7.02 Fall 2001 Genetics Day 6

I. Mapping *ara::*mini-Tn*10* with respect to *leu* and *thr* operons

A. Transduction of mapping strain, C600, with P1 lysate from Ara- mutants

1. Phenotype of C600: Leu-, Thr-, Ara+, Kan^S

Why can t we use the BW140 to map our mutation? (We re measuring the rate at which different genes are transferred and in order to do this we must be able to distinguish between donor and recipient genes. BW140 has the same genotype and phenotype as our mutant strains with respect to *leu* and *thr*.)

B. Characterization of P1 transductants for Cotransduction mapping **(VI-A Part 2 and VI-B)**

1. Protocol tells you to patch 20 transductants from your 4 donor strain transductions on to 5 different plates
   a. If you don t have enough transductants on one plate, you can borrow from one of your other plates
   b. If you have patched all your transductants from your four plates and still don t have 80 transductants, you can see if other groups have extra mutants
      - It s really important that you not patch a transductant that another group has already used because counting the same transductant twice will result in incorrect cotransduction frequencies.
   c. The five different plates you are using are:

	Kan^R	Kan^S	Leu+	Leu-	Thr+	Thr-
M9 Glu	+	+	+	-	+	-
M9 Glu Leu	+	+	+	+	+	-
M9 Glu Thr	+	+	+	-	+	+
M9 Glu Leu Thr	+	+	+	+	+	+
LB Kan	+	-	-	-	-	-

+ = growth, - = no growth; assume remainder of phenotype is wt unless otherwise stated

   d. You also need to patch controls on both sets of plates patched
      - Controls allow you to compare the appearance of your transductants with cells of known phenotype
      - What would make a good control for your plates?
        * A Kan^S and Kan^R strain
        * A Leu+ and Leu- strain
        * A Thr+ and Thr- strain
        * C600 = Kan^S Leu- Thr-
        * BK1, EJ1, H24, JET2 = Kan^R Leu+ Thr+
   e. On Day 7 you will need to interpret the results from all your patching. You should make a chart similar to the one in your manual for analysis of your transductants.
      (Do this before Genetics Day 7/PBC Day 1)

f. We will go through the first example presented in your lab manual to help you understand how to analyze phenotypes, determine transductant classes and cotransduction frequencies

Grid #	Donor Strain	M9 Glu	M9 Glu Leu	M9 Glu Thr	M9 Glu Leu Thr	LB Kan	Can this data be used?	Leu Class	Thr Class
1	**XM5**	**-**	**-**	**-**	+	+	Yes	-	-
2	**XM5**	**-**	**-**	+	+	+	Yes	+	-
3	**XM5**	**-**	**-**	**-**	**-**	+	No		
4	**XM5**	**-**	**-**	**-**	+	+	Yes	-	-
5	**XM5**	**-**	+	**-**	+	+	Yes	-	+
6	**XM10**	+	+	+	+	+	Yes	+	+
7	**XM10**	**-**	**-**	+	+	+	Yes	+	-
8	**XM10**	**-**	**-**	+	+	+	Yes	+	-
9	**XM10**	+	**-**	**-**	+	+	No		
10	**XM10**	**-**	**-**	**-**	+	+	Yes	-	-

- These transductants were selected on LB Kan plates.
- Strategy for analysis suggested in lab manual.
  * Check M9 Glu Leu Thr plate first. This is a positive control, if transductants don t grow, they should be disregarded.
  * Check the M9 Glu Thr plate (if you selected for Leu+) or the LB Kan plate (if you selected for Kan^R). You would expect all your transductants to grow on these plates because this is what you selected for originally. If they don t they should be disregarded.
  * Check the M9 Glu plate. If transductants grow here and not on all the other M9 plates with glucose and amino acid supplements, they should be disregarded.
- Phenotype from the transductant in grid 3 can t be used because it does not grow on M9 Glu Leu Thr indicating something other than Leu- or Thr- is inhibiting growth on these plates.
- Phenotype from the transductant in grid 9 can not be used because it grows on M9 in the absence of both amino acids, but not in the presence of either amino acid singly, which wouldn t logically happen.
- So you have 8 different transductants whose phenotypes you can score

3/8 Leu- Thr- 3/8 Leu+ Thr- 1/8 Leu- Thr+ 1/8 Leu+ Thr+

Could use these numbers to calculate the cotransduction frequency between Kan^R and Leu+/Thr+. (Obviously you wouldn t use any number of transductants this small when calculating cotransduction frequencies, you ll calculate co-transduction frequencies from the class data.)

Cotransduction of Kan^R and Leu+ = $\frac{\#\ Kan^R\ Leu+}{}$ = 4/8 = 0.5

$$\text{Contranduction of Kan}^R \text{ and Thr+} = \frac{\text{\# Kan}^R \text{ Thr+}}{\text{Total \# of Kan}^R} = 2/8 = 0.25$$

You don t have a rare recombinant class in your data to allow you to differentiate between

ara------leu-----thr and *thr----------ara------leu*

However, when you calculate cotransduction frequencies from the class data, a rare recombinant class should emerge. You will also use the data generated from the opposite day s cotransduction experiments to substantiate your predicted map order.

II. Stabilization of *ara::lacZ* fusion

A. General characterization of P1 Transductants

1. The other patching you will be doing today involves both your C600 and BW140 transductants.
2. You will patch 4 colonies from each transductant on to five plates

	Phenotype differentiated	**Controls**
M9 Glu Thr	Leu+/Leu-	Leu- (C600) Leu+ (any other strain we use)
LB Kan	Kan^R/Kan^S	Kan^S(C600/BW140)Kan^R (other strains)
Mac Ara Kan	Ara+/Ara-	Ara+ (C600/BW140/JET2) Ara- (BK1, EJ, H24)
LB X-gal Kan	LacZ+ constitutive	LacZ(I)(BK1) LacZ(C) (H24) LacZ- (BW140, JET2, EJ1)
LB Ara X-gal Kan	LacZ+	LacZ+ (BK1, H24) LacZ- (same as above)

3. You will also need controls for this. Based on these criteria, you should determine the minimum number of strains you need to streak as controls. (i.e. C600, JET2, BK1, H24)
4. On day seven you will also record your data from this set of patching. (Make a chart ahead of time.) You will also take one or more of your Ara- LacZ+ strains from the LB Kan patching plate and streak for singles on a fresh LB Kan. You will use map the insertion of the transposon in these strains with PCR during the RDM module.
5. Congratulations you have successfully created, isolated and stabilized *ara::lacZ* fusions.

Office Hours: If you have questions as you are preparing your lab report, feel free to ask. I will be in the lab during the Protein Biochemistry module or you can contact me by e-mail (jen17@mit.edu) if you would like to arrange a time outside of class to discuss your questions.