

## Homework set - Biotechnology

1. A preparation of linear DNA is digested to completion with the following combination of enzymes. Gel electrophoresis reveals the size of the fragments after digestion. Using the information given below, construct a restriction map of this viral DNA.

Enzyme used	Fragment sizes (kb)*
<i>Bgl II</i>	5 and 10
<i>Hga I</i>	5 and 10
<i>Sma I</i>	2 and 13
<i>Bgl II</i> and <i>Hga I</i>	5
<i>Bgl II</i> and <i>Sma I</i>	2,5 and 8
<i>Hga I</i> and <i>Sma I</i>	2, 3 and 10

\*kb is kilobase pairs

2. A preparation of linear viral DNA is digested to completion with the restriction endonuclease *Sma I*. Gel electrophoresis of the digest reveals five fragments of the following sizes in kilobases (1 kb = 1000 np): 10 kb, 7 kb, 6 kb, 3 kb and 2 kb. A partial digest of the intact viral DNA is then made with *Sma I* and the fragments produced are separated by gel electrophoresis and recovered from the gel. Five fragments, labeled A through E in decreasing size, are observed. Each is redigested to completion with *Sma I* and analyzed by gel electrophoresis. The results are given below. Construct the *Sma I* restriction map of this viral DNA.

Size classes in partial	Fragment sizes (kb) observed after redigestion to completion
<b>A</b>	10.0, 6.0, 2.0
<b>B</b>	7.0, 6.0, 2.0
<b>C</b>	10.0, 3.0
<b>D</b>	7.0, 2.0
<b>E</b>	6.0, 2.0

3. The following is a single strand of DNA. Find all potential restriction sites that would exist in the double stranded version.

5'TAGAATTCGACGGGATCCGGGGGGCATGCAGATCA3'

4. Draw the expected gel pattern derived from the dideoxy sequencing method for a template strand that has the following sequence:

5'CAGCGAATGCGGAA3'

5. A circular plasmid is incompletely digested with *Hae III* and fragments of 10 kb, 12 kb, 22 kb and 32 kb are recovered. Draw the restriction map for *Hae III*.

6. The DNA for a gene yields fragments of 1.7 kb, 2.1 kb, and 3.2 kb when digested with *Bg III*. When DNA is isolated from a mutant for this gene, the *Bg III* fragments recovered are 3.2 kb and 3.8 kb in length. Explain what happened.

7. A pregnant 30-year-old woman learns her father has Huntington's disease. There is no history of the disease in her husband. Using a genetic test that can detect the disease is used on the her father, husband, fetus and on her. the results are shown below.

<b>Father</b>	<b>Husband</b>	<b>Woman</b>	<b>Fetus</b>
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Banding patterns on X-ray film.  
 Will the woman develop the disease?  
 Will the child develop the disease?

8. A linear DNA molecule is labeled with  $^{32}\text{P}$  at its 5'prime ends. It is then digested to yield the following fragments (\*glabellad fragments):

<i>Hind III:</i>	3.0*, 4.0, 5.0*
<i>BG III:</i>	1.8*, 2.2*, 2.7, 5.3
mix:	1.2, 1.5, 1.8*, 2.2*, 2.5, 2.8

9. Many human genes contain introns. Since bacteria cannot excise introns from mRNA, how can bacteria be used to make large quantities of a human protein?

10. The following sequence is the coding strand of a DNA molecule's open reading frame. Draw the mRNA molecule that would be transcribed from this DNA.  
**5'TTCGCATGATCCCCAAAATCTTTTAT3'**

Next, give the first four amino acids coded for by the transcript.

11. The following sequence is the template strand of a DNA molecule's open reading frame. Draw the mRNA molecule that would be transcribed from this DNA.  
**5'TAAAAGATTTTGGGGATCATGCGAA3'**

Next, give the first four amino acids coded for by the transcript.

12. You want to clone a DNA fragment into a plasmid that has antibiotic resistant genes for streptomycin and tetracycline. You choose to insert the fragment into a restriction site in the streptomycin resistant gene.

a) How would you get the DNA fragment into the chosen site? Include the procedures from the point where you have grown bacteria that have the plasmid that will get the insert.

