Biotechnology

A. Objectives

Become familiar with methods used for

- Creating genomic libraries
- Detecting specific DNA sequences
- DNA sequencing;
- Analyzing gene expression

B. Before coming to lab

Read chapter 20 in Campbell et al. *Biology.* In particular, study

- Figure 20.5 on genomic libraries
- Figure 20.7 on nucleic acid probe hybridization;
- Figure 20.10 and 20.11 on restriction fragment analysis and Southern blotting
- Figure 20.12 on DNA sequencing;
- Figure 20.13 and 20.15 on analysis of gene expression levels;
- Figure 20.21 on genetic markers for disease-causing genes
- Figure 20.24 on DNA fingerprinting

Fill in as many of the answers to the worksheet questions as you can.

C. During lab

Your instructor will explain the techniques listed under objectives with the aid of computer animations. Fill out the remaining answers in the worksheet in part E. Make sure not to leave the lab without having all the answers. This material is relevant for the final lecture exam.

E. Worksheet

Creating genomic libraries

During our genetic engineering laboratory, you became familiar with transforming bacteria with transgenic plasmids. The same methods are used to **clone** eukaryotic or human genes (Campbell p. 399, Figure 20.4). Cloned genes can be stored in libraries.

1. How are genomic and cDNA libraries made (Fig. 20.5 and 20.6)? Outline and number the steps.

2. What is the difference between cDNA and genomic DNA?

3. List advantages and disadvantages of plasmid, phage, BAC, and cDNA libraries.

Detecting specific DNA sequences

4. Describe how a specific gene can be detected in a genomic or cDNA library (Fig. 20.7) by **nucleic acid hybridization**. Number the steps.

5. Researchers can distinguish between the “normal” beta-globin gene and the mutated allele that causes sickle-cell anemia (Figure 20.10 and 20.11) by **restriction fragment analysis** and **Southern blotting**. Explain how these techniques work using the terms **single nucleotide polymorphism** and by outlining the steps. Number the steps.

6. HIV can be detected by ELISA and by RT-PCR. What exactly do those technologies detect?

7. Explain how forensic scientists create a **genetic profile** with **short tandem repeats** (Figure 20.24)

8. Name two other applications of STR analysis.

9. Why do scientists use 13 STR markers to create a genetic profile?

10. What are (two) advantages of **STR analysis** over **RFLP analysis?**

11. A bloody crime has occurred. Police have collected blood samples from the victim, two suspects, and blood found from the scene. Briefly list the steps the lab went through to produce the following autoradiograph.

a)

b)

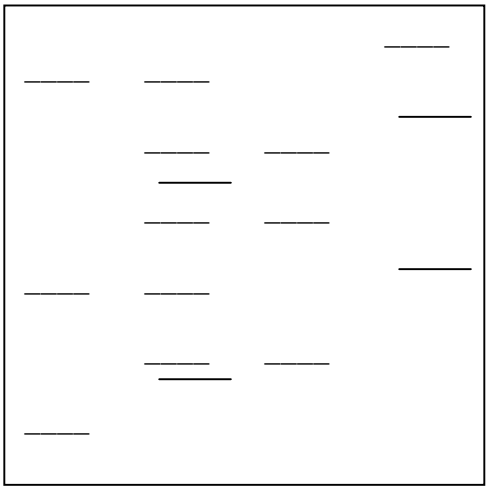
c)

d)

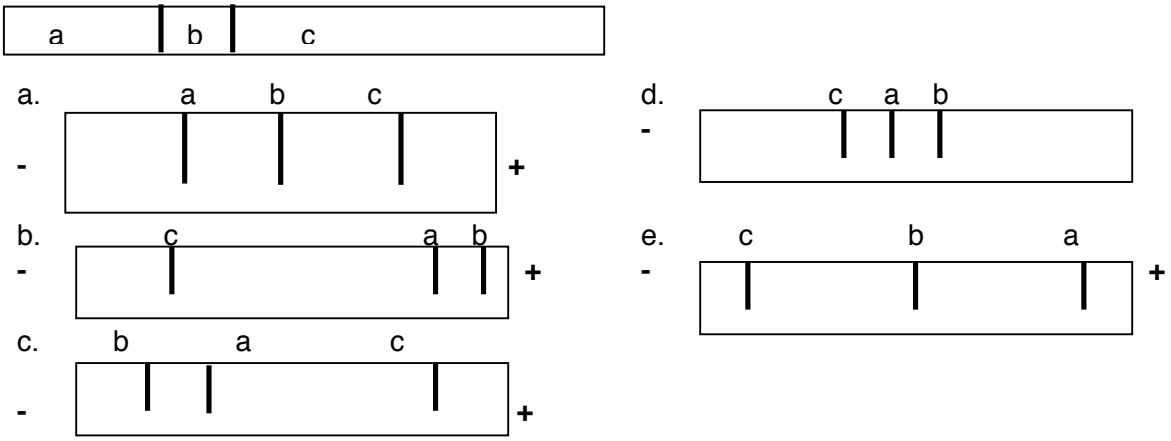
e)

Which suspect would you charge with the crime?

Victim Crime Scene Suspect 1 Suspect 2



12. The following segment of DNA has restriction sites I and II which create restriction fragments a, b, and c. Which of the following gel(s) produced by electrophoresis would represent the separation and identity of these fragments?



DNA sequencing

13. What is the name of the most commonly used method of DNA sequencing?

14. This method, also called _____ involves _____ separate reactions.

15. Each sequencing reaction contains

    a)

    b)

    c)

    d)

    e)

16. How do ddNTPs differ from dNTPs?

17. What is the consequence of this difference?

18. Compare and contrast ddNTPs and acyclovir.

19. Name three commonalities between PCR and DNA sequencing.

20. At the end of the four sequencing reactions, each tube contains

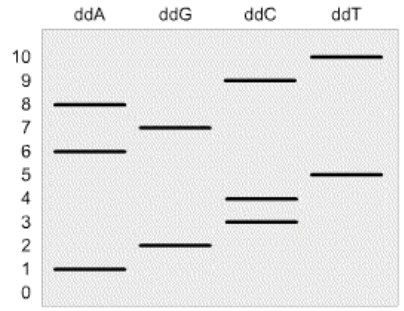
    _____

    _____

21. How are the fragments analyzed?

22. This gel was produced after sequencing a DNA strand with the Sanger method. What is the sequence of nucleotides in the **original single-stranded DNA fragment**? Provide your answer 5' to 3'.

Autoradiogram of a dideoxy sequencing gel



Analyzing gene expression

23. What is the difference between **Southern blott**ing, **Northern blotting** and **RT-PCR analysis** (Figure 20.13)**?**

24. What is the purpose of the **DNA chip technology** discussed in our class?

25. Describe the steps involved in this type of DNA chip technology (Figure 20.15)

a)

b)

c)

d)