Chapter 20: DNA Tools and Biotechnology

20.1 Describe DNA sequencing, DNA cloning and the polymerase chain reaction.

20.2 Identify techniques that allow us to study the expression and function of one or more genes.

20.3 Explain how multicellular organisms can be cloned, and identify ways to obtain animal stem cells, then describe the uses of stem cells.

20.4 List some practical applications of DNA-based technology.

Biotechnology is a rapidly changing field, and breakthroughs in this area are providing changes in numerous fields of biology, including evolution and medicine. This chapter will use your conceptual understanding of the underlying biology to make sense of the techniques. 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.

Study Tip: The opening figure shows several of the important techniques of biotechnology and their useful applications. As each is introduced in the coming pages, you will apply the knowledge you have gained in the preceding chapters to understand how these techniques work. When you complete this chapter, you should return to this image and explain both the methods and the applications shown here.

Concept 20.1 DNA sequencing and DNA cloning are valuable tools for genetic engineering and biological inquiry

LO 20.1: Describe DNA sequencing, DNA cloning and the polymerase chain reaction.

1. As we begin this chapter, you will need to acquire some new vocabulary. To start this chapter, define:

   DNA technology

   nucleic acid hybridization

   genetic engineering

   DNA Sequencing

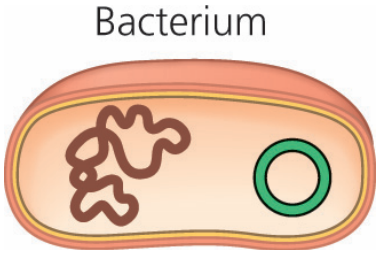
2. What is accomplished in *DNA sequencing?*

3. DNA sequencing techniques are changing rapidly, and your textbook describes three different "generations." Each technique is faster and less expensive than the previous one. The chapter opening photograph shows a single strand of DNA being moved through a *nanopore* in a membrane and the bases are identified one by one. Figure 20.3 in your text describes "next-generation sequencing." This procedure is an example of "high-throughput" DNA technology, and is currently the method of choice for studies where massive numbers of DNA samples are being sequenced. An interesting task is presented in the INTERPRET THE DATA question. Thoroughly study the figure for technique, then place the first 25 nucleotides seen in the flow-gram in the space below.

Making Multiple Copies of a Gene or Other DNA Segment

4. Isolating a single gene is like finding a needle in a haystack. To study a specific gene, scientists often use *DNA cloning*. What is this?

5. *Plasmids* are important in biotechnology. Label the plasmid and the bacterial chromosome in the sketch. What are plasmids? How are they different from the main bacterial chromosome?

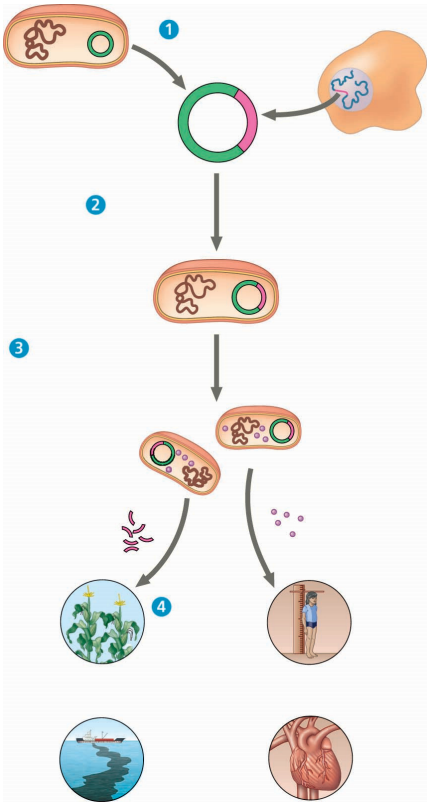


6. What is a *cloning vector*?

   a. Why are bacterial plasmids widely used as cloning vectors?

   b. Plasmids may be altered by genetic engineering to create *recombinant DNA molecules*. What does this mean?

7. Using Figure 20.4 in your text, label this diagram and explain the four steps in this preview of *gene cloning*.



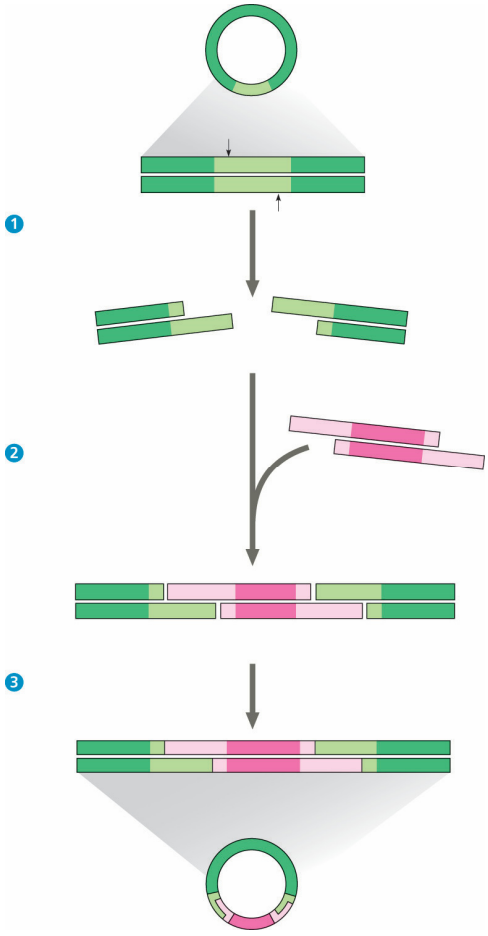
8. Give two specific uses of gene cloning.

