

1. Bacterial promoters have the following consensus sequence. Use the sequence below to answer the following questions (20 points).

$\begin{array}{ccc} -35 & & -10 \\ \text{TTGACAT} & & \text{TATAAT} \end{array}$

A. What protein binds the -35 and -10 sequences?

B. Given the following two promoter sequences, which do you predict will be the stronger promoter? (**Circle one**)

$\begin{array}{c} -35 \\ \text{TAGATAT} \end{array}$	$\begin{array}{c} -10 \\ \text{TAGATT} \end{array}$	$\begin{array}{c} -35 \\ \text{TTGACTT} \end{array}$	$\begin{array}{c} -10 \\ \text{GATAAT} \end{array}$
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C. Listed below is the sequence of the Lactose operon promoter:

$\begin{array}{ccc} -35 & & -10 \\ \text{TTTACAC} & & \text{TATGTT} \end{array}$

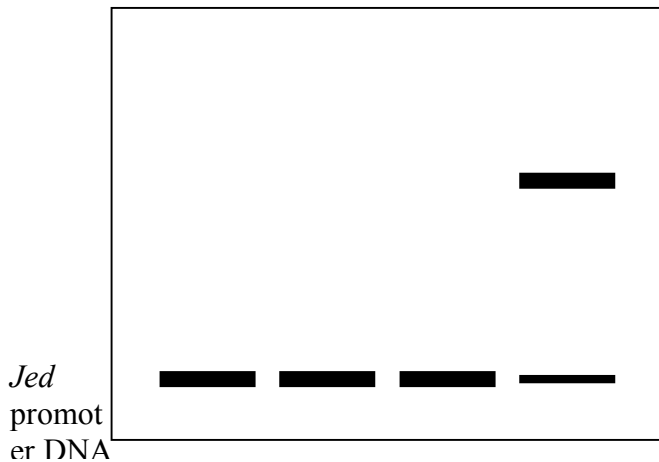
- Rewrite the promoter showing a mutation that would make the promoter stronger
- Rewrite the promoter showing a mutation that would make the promoter weaker

C. Briefly describe the biochemical principle underlying the relative strength of bacterial promoters. How does the degree of similarity between a promoter and the “ideal” consensus sequence govern the rate of transcription?

2. You have identified a new bacterial operon that is activated only in the presence of the dark side of the force. You name it the *Jedi* (*jed*) operon. As part of your studies of the operon you identify two transcriptional activator proteins, LUKE (LK) and LEAH (LEH), that you believe are essential for the induction of the *jed* operon. To study the function of LK and LEH you perform a gel-shift assay and a transcriptional reporter assay. The results of those experiments are shown below. (20 points)

- You use purified LK and LEH protein to measure binding to the *Jed* promoter region *in vitro* using gel mobility shift assays. You use radio-labeled dsDNA that contains ~200 base-pairs upstream of the *jed* operon as a substrate for DNA binding. The results of your assay are shown below on the left.
- To be sure that these proteins are involved in activation of the *jed* operon, you fuse the promoter region of the operon to the LacZ gene and test the levels of expression in cells that have mutations that fail to produce the LK and/or LEH proteins. You get the results shown on the left

LEH - + - +
 LK - - + +



LK Gene	LEH Gene	Transcription Units
Wildtype	Wildtype	2000 Units
Mutant	Wildtype	50 Units
Wildtype	Mutant	50 Units
Mutant	Mutant	50 Units

Based on these data, what can you conclude about how LK and LEH function at the *Jedi* promoter? Be sure to include a biochemical mechanism for how the two activators likely increase transcription rates of RNA polymerase.

Finally, you have identified two mutant strains of bacteria that fail to induce the *Jed* operon in the presence of the dark side of the force. A close inspection of the promoter region in these two strains shows that they are insertion mutants. Mutant strain 1 has 5 extra base-pairs between the LK and LEH binding sites while mutant strain 2 has 30 extra base-pairs between the LK and LEH binding sites. Explain why these two strains fail to activate transcription.

Mutant Strain 1:

Mutant Strain 2:

In class this past week we discussed the logic of bacterial gene expression control using the Lactose operon. This set of problems will have you think further about how Jacob and Monod used bacterial genetics to work out the regulation of the operon. But first a review of some terms from your Genetics course:

- In the following problem, a “+” indicates a wildtype gene and a “-“ indicates a loss of function mutation in the gene. For example, Z+ is a normal β -Galactosidase gene and Z- is a gene that no longer produces a functional protein

For each of the genotypes listed below, indicate whether β -Galactosidase (LacZ) and/or the Permease (LacY) proteins will be functional in the presence of IPTG. IPTG is an artificial inducer of the Lac operon that can enter cells and cause the release of repression by binding to the Lac repressor.

- I+ is a normal repressor gene; I^s is a mutant of the Lac Repressor which represses the Lac operon even in the presence of inducer (Lactose or IPTG)
- O+ is a normal operator sequence; O^c is the constitutive operator mutant which prevents repressor binding.
- P+ is a normal promoter sequence, P- is a promoter sequence that prevents RNA polymerase from binding

A. The Following are haploid strains of bacteria in which individual mutations can be studied. Fill in the chart using “+” to indicate that the functional proteins would be produced or a “-“ to indicate that no functional protein would be produced in the presence or absence of the IPTG inducer

Genotype	No IPTG		IPTG Added	
	β -Gal	Permease	β -Gal	Permease
I+ P+ O+ Z+ Y+				
I- P+ O+ Z+ Y+				
I+ P+ O+ Z- Y+				
I+ P+ O+ Z+ Y-				
I^s P+ O+ Z+ Y+				
I+ P+ O^c Z+ Y+				
I+ P- O+ Z+ Y+				

B. The following are partial diploid strains that are designed to test the relationship between mutations in the operon.

Genotype	No IPTG		IPTG Added	
	β -Gal	Permease	β -Gal	Permease
I+ P+ O+ Z+ Y+ / I+ P+ O+ Z+ Y+				
I- P+ O+ Z- Y+ / I+ P+ O+ Z+ Y+				
I+ P+ O+ Z+ Y- / I+ P+ O+ Z+ Y+				
I^s P+ O+ Z+ Y+ / I+ P+ O+ Z+ Y+				
I+ P+ O^c Z+ Y+ / I+ P+ O+ Z+ Y+				
I+ P- O+ Z+ Y+ / I+ P+ O+ Z+ Y+				

Genotype	No IPTG		IPTG Added	
	β -Gal	Permease	β -Gal	Permease
I+ P+ O+ Z+ Y- / I+ P+ O+ Z- Y+				
I- P+ O+ Z+ Y- / I+ P+ O+ Z- Y+				
I+ P- O+ Z+ Y- / I+ P+ O+ Z- Y+				

I+ P+ O^c Z+ Y- / I+ P+ O+ Z- Y+		
I^s P+ O+ Z+ Y- / I+ P+ O+ Z- Y+		
I- P- O+ Z+ Y- / I- P+ O+ Z- Y+		
I- P+ O^c Z+ Y- / I- P+ O+ Z- Y+		
I- P- O^c Z+ Y- / I- P+ O+ Z- Y+		
I+ P- O+ Z+ Y- / I- P+ O+ Z- Y+		
I+ P+ O^c Z+ Y- / I- P+ O+ Z- Y+		
I^s P- O+ Z+ Y- / I+ P+ O+ Z- Y+		
I^s P+ O^c Z+ Y- / I+ P+ O+ Z- Y+		