

Name _____

Section [Circle One: (Mon Weds) (Tues Thurs)]

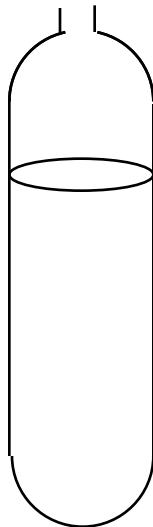
Genetic Engineering - Exam 2

100 points

1. (20) Full Scale Plasmid Preps

- a. List the steps involved in lysis of your culture and set-up of the gradient and state what each does. Do not give times, volumes, etc. **Do not describe the gradient in this part.**

- b. Draw a diagram of a completed gradient showing the positions of the principal macromolecules.



- c. Explain why chromosome and plasmid separate

- d. How is the DNA “cleaned up” for subsequent use?

- e. You dilute a DNA sample 1/20 and then measure the OD₂₆₀ and OD₂₈₀. The first reading is 0.298 and the second is 0.166. What do these numbers tell you? Why?

2. (10) Transformation in *Bacillus*

_____ shows that early in transformation DNA is tightly bound to the outside of the cell

_____ is the difference between GMI and GMII growth media

_____ is used to shock *Bacillus* out of competence

_____ happens to DNA in the cytoplasm

_____ is the definition of the eclipse period

_____ is the reason why it difficult for *Bacillus* to be transformed by recombinant DNA

_____ is how this difficulty is overcome

_____ is the period of the cell cycle during which *Bacillus* is most readily transformed

_____ is another phenomenon that occurs, or a product that is made at this time

_____ is the term for cell products that are formed at this time.

3. (5) Plasmids

_____ means that two plasmids are closely related.

_____ means that a plasmid exists in high copy number

_____ means that a plasmid can integrate into the chromosome

_____ means that a plasmid can be transferred from cell to cell

_____ means that a plasmid exists in low copy number.

4. (10) Complete the following table:

Plasmid	Selection	Screening
ColEI		
RSF2124		
pSC101		
pBR322		
pUC9		

5. (10) Describe the strategy for synthesizing an oligonucleotide. What would you do differently if you were synthesizing an entire gene?

6. (20) pUC plasmids and M13mp phages
- a. Draw a diagram showing the principal components of pUC9 and indicate where they came from.
 - b. What is the difference between the various pUC plasmids? How were these differences constructed?
 - c. Briefly describe \square complementation
 - d. How was JM83, the host strain for the pUC plasmids modified to become lac⁺ when transformed with pUC?
 - e. What is the relationship between M13mp phages and pUC plasmids? Why was this done?
 - f. Using diagrams, describe the life cycle of bacteriophage M13.

8. (15) cDNA cloning

What do we call mRNA in the nucleus?	
What do we call mRNA in the cytoplasm?	
List the three post-transcriptional modifications of mRNA	
What are the barriers that prevent eukaryotic genomic clones from being expressed in prokaryotes?	
Draw a simple diagram showing how mRNA is primed to make the first strand of DNA	
What enzyme is used to make the first strand?	
How do you get rid of the RNA in the DNA/RNA hybrid?	
Draw a simple diagram showing how the second strand is synthesized.	

Extra Credit (5) Describe the three activities of DNA polymerase I and show how they are organized on the protein. What is the Klenow fragment and why is it useful?