Using Restriction Enzymes to Make a Recombinant DNA Plasmid

9. What is a *restriction enzyme*?

10. What is the source and original function of restriction enzymes?

11. Figure 20.5 in your text shows how a plasmid is engineered. Label and explain each step of Figure 20.5.



12. Explain the following key points about making a recombinant plasmid:

    a. What is an example of a *gene of interest* that might be engineered into a plasmid?

    b. What is a *restriction site*?

    c. What are *sticky ends*?

    d. Why are both the gene of interest and the plasmid cut with the *same* restriction enzyme?

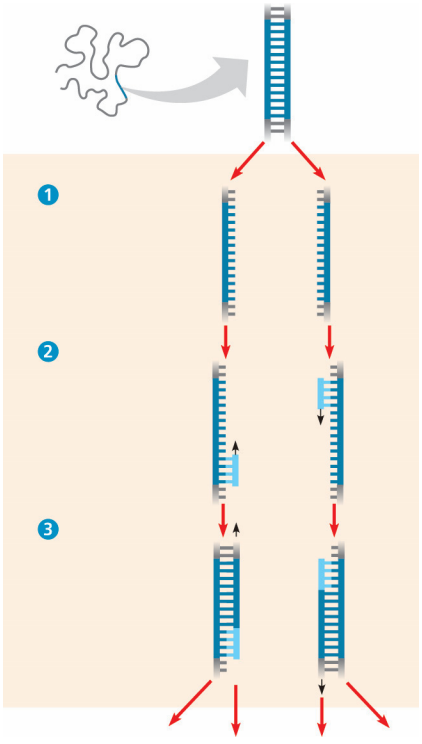
    e. What is the role of *DNA ligase* in this process?

13. When DNA is cut with restriction enzymes, DNA fragments result. Carefully study Figure 20.6 in your text to learn about *gel electrophoresis*. Why is the DNA sample to be separated by gel electrophoresis always loaded at the cathode or negative end of the power source?

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

Amplifying DNA: The Polymerase Chain Reaction (PCR) and Its Use in DNA Cloning

15. 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. Label this figure and explain the three initial steps that occur in cycle 1 of PCR.



16. What is the purpose of the *primers*? (Recall what you know about DNA synthesis.)

17. Why was the discovery of *Taq polymerase* a breakthrough for this process?

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

19. What are four important applications of PCR?

Expressing Cloned Eukaryotic Genes

Getting a cloned eukaryotic gene to function in bacterial host cells can be difficult because of some differences in gene expression in prokaryotic and eukaryotic cells. Let's examine some solutions.

20. What is an *expression vector*?

21. Why can only *complementary DNA (cDNA)* be used in engineering a plasmid that will be inserted into a bacterial cell?

22. What advantages does use of yeast have over bacterial cells for an expression system?

23. What are two techniques besides use of cloning vectors that can be used to introduce recombinant DNA into eukaryotic cells?

24. We tell our students "DNA is DNA is DNA." Cite an example from the *EVOLUTION* heading to explain what we mean.

Concept 20.2 Biologists use DNA technology to study gene expression and function

LO 20.2: Identify techniques that allow us to study the expression and function of one or more genes.

The last concept introduced you to some of the tools of DNA technology, including how DNA is sequenced and genes cloned. Once a gene is isolated, a subsequent question may be "what does it do?" Since genes produce mRNA, there are several methods to determine where the mRNA produced by a gene is found in an organism, or at what stage of its development it is produced. Your instructor may introduce you to all the methods described in the text, but we are selecting just a few for a deeper study.

25. Question 21 asked about cDNA. Using Figure 20.10 in your text, completely label the following figure and describe the five steps in the production of cDNA. Recall that cDNA is what is inserted into recombinant plasmids.

