

Genetics Day 5 Interpretation Questions (and Answers)

1. During the GEN 4 Interpretations, you explained the advantages of using either high or low MOIs during different experiments. Today, during the P1 transduction, did you want a high or low MOI and why?

Today we want a LOW MOI. The P1 lysate that you used to infect the recipient cells today contains different kinds of DNA; 99.9% of the phage contain P1 DNA (which will lyse the cells) and 0.1% of the phage contain bacterial DNA. We want the phage containing bacterial DNA, *specifically* that containing the *ara::lacZ* fusion, to enter the cells and recombine, thereby resulting in Kan^R colonies. If we had a HIGH MOI, there is a greater chance that an additional phage will inject its DNA into the transductant and lyse the cell. Thus, we would be unable to identify the colony that would have grown on the LB Kan plate.

2. You infected KBS1 with your P1 lysates in lab today. If our reagents were working correctly, you would have also infected C600. Explain what we were trying to accomplish by infecting each strain with our P1 lysates, including the key phenotypes that each strain must have to make the experiments work.

KBS1 is used to stabilize the mutation. It does not contain pNK (which carries the transposase gene) and thus the transposon will not excise and hop around the genome. For our experiments to work, the KBS1 "recipient" strain must have the opposite phenotype as our "donor" strain (our mutants); thus, KBS1 must be Ara⁺ and Kan^S. (In addition, when using the KBS1 strain, we can see if the *ara* and the *lacZ/kan* genes are genetically linked.)

C600 contains genetic markers that can be used map the mutation. Again, its phenotype must be opposite of the donor's for the genes of interest. C600 is Thr⁻, Leu⁻ (Cm^S), Ara⁺, and Kan^S.

3. In haste, the TAs accidentally aliquoted out the pNK/KBS1 strain used on Day 2, rather than the KBS1 strain. Why would this present a problem in the experiment?

We are using the KBS1 strain because it does not contain the pNK plasmid with the transposase gene. By moving the mini-Tn10 transposon away from the transposase gene, we are stabilizing the transposon and preventing its haphazard movement within the bacterial genome.