

GEN Day 6 Interpretation Questions (and Answers)

Part I: General Characterization of P1 transductants

1. Interpret your GEN Day 5 results. What did you expect to see on your transductant plates? Did your results match what you expected? If not, propose an explanation.

The students should have expected to see some transductants on each of their "infected" plates, and "no growth" on their uninfected controls. Many student groups had "small" colonies on their uninfected plates, and many groups had no transductants with some of their lysates. One possible explanation for the lack of transductants is the question of MOI in their transduction experiment (remember, we didn't actually calculate it beforehand). If they used too many phage, they likely had lysis of their potential transductants. If they used too few phage, it is unlikely that many cells were infected by a phage containing the *ara* operon (and the miniTn10 transposon).

2. You make a P1 lysate from your mutant strain Q1W1, which has the phenotypes Ara-, LacZ+-inducible, Kan^R, and use it to infect KBS1 cells. After selecting for Kan^R transductants, you patch on the same set of plates used on Day 3 (since you are testing for Ara, Kan, and Lac phenotypes as before). When you analyze your patching plates the next day, you are confused to note two classes of transductants. One class is Ara-, LacZ+-inducible, Kan^R, (the same as Q1W1), which is the result you had hoped for. However, the plates also show a second class, which is phenotypically Ara+, LacZ-, Kan^R. You go back through your previous days' calculations and realize the MOI you calculated for the transposon mutagenesis experiment that gave rise to the Q1W1 mutant was 5, instead of the 0.1 you had aimed for. Interpret the results of this experiment by describing the location and orientation/reading frame of any transposon insertions that must have existed in your original mutant strain (Q1W1).

At an MOI of 5, there is a high probability of getting multiple phage infections, and thus multiple transposon insertions. In this case, it is likely that your original strain (Q1W1) had two transposon insertions. One transposon was inserted in either the *araA* or *araB* gene in the correct reading frame and orientation to give a successful *ara::lacZ* translational fusion. When this transposon was moved into the KBS1 cells by transduction, this caused the cells to become Ara-, LacZ+ (inducible), Kan^R. The other transposon was inserted in another, non-*ara* gene. This transposon did not insert in the correct reading frame and/or orientation. When this transposon was moved into the KBS1 cells by transduction, the cells remained Ara+ (no change in this phenotype), but became LacZ- and Kan^R when the transposon and surrounding genes were integrated by homologous recombination.

GEN Day 6 Interpretation Questions (and Answers) (continued)**Part II: General Characterization of P1 transductants**

1. Using the class data provided in lab, determine the map order of the *ara*, *leu* and *thr* genes. Be sure to show your calculations!

Starting with the Cm selection data, you should disregard the classes with "25 colonies" (these do not grow on the M9 Glu Leu Thr plate) and "45 colonies" (these are red on Mac Ara Kan, suggesting that the KanR phenotype is not linked to Ara).

Next, you can calculate the cotransduction frequency between CmR and KanR and CmR and Thr+:

$$\text{CmR and KanR} = \frac{\# \text{ CmR and KanR}}{\text{total CmR}} = \frac{20 + 710}{1170} = 62.4\%$$

$$\text{CmR and Thr+} = \frac{\# \text{ CmR and Thr+}}{\text{total CmR}} = \frac{20 + 0}{1170} = 1.7\%$$

From this data, you know that CmR and KanR are closer together than CmR and Thr+ (high CTF = closer together; low CTF = farther apart). Thus, you can eliminate the order "*leu* (Cm)–*thr*–*ara* (Kan)"

To distinguish between the two remaining orders—*leu-ara-thr* or *ara-leu-thr*—look at the "rare class," which is CmR, Thr+, KanS (0 colonies). It takes a quadruple crossover to get this class if the order is *leu-ara-thr*, while only a double crossover is required if the order is *ara-leu-thr*. Since "rare classes" arise from "rare events"—and a quadruple crossover is more rare than a double crossover—the likely gene order is *leu-ara-thr*.

Using the KanR selection data, you should disregard the classes with "105 colonies" (these do not grow on the M9 Glu Leu Thr plate or the LB Kan plate) and "55 colonies" (these are red on Mac Ara Kan, suggesting that the KanR phenotype is not linked to Ara).

Next, you can calculate the cotransduction frequency between KanR and CmR and KanR and Thr+:

$$\text{KanR and CmR} = \frac{\# \text{ CmR and KanR}}{\text{total CmR}} = \frac{830}{1340} = 61.9\%$$

$$\text{CmR and Thr+} = \frac{\# \text{ CmR and Thr+}}{\text{total CmR}} = \frac{0 + 60}{1340} = 4.5\%$$

As you can see, the CTF for KanR and CmR stays the same regardless of what marker you select for initially (CmR or KanR). Using this selection, though, you cannot eliminate one gene order using the "rarest class." In either order (*leu-ara-thr* or *ara-leu-thr*), obtaining the "rare class" (CmR, KanR, Thr+) requires a double crossover event that integrates all three markers. Using the CmR selection data is the best way, therefore, to determine the gene order.