Exam II **NAME:_____**

Imagine that you isolate a new bacterial strain from a bovine gut and want to check whether it uses a sigma factor similar to the housekeeping sigma-70 factor (σ^{70}) of *E. coli*. PCR would be a cheap, fast method to start with.

1. **Design primers (25 bp in length) that will amplify the sigma 70 factor from *E. coli* K-12 MG1655.**

   Forward primer 5'-

   Reverse primer 5'-

To your delight, the PCR reaction indicates that your new bacterial strain contains a gene for a sigma 70 factor that is similar enough to the *E. coli* gene to be amplified by your primers. You would like to determine whether the sigma in your new bacterial strain functions in a similar mechanism to that of *E. coli*. In *E. coli* sigma 70, Tyrosine 430 (Y430) has been shown to participate in the separation of the template strand from the nontemplate strand. Mutation of the Y430 to Alanine significantly decreases the rate of strand separation during transcription initiation.

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              427 428 429 430 431 432 433
Wildtype  5′ TTC TCC ACC TAC GCA ACC TGG 3′
          N-  F   S   T   Y   A   T   W —C

              427 428 429 430 431 432 433
Mutant    5′ TTC TCC ACC GCG GCA ACC TGG 3′
          N-  F   S   T   A   A   T   W —C
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2. **List a DNA endonuclease (restriction enzyme) that would allow you to test for the presence of the Y430 to Alanine mutant (the DNA sequence switch of TAC to GCG):**

3. **Draw a picture of the result that you expect if you react wildtype and mutant PCR product with your chosen DNA endonuclease and resolve the products on an agarose gel.** You should include lanes for: (i) digested wildtype; (ii) digested mutant; and (iii) a 100-bp DNA ladder. **Include the polarity of the electric field in your drawing.** [Note: Don't forget to consider the length of your full PCR product and the total number of times that the endonuclease will cut your products]

4. Imagine that you have 75 uL of PCR product that you would like to digest with 5 uL of your chosen DNA endonuclease. You are supplied with a 10x (ten-times concentrated) stock of reaction buffer. **How much 10x stock reaction buffer should you add to the reaction to achieve a final solution at 1x? Show all your work.**

5. Compare the nucleophile, electrophile, and leaving group for reactions catalyzed by DNA polymerase, DNA endonucleases, RNA polymerase, and ribosomes [peptidyl-transfer reaction only; i.e. peptide bond formation]

	Electrophile	Nucleophile	Leaving Group
DNA polymerase			
DNA endonuclease			
RNA polymerse			
Ribosome			