

GEN Day 3 Interpretation Questions and Answers

Experiment III-A: Phenotypic Characterization of Ara⁻ Colonies

1. What is the difference between “LacZ inducible” and “LacZ constitutive”? What do these phenotypes tell you about the location of the transposon in the *ara* operon? (Hint: see pages 64 and 65 in the manual appendix!)

LacZ constitutive strains transcribe *lacZ* and produce β -galactosidase regardless of whether or not an inducer is present, whereas LacZ inducible strains only transcribe *lacZ* if an inducer is present. By determining whether our translational *ara::lacZ* fusion is LacZ constitutive or inducible we should be able to tell if the *lacZ* gene was inserted into *araC* (LacZ-constitutive) or *araBA* (LacZ-inducible). (0.5 points)

1. When group Q2 gets back their patching results, they find that one of their "white" mutants failed to grow on both the M9 Ara Leu Kan and M9 Glu Leu Kan plates. Can you say for sure that this mutant is Ara⁻? Why or why not?

You cannot say for sure that this mutant is Ara⁻. An Ara⁻ mutant should not be able to grow on the M9 Ara Leu Kan plate (cannot use arabinose as a carbon source), which is consistent with group Q2's observations. However, an Ara⁻ should have no problem using glucose as a carbon source, and should have grown on the M9 Glu Leu Kan plate. (It may be that this strain has a mutation that prevents growth on minimal media.) (0.5 points)

Experiment II-C: Demographics of the Mutant Hunt

1. Interpret your data.
 - a) What is the expected ratio of Ara⁻ to Kan^R colonies on the Mac Ara Kan plates? Is the ratio for your plates different?

The probability that the transposon inserts into *araA*, *araB*, or *araC* (*araD* insertions are lethal) instead of any other of the ~4,000 genes in the *E. coli* genome is 3/4000. (FYI: They learned that *E. coli* has ~4000 genes at Prof. Guarente's lecture on 2/3/05.) If their ratio is different, they will hopefully try to offer an explanation of why this might be. (1 point)

- b) What is the expected ratio of blue colonies to Kan^R colonies on the LB X-gal Kan plates? Why is this different from the ratio of Ara⁻ to Kan^R colonies on Mac Ara Kan?

Any colony that received a transposon insertion—regardless of where or how the transposon inserts in the genome—will become Kan^R. (The Kan^R gene has its own promoter, start codon, and RBS.) However, in order for colonies to break down X-gal (i.e. become LacZ⁺) *lacZ* must have inserted in the correct location (behind a promoter), orientation, and reading frame, an event that has a probability of ~1/6. Thus the ratio of blue colonies to Kan^R should be approximately 1/6. This ratio is higher than the ratio of Ara⁻/Kan^R because you are not trying to mutate a particular gene here; insertion of the transposon into ANY gene in the correct orientation/reading frame will result in LacZ⁺.

Experiment II-D: Titration of lambda1205 phage

1. You decide to repeat the transposon mutagenesis experiment from Day 2, but instead use pNK/LE392 cells as your starting strain. Do you expect to get more or less total colonies on your Mac Ara Kan plates? Explain your answer briefly.

You should see less colonies on your Mac Ara Kan plates, perhaps even NONE. When lambda1205 infects pNK/LE392 cells, the phage is capable of undergoing the lytic life cycle. Thus, most of the infected cells will get lysed, and will not be able to give rise to colonies (even if a transposon insertion has occurred). (1 point)