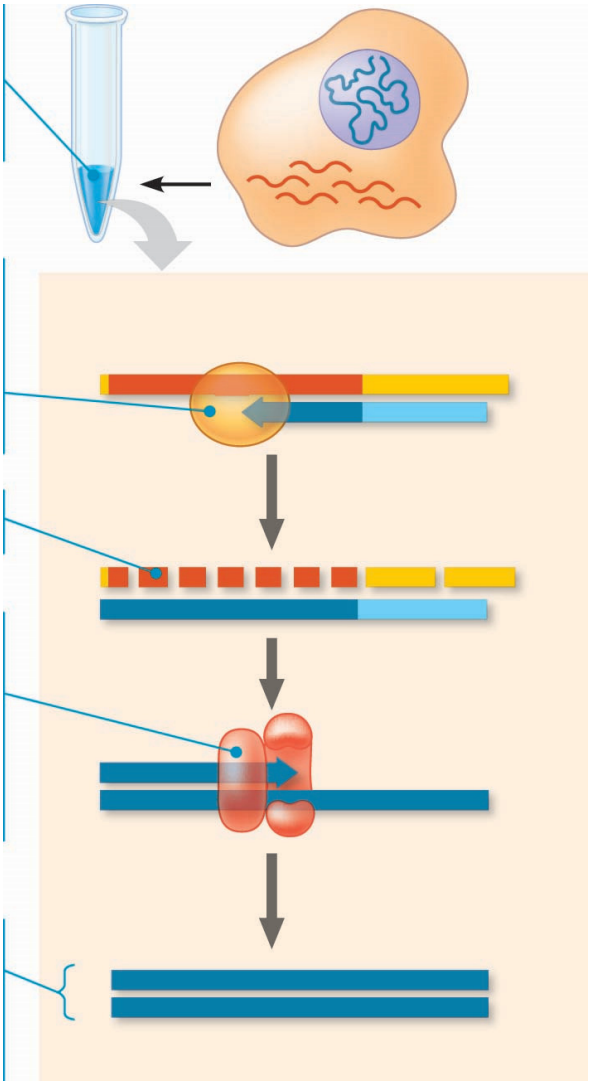
1.

2.

3.

4.

5.



26. On February 4, 2020, the Centers for Disease Control and Prevention (CDC) released *2019-nCoV Real-Time RT-PCR Diagnostic Panel* tests for emergency use to diagnose patients suspected to have the COVID-19. RT-PCR tests are able to detect RNA from SARS-CoV2 (the coronavirus that causes COVID-19 disease). What are the three tools of biotechnology that are employed in RT-PCR? (Refer to Figure 20.11 in your text.)

27. What genes are active (producing mRNAs) in a particular tissue? *RNA sequencing*, or *RNA-seq*, is one method used to study gene expression across an entire genome. Study Figure 20.12 in your text to see how this is done.

    a. What occurs in step 1?

    b. What occurs in step 4?

    c. What does the resulting data reveal?

28. What are the two advantages RNA-seq has over older methods?

29. *DNA microarray assays* can be used to determine a pattern of gene expression, such as what genes are expressed in an embryo on day 2 of development compared to day 5. The expression of thousands of genes can be measured at one time. Turn back to Figure 18.27 in your text and explain how microarrays are used to determine appropriate treatment for different types of breast cancer.

30. Briefly describe two ways genes may be edited or silenced.

31. What are *SNP*s? How are they used to help screen for certain diseases? What are some examples of diseases for which there are genetic markers?

Concept 20.3 Cloned organisms and stem cells are useful for basic research and other applications

LO 20.3: Explain how multicellular organisms can be cloned, and identify ways to obtain animal stem cells, then describe the uses of stem cells.

32. What are *stem cells*?

33. What is a *totipotent* cell?

34. In early cloning experiments, John Gurdon and his colleagues transplanted nuclei from frogs into enucleated eggs (*nuclear transplantation*). What did they conclude from this work?

35. Cloned mammals were found to not always be identical to their parents or siblings. Often the cloned animals exhibit defects. What seems to be the reason for the high incidence of abnormalities?

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

37. What are *induced pluripotent stem cells (iPS)*?

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

39. What are two potential uses for human iPS cells?

Concept 20.4 The practical applications of DNA-based biotechnology affect our lives in many ways

LO 20.4: List some practical applications of DNA-based technology.

40. In January 2020, as this guide is being written, a new coronavirus has emerged in China. At this date, diagnosis is confirmed using RT-PCR. Give two examples of ways biotechnology is used in the treatment or diagnosis of diseases that are of interest to you.

41. What are three applications of *personal genome analysis*?

42. What is the promise of *personalized medicine*?

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

44. The CRISPR-Cas9 offers a promising route to correct certain genetic defects without the associated problems of gene therapy. At present, what are the major concerns about this method?

45. DNA technology has been used in the development of certain drugs such as Gleevec that have been highly effective in cancer treatment. In many cases, persons appear to be in nearly complete remission only to have a relapse. Explain what occurs in these cases.

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

47. Describe how *short tandem repeats (STRs)* can produce a sensitive *genetic profile*. Don't miss the information about Earl Washington that goes with Figure 20.24 in your text.

48. Cite four examples of *genetically modified organisms (GMOs)*. Why are GMs controversial?

Test Your Understanding, p. 440

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

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

7. _____

10. Use the space below for the drawing.