Name Key **Period** _____

Chapter 20: Biotechnology

The AP Biology exam has reached into this chapter for essay questions on a regular basis over the past 15 years. Student responses show that biotechnology is a difficult topic. This chapter requires a strong conceptual understanding of the technological processes and the underlying biology that guides the procedure. With a little careful work, this chapter will give you insights into the incredible advancements already made and a basis for understanding the new marvels yet to be discovered in biotechnology.

Overview

1. It is important to understand the meaning of the three terms in bold to start this chapter.

recombinant DNA DNA molecules formed when segments of DNA from different sources are combined

biotechnology manipulation of organisms or their components to make certain products

genetic engineering
direct manipulation of genes for practical purposes

Concept 20.1 DNA cloning yields multiple copies of a gene or other DNA segment

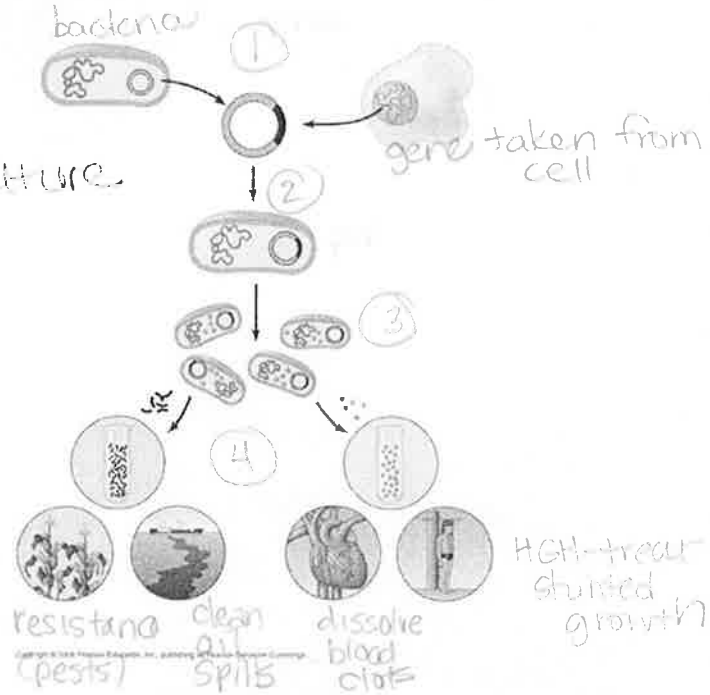
2. Plasmids are important in biotechnology. Give a full and complete definition of *plasmid.*

Small Circular DNA pieces that replicate separately from a bacterial chromosome

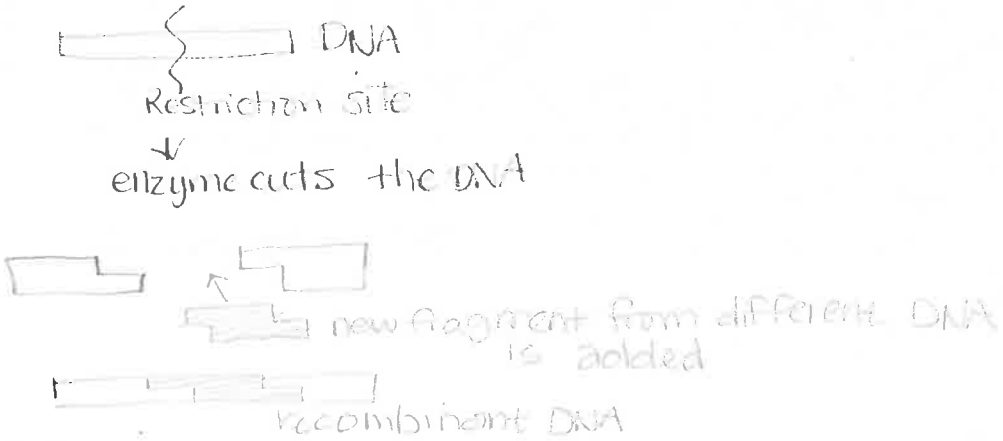
3. The production of multiple copies of a single gene is called gene cloning.