- b) How do you get the modified plasmid to replicate?
- c) How will you select? How will you screen??
13. What will be the average size of fragments if a large linear DNA molecule is digested to completion with a restriction enzyme that recognizes the site AACGTT, if the amount of the base A is found to be 10%?
14. If an organism is found, which has codons of only 2 bases in length, how many different codons could be generated?
15. What is the site of protein synthesis?
16. What couples transcription and translation, allowing the transcript to be used as instruction for the amino acid sequence found in the protein?
17. Describe how eukaryotic mRNA can be turned into cDNA.
18. You have cloned a fragment of DNA into a plasmid, hoping that it is from a human Y-chromosome. Explain in step by step detail how you would verify or not that the fragment is y-chromosomal, using the dot blot method.

19. You have found blood at the scene of a crime and it is not the same blood type as that of the victim. A suspect has been apprehended. Explain in detail, sequentially, how it can be determined if the blood from the crime scene matches the blood of the suspect. Use the RFLP and southern blot method.
  
20. A complete digest of a 20kb DNA fragment with Hae II, yields smaller fragments of 3, 4, 5 and 8kb. An incomplete digestion yields fragments of 4, 8, 9, 11 and 12kb. Draw the restriction map using this information.
  
21. What is aminoacyl tRNA synthetase?
  
22. What is a palindromic DNA sequence? Draw one.
  
23. Describe PCR in detail starting with the necessary components. Continue with a step by step description of the process of amplification. After amplification, how would the products be visualized? (Write on back.)

**SAMPLE EXAM:**

Genetics 410-01; Exam on Chapters 8, 9 and 10; July 27, 1998

Name:

Remember to print legibly.

1. Draw the basic structure that all amino acids have in common. (5 points)
  
2. What is the name of the kind of reaction that occurs during the formation of proteins from amino acids? (5 points)

3. What is the difference between RNA and DNA molecules, excluding the difference with thymine and uracil? (Draw the sugars of each, including the numbering of the bases and the *difference*.) (5 points)
  
4. Describe the nature of a transcription terminator in prokaryotes. (5 points)
  
5. What are the following components made of? (32 points)

**Promoter**

**corepressor** for the tryptophan operon

**repressor**

**inducer** for the *lac* operon

**transcription terminator**

**translation terminator**

**restriction enzyme**

**plasmid**

**ribosome**

**tRNA**

**mRNA**

**intron**

**exon**

**codon**

**operator**

**cyclic AMP**

6. What is an inducible operon? Give an example. (5 points)
7. What is a repressible operon? Give an example. (5 points)
  
8. Describe in detail all aspects of how the *trp* operon works. (5 points)

9. Describe the CRP-cAMP regulation aspect of the *lac* operon. (5 points)

**10. Genotype**

**Constitutive or Inducible (say which)**

Synthesis of *lac* mRNA

F' *lacO<sup>c</sup> lacZ<sup>+</sup> / lacO<sup>+</sup> lacZ<sup>+</sup>*

F' *lacO<sup>+</sup> lacZ<sup>+</sup> / lacO<sup>c</sup> lacZ<sup>+</sup>*

F' *lacI<sup>-</sup> lacZ<sup>+</sup> / lacI<sup>+</sup> lacZ<sup>+</sup>*

F' *lacI<sup>+</sup> lacZ<sup>+</sup> / lacI<sup>-</sup> lacZ<sup>+</sup>*

F' *lacO<sup>c</sup> lacZ<sup>+</sup> / lacI<sup>-</sup> lacZ<sup>+</sup>*

F' *lacO<sup>c</sup> lacZ<sup>-</sup> / lacO<sup>+</sup> lacZ<sup>+</sup>*

F' *lacO<sup>c</sup> lacZ<sup>+</sup> / lacO<sup>+</sup> lacZ<sup>-</sup>*

(7 points)

11. Describe in detail PCR including all necessary components and how results are observed. (5 points)

12. The following DNA template is sequenced. Draw the gel with the bands that would be expected to be seen. Describe the reactions that would need to be run. (5 points)  
5' ACCCTTTCAAACCCTTCGAC3'

13. Explain the difference between selection and screening for identification and location of bacteria that have taken up a plasmid with an insert. (5 points)

14. A preparation of linear DNA is digested to completion with the following combination of enzymes. Gel electrophoresis reveals the size of the fragments after digestion. Using the information given below, construct a restriction map of this viral DNA. (5 points)

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15. Many human genes contain introns. Since bacteria cannot excise introns from mRNA, can bacteria be used to make large quantities of a human protein? If so, how? (5 points)