c. Mix by shaking gently and incubate on bench for 30 min.
   d. Practice phage plating with your two control strains
      - Suggest adding 3 ml top agar to tubes already in heating blocks
      - Add 0.3 ml uninfected LE392 to 3 ml melted top agar (in heating block)
      - Remove tube from heating block, roll tube briskly in hands to mix
      - Flame top of tube and quickly pour contents on to plate
      - Spread agar evenly by swirling plate
      - Set aside to allow to solidify. Note the time that it takes.
      - Repeat procedure except add 0.1 ml of 10^{-5} to top agar and plate on phage alone plate
      - What do you expect to see from these two plates?
   e. Plate your bacterial cells infected with different phage dilutions by removing 0.4 ml of culture and adding it to two tubes of your top agar.
   f. After all 4 plates have solidified, incubate overnight at 37°C

F. Why is phage tittering important?
1. We want to infect our cells with a certain number of phage.
2. Why? We want to minimize the chance that more that one phage will infect our cells, so that we don t end up with multiple transposon insertions
   a. Why is it important that we don t end up with multiple transposon insertions? Want to characterize a phenotype that we know to be result of one mutation.
3. How do we determine this? First we need to calculate a multiplicity of infection, which is equal to the # of phage particles/number of bacterial cells

4. Once we have done this, we can use the Poisson distribution (a discrete distribution that is often used to model the number of events that will occur in a specific time period) to model how often we expect a certain number of phage to infect one cell at a given moi.
5. The formula for the Poisson distribution (also given in your appendix)

$$P(n) = \frac{m^n e^{-m}}{n!}$$

n=number of phage, m = m.o.i.

6. Using the Poisson distribution, we can determine the percentage of cells that would be infected by more than one phage at a given m.o.i.

   At an m.o.i. of 1 this is equal to 0.26, which is $P(>1) = 1-(P(0)+P(1))$

   At an m.o.i.of 0.1, this is equal to 0.01

G. You will use the phage titer you determine today along with the CFU/ml calculation from day 2 to determine the m.o.i of your transposon mutagenesis
H. You will also count the number of cells that you screened during your transposon mutagenesis to calculate the frequency of transposition events resulting in Ara- mutants. (You do not need to count all your plates, just a representative sample)