4. Using Figure 20.2, label and explain the four steps in this preview of *gene cloning*.

(1) gene inserted into plasmid
(2) plasmid put in bacterial cell
(3) Host cell grown in culture
(4) research + application



5. Read the description of *restriction enzymes* on page 398 carefully. Then draw and explain each step of Figure 20.3. When you finish, you should have recreated Figure 20.3 in the space below.



6. What is a *cloning vector*?

DNA molecule that carries foreign DNA into a host cell + replicates there

7. Figure 20.4 is a more detailed discussion of the gene cloning procedure shown in Figure 20.2. Explain the following key points.

   a) Explain why the plasmid is engineered with amp^R and *lacZ*.

   amp^R - makes E. coli cells resistant to amplicillan
   lac Z - hydrolyzation of lactose (distinguish)

   b) After transformation has occurred, why are some colonies blue?

   colonies with nonrecombinant plasmids will be blue because they can hydrolyze X-gal (lactose like product) - a that forms a blue product

   c) Why are some colonies white? Why is this important?

   Colonies with recombinant plasmid (lac Z disrupted) will be white because they can't hydrolyze X-gal
   Distinguishing recombinants

8. The cloning procedure described in question 7 and Figure 20.4 will produce many different fragments of hummingbird DNA. These fragments may be stored in a *genomic library*.

   a) What is the purpose of a *genomic library*?

   complete set of plasmid-containing cell clones, each carrying copies of a particular segment → complete genome of an organism

   b) Explain how a *bacterial artificial library (BAC)* and a *cDNA library* are formed.

   bacterial artificial library - like above procedure - plasmids are large
   cDNA library - use mRNA as template for 1st strand = no introns

9. Once the hummingbird DNA is cloned, we have the problem of finding the piece of DNA that holds our gene of interest. Explain how *nucleic acid hybridization* will accomplish this task.

   detect a genes DNA by its ability to base-pair with a complementary sequence on another nucleic acid molecule
   (know part of the nucleotide sequence of the gene of interest) → synthesize a "probe"

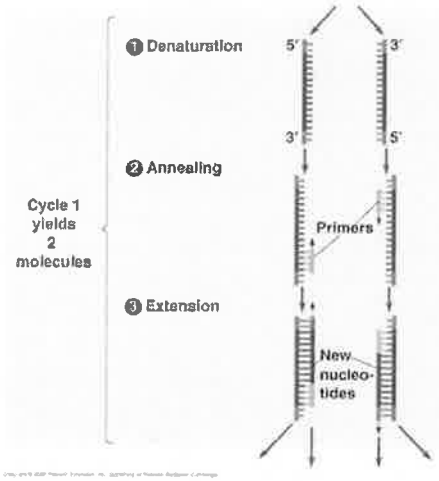
10. Describe how a radioactively labeled *nucleic acid probe* can locate the gene of interest on a multiwell plate. (Use Figure 20.7 to guide your response.)

    - cells are applied to a nylon membrane - breaks cells + denatures DNA
    - ssDNA sticks to membrane
    - membrane incubated in solution of radioactive probe molecules
    - can find location on the membrane of DNA that hybridized to the probe

11. What are two problems with bacterial gene expression systems?

1. certain aspects of gene expression are different in eukaryotes & bacteria
2. presence of introns in eukaryotic cells - prevent expression of gene by bacterial cells

12. The *polymerase chain reaction (PCR)* is a Nobel Prize–winning idea that is used by scientists to amplify DNA, particularly when the quantity of DNA is very small or contaminated. Explain the three initial steps that occur in cycle 1 of PCR.



1. breaking apart DNA
2. Adding primers
3. extending DNA

13. How many molecules will be produced by four PCR cycles?

2^n 2^4 $= 16$

Concept 20.2 DNA technology allows us to study the sequence, expression, and function of a gene

This section begins with a discussion of *gel electrophoresis*, a technique covered in AP Biology Lab 6. It is important to understand the principles of gel electrophoresis.

