

7.03 Review Session for Exam 2
Session lead by Alice Chi and Nate Young

Question 1.

You have isolated a Tn5 insertion that is linked to but not inside LacI and the lac operon. You use this Tn5 insertion to order two LacI- mutations, LacI1- and LacI2-. You grow phage on a **Tn5 LacI1-** strain and infect a **LacI2-** strain, selecting for Kan^R. You get 5 transductants that show normal LacZ regulation, and 495 transductants that are constitutive.

Then you grow phage on a **Tn5 LacI2-** strain and infect a **LacI1-** strain, selecting for Kan^R. You get 500 transductants that are constitutive.

a. Draw out the transduced DNA aligning with the chromosomal DNA in each of these two transduction experiments. Do a separate drawing for each potential map order.

b. For each possible order, write the phenotypes you would get from each double and quadruple crossover event. Do this for both transduction experiments.

c. Draw a map of this region of the genome, showing the correct relative order of the **Tn5** insertion site and the **LacI1** and **LacI2** loci.

Question 2.

a. A codon for serine is 5'-UCG-3'. Draw an mRNA with this codon in it, and draw ser-tRNA with its anticodon base pairing with that codon.

b. Draw both strands of the gene that encodes the ser-tRNA, showing the sequence of the portion of the gene that encodes the anticodon. Label the strand that is used as a template for transcription.

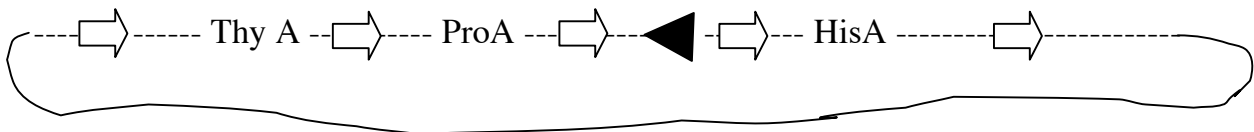
c. The amber stop codon is 5'-UAG-3'. Draw an mRNA with this codon in it, and draw a mutant ser-tRNA recognizing that codon and suppressing the amber mutation.

d. Draw both strands of the mutant gene that encodes the suppressor ser-tRNA, showing the sequence of the portion of the gene that encodes the anticodon. Label the strand that is used as a template for transcription.

e. The ochre stop codon is 5'-UAA-3'. Is it more likely to isolate an amber-suppressing allele or an ochre-suppressing allele of the gene for this ser-tRNA?

Short Answer Questions

- a. If a protein is normally 50 kDa, can you get a 20 kDa protein as a result of:
a missense mutation? _____ a silent mutation? _____
a nonsense mutation? _____ a frameshift? _____
- b. How do you do a complementation test in bacteria?
- c. Are loss of function mutations normally recessive or dominant?
- d. Does an F plasmid have to integrate into the genome to be stably maintained?
- e. By what sequences does an F⁺ integrate into the chromosome? _____
By what sequences does an F' integrate? _____
- f. If it is hard to assay the enzyme being regulated in your system, what can be done to measure its expression?
- g. Can you map the site of a dominant mutation by using:
a wildtype transposon library? _____
a wildtype plasmid library? _____
- h. In the Hfr below, does ProA transfer early or late? _____
If you isolated an F' with ProA on it, would it also contain ThyA? _____



- i. You isolate a dominant, uninducible allele (called X*) of geneX, which encodes a regulatory protein. You have a haploid *E. coli* strain that has a transposon insertion in geneX and shows an uninducible phenotype. What type of allele is X*?