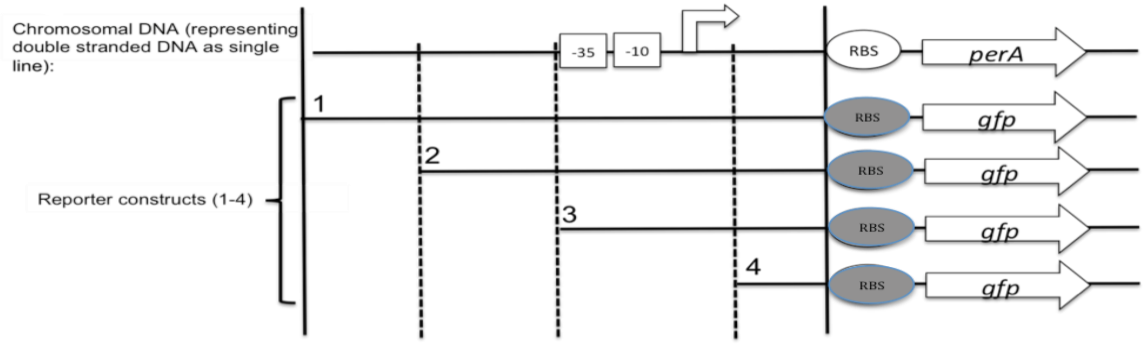
Name:____________________________________

Biol 3530 Individual Problem Solving



1. In the diagram above, the area between the solid vertical lines was copied from the chromosomal DNA template shown (at the top) and placed upstream of the *gfp* gene and its ribosomal binding site (RBS) to create “Reporter construct 1.” Shorter pieces of DNA were copied from the template to make reporter constructs 2, 3, and 4. GFP protein exhibits green fluorescence.

A) Assuming that the *perA* promoter (-10, and -35 boxes) is active under normal growth conditions, and given only the information in the diagram, which of the 4 reporter constructs shown above will be active (i.e. cause cells to exhibit green fluorescence)? 9 pts

B) Now forget all of your expectations from part A. You grow your cells in standard laboratory conditions, and cells carrying constructs 1 and 2 are bright green, while cells with construct 3 are very faintly green, despite being grown in the same conditions for the same amount of time. What might these results tell you about the control of *perA* gene expression? 10 pts

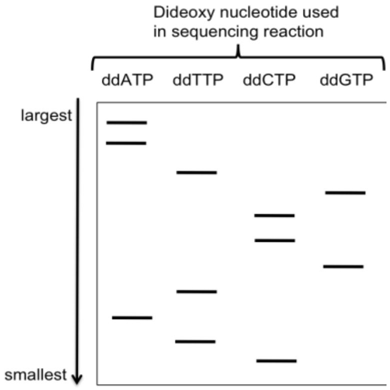
2. You are working with Sydney Brenner’s T4 bacteriophages with various mutations in the rII region of the chromosome. Which of the following mutants would be LEAST likely to produce daughter viral particles (a sign of successful infection) in an *E. coli K* **opal suppressor** strain (suppresses opal mutants only), and why? 8 pts

A. T4 with an opal mutation in rII
B. T4 with a missense mutation in rII
C. T4 with a silent mutation in rII
D. All can infect the *E. coli K* strain similarly, because rII is not required to infect that host (permissive host)

Answer and rationale:

3. Below is the result of a DNA (Sanger) sequencing reaction using the primer indicated on the right.

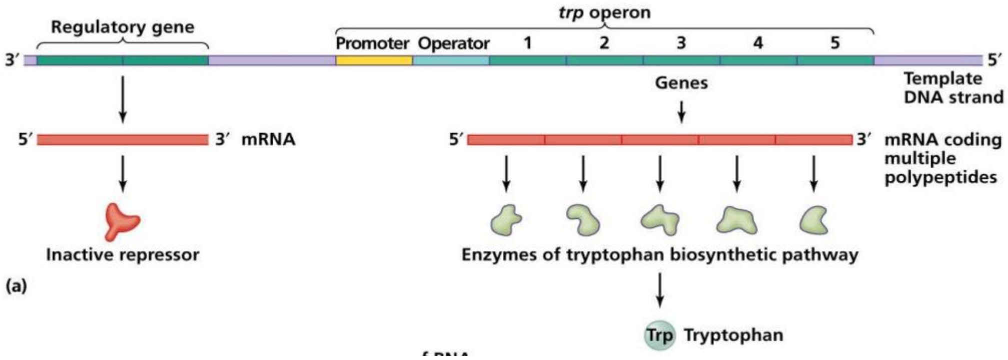
A) Complete the DNA sequence by adding to the primer sequence below the image. Make sure you add the bases to the correct side of the primer! 14 pts