14. Gel electrophoresis is a technique used to separate nucleic acids or proteins that differ in size or electrical charge.

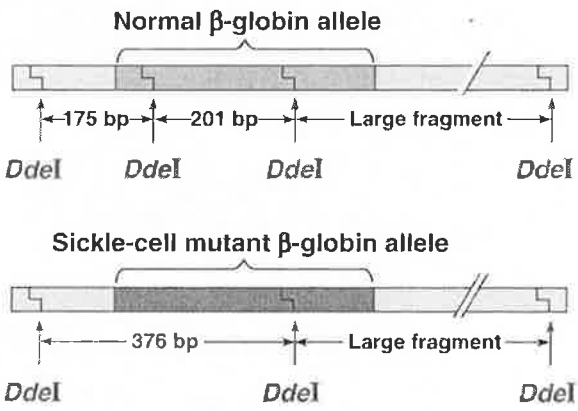
15. Why is the DNA sample to be separated by gel electrophoresis always loaded at the cathode or negative end of the power source?

Because nucleic acids are negatively charged (PO_4^-), they travel toward positive end

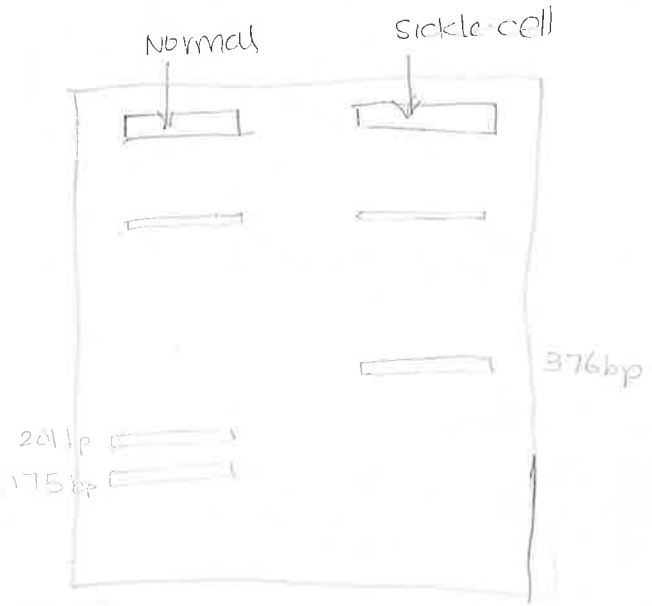
16. Explain why shorter DNA molecules travel farther down the gel than larger molecules.

Thick agarose fibers impede longer molecules more than short ones - short moves faster & to end.

17. To the right of the β-globin alleles, draw a gel showing the different pattern obtained from a normal patient and a sickle-cell patient. For help, examine Figure 20.10.

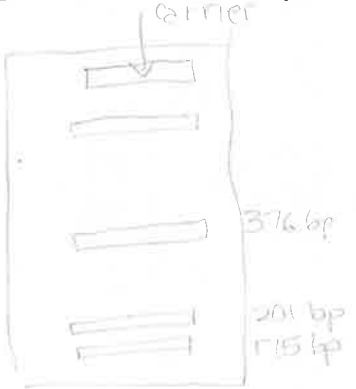


(a) *DdeI* restriction sites in normal and sickle-cell alleles of β-globin gene



18. A patient who is a carrier for sickle-cell anemia would have a gel electrophoresis pattern showing four bands. Add this pattern to your gel in number 17 and explain why the gel shows a four-band pattern.

regular alleles AND sickle cell alleles



19. What is the purpose of a *Southern blot*?

compare restriction fragments produced from different samples of genomic DNA

20. What two techniques discussed earlier in this chapter are used in performing a Southern blot?

- gel electrophoresis
- blotting with a membrane
- hybridization with a probe

21. In working toward the general idea of how DNA sequencing was mechanized, look at Figure 20.12 to answer the following general questions about the *dideoxy chain termination method* for sequencing DNA.

a) Why does a dideoxyribonucleotide terminate a growing DNA strand? (You may need to refer to Figure 16.14, as suggested in the text, to answer this question).

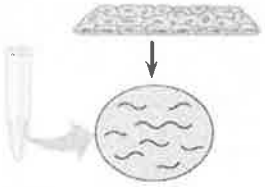
because it lacks a 3'-OH group (attachment site for another nucleotide

b) Why are the four nucleotides in DNA each labeled with a different color of fluorescent tag?

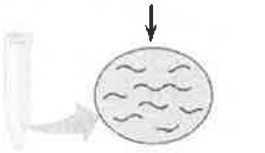
to be able to identify the ending nucleotides + ultimately the entire original sequence

Use unlabeled Figure 20.15 to explain the four steps of *DNA microarray assays*.

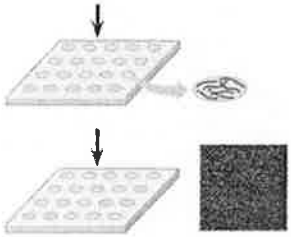
(1) isolate mRNA



(2) make cDNA by reverse transcription (fluorescently labeled nucleotides)



(3) apply the cDNA mixture to the microarray. The cDNA hybridizes with any complementary DNA on the microarray.



(4) Rinse off excess cDNA, fluorescence represents a gene expressed in the tissue sample

22. Explain how microarrays are used in understanding patterns of gene expression in normal and cancerous tissue.

look for base pair variations (SNPs)
"genetic markers" for certain conditions - like cancer

Concept 20.3 Cloning organisms may lead to production of stem cells for research and other applications

23. What is a *totipotent* cell?

cell with the potential to "dedifferentiate" and then give rise to all the specialized cell types

24. How is *nuclear transplantation* performed in animals?

remove egg cell from ovary, remove nucleus
fuse with cultured mammary cell

25. Use unlabeled Figure 20.18 to explain the six steps in reproductive cloning for mammals.

(1) arrest development of cultured mammary cells
(2) nucleus removed from egg cell
(3) fuse cells
(4) grow them in culture
(5) implant into surrogate
(6) clone born

26. What are *stem cells*?

unspecialized cell that can reproduce itself indefinitely and differentiate into specialized tissues

27. What is the major difference between *embryonic stem cells* (*ES*) and *adult stem cells*?

↓ can give rise to all cell types

↓ can only give rise to the cells of the origin tissue type

28. How might *induced pluripotent stem cells* (iPS) resolve the debate about using stem cells for medical treatments?

pluripotent, but dont involve use/ destruction of an embryo

Concept 20.4 The practical applications of DNA technology affect our lives in many ways

29. In question 17, you used two ideas that are featured in the first part of this concept. Explain how *single-nucleotide polymorphisms (SNPs)* and *restriction fragment length polymorphisms (RFLPs)* were demonstrated in analyzing sickle-cell alleles.

looking for indication of disease

SNPs- presence or absence of disease causing mutation

RFLP- variations in DNA sequence

30. Explain the idea of *gene therapy*, and discuss the problems with this technique as demonstrated in the treatment of SCID.

introducing genes into an afflicted individual for therapeutic purposes

- can cause cancers, other issues w/ normal cell function

31. Explain how *transgenic* "pharm" animals might be able to produce human proteins.

insert human gene into their genome; then transgenic animal can express the gene

32. Describe how *short tandem repeats* can produce a sensitive *genetic profile*.

variations in marker lengths, which varies from person to person : identification

33. How does the *Ti plasmid* make genetic engineering in plants a possibility?

Tiplasmid is from the soil bacterium Agrobacterium tumefaciens
Integrates its TDNA into the chromosomal DNA of its host's plant cells

34. What are *genetically modified organisms*, and why are they controversial?

organism that has aquired by artificial means, one or more genes from another species
- safety
- environmental consequences

Testing Your Knowledge: Self-Quiz Answers

Now you should be ready to test your knowledge. Place your answers here:

1._____ 2._____ 3._____ 4._____ 5._____ 6._____ 7._____ 8._____

9. Use the space below for the drawing.