3'-A A T T G C G-5'

B) Now take the sequence you wrote above and make it double stranded (you don't need to include the primer). Label all 3' and 5' ends. 10 pts

4. The *trp* operon (below) encodes enzymes essential for the production of the amino acid Tryptophan.



A) Armed with ONLY the information in the previous sentence, is there a way to **SELECT** for spontaneous mutations that eliminate production of Tryptophan (Trp$^-$ phenotype)? If so, how, and if not, why not? Look up "selections versus screens" if you need a reminder… 10 pts

Continued on next page…

B) The *trp* operon is controlled by a repressor protein (the Trp repressor), which can only bind DNA when Tryptophan is readily available and associates with the repressor. Fill in the following table to indicate relative levels of *trp* operon expression from each strain under each described condition. You can use "-" to indicate little to no expression, and "++" to indicate higher expression levels. Assume, for now, that all cells are capable of survival in laboratory media with and without tryptophan. 9 pts

Strain	*trp* Expression in media lacking Tryptophan	*trp* Expression in media supplemented with Tryptophan
Wild type		
P^+O^- mutant		
P^-O^+ mutant		

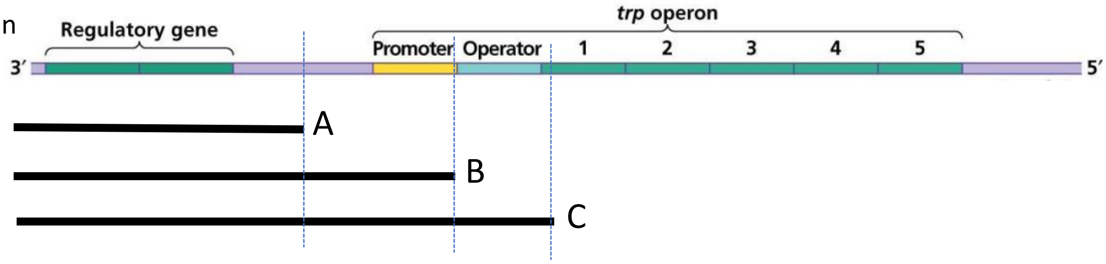
C) In reality, it isn't a safe assumption that each of the mutants will be able to survive under each of the conditions listed above. Which strain(s) would probably be unable to survive under one or more of the conditions listed above, and why? Remember that Tryptophan is an amino acid, and cells need to either make or receive Tryptophan to survive. Be sure to clarify both the strain(s) and the growth condition(s) that would result in death. 10 pts

D) Now, can you envision a way to SELECT for revertants of the mutation listed above (answer to part C)? Remember, these revertants would have a wild type phenotype. If so, how, and if not, why not? 10 pts

5. Using a mutagen known to induce base substitutions, you generate a mutant strain of bacteria with a "non-repressible" phenotype for *trp* operon expression, which is either an O- or repressor- mutation. You want to know which it is. You conduct a deletion mapping experiment by analyzing the phenotypes of f- bacterial strains carrying the mutation of interest after hfr-mediated donation of the deletion constructs shown below. In other words, you are looking for recombination between each of the linear DNA fragments below (A, B, and C), and your point mutant strain.

F$^-$ (point mutation in regulatory gene or operator)

hfr (contain wild type DNA, for ONLY the regions shown in black)



Describe how the results of this deletion mapping experiment would differ with each potential original mutation (O- or repressor-). Be specific about what you would measure as the outcome of this experiment. 10 pts

If the original point mutation is O-, I would expect:

If the original point mutation is repressor-, I would expect: