

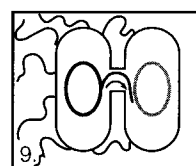
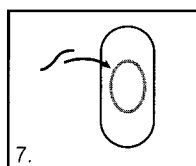
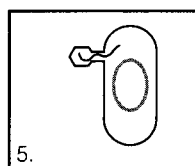
In recent years—within the lifetimes of most students reading this textbook—biologists have learned to construct genes and use them to alter organisms. A new toolkit consisting of enzymes, radioactive isotopes, cell culture techniques, and “gene machines” enables us to find genes, read them, compare them, insert them into new organisms, and harvest their products. Genetic engineering has revolutionized our understanding of genes and promises to transform our lives in many ways, from medicine to agriculture. The Human Genome Project has mapped our DNA, giving us new insights into human evolution, development, health, and disease. But our new understanding of genes and our ability to manipulate them are accompanied by some risks and numerous social and ethical questions. This chapter concerns DNA technology—its methods, promise, and potential problems.

Organizing Your Knowledge

Exercise 1 (Modules 12.1 – 12.2)

Bacteria can transfer DNA via conjugation, transformation, and transduction. Match the following statements with one of the methods of bacterial DNA transfer. (Some statements are true of all methods of DNA transfer.)

- | | |
|---------------------------|---|
| A. Conjugation | _____ 1. What happened in Griffith’s experiment with pneumonia bacteria |
| B. Transformation | _____ 2. DNA may be integrated into chromosome of recipient |
| C. Transduction | _____ 3. Taking up of DNA from the fluid surrounding a cell |
| D. All three of the above | _____ 4. Alters genetic makeup of recipient cell |
| | _____ 5. Figure 5 below |
| | _____ 6. Male and female cells joined by sex pili |
| | _____ 7. Figure 7 below |
| | _____ 8. Bacterial “mating” |
| | _____ 9. Figure 9 below |
| | _____ 10. Creates a recombinant cell |
| | _____ 11. Transfer of genes by a bacteriophage |
| | _____ 12. May involve transfer of genes by a plasmid |
| | _____ 13. Usually controlled by a piece of DNA called an F factor |



Exercise 2 (Modules 12.3 – 12.9)Web/CD Activity 12A *Restriction Enzymes*Web/CD Activity 12B *Cloning a Gene in Bacteria*

Plasmids can be used to engineer bacteria to produce desired genes or proteins. Review techniques used to splice and clone genes by filling in the blanks.

Gene engineers use plasmids as ¹_____ to insert genes into bacteria or eukaryotic cells. Imagine that you wanted to build a bacterium capable of making large quantities of human growth hormone (HGH), which is a protein. Your first step would be to obtain the ²_____ that codes for HGH. One way to do this is to use a ³_____ enzyme to cut up all the DNA in a human cell. The enzyme recognizes short nucleotide ⁴_____ within DNA molecules and cuts the DNA at specific points in these ⁵_____ sequences. Restriction enzymes cut the two DNA strands unevenly, leaving single-stranded ends that can hydrogen-bond with complementary single-stranded ⁶“_____ends.” A restriction enzyme can chop up a cell’s DNA into thousands of pieces, each consisting of a few genes.

The next step in making human growth hormone is isolating a supply of ⁷_____ to use as vectors, for carrying the DNA fragments into bacteria. These are treated with the same restriction enzyme that was used to cut up the human DNA, producing plasmids with sticky ends that are ⁸_____ to sticky ends of the human DNA fragments.

Now the human DNA fragments are mixed with plasmids. The sticky ends on the fragments base-pair with the sticky ends on the plasmids, but these connections are weak and temporary. An enzyme called DNA ⁹_____, which normally functions in DNA ¹⁰_____, is used to catalyze the formation of covalent bonds between adjacent nucleotides in the DNA fragments and plasmids. This forms ¹¹_____ DNA, a DNA molecule with a new, human-made combination of genes.

In the next step, each recombinant plasmid is added to a bacterium. Under specific conditions, a bacterium will take up the plasmid DNA from solution by the process of ¹²_____. The bacterium, with its recombinant plasmid, is allowed to grow and reproduce on a nutrient medium. Each bacterium replicates its own DNA and the plasmid DNA and then divides repeatedly. Each bacterium grows into a colony of identical cells, all containing the recombinant DNA. This production of multiple copies of the genes is called gene ¹³_____. Cloning all the different DNA fragments obtained from the human cell produces a genomic ¹⁴_____ of DNA segments. Because this procedure does not target a particular gene (at least so far), it is called the ¹⁵“_____ approach” to gene cloning. (DNA fragments can also be spliced into phages, ¹⁶_____ that infect bacteria. The phages reproduce in bacteria to produce libraries of cloned DNA pieces.)

There are a lot of genes to sort through in a library produced from an entire eukaryotic genome. Plus, eukaryotic genes contain noncoding ¹⁷_____, which must be removed before bacteria can read them. It is often better to start with the genes expressed in a particular kind of cell, using the enzyme ¹⁸_____ transcriptase to

produce intron-free genes. If you wanted to obtain a human growth hormone gene, the place to start would be a cell from the pituitary gland, where HGH is made. In the cell, the HGH gene (and others) is transcribed into RNA. Enzymes then remove the introns from the RNA and splice the remaining ¹⁹ _____ together to make mRNA. The mRNA is then extracted from the cell, and reverse transcriptase (obtained from a ²⁰ _____) is added. The reverse transcriptase transcribes a strand of DNA along the mRNA molecule. The RNA is then broken down, and a second DNA strand is synthesized, producing double-stranded DNA. This artificial gene lacks introns, so it is more manageable than the original gene. It also can be transcribed and translated by ²¹ _____, which lack the ability to splice RNA. Complementary DNA produced in this fashion is cut and pasted into plasmids, using restriction enzymes and ligase, and then cloned in bacteria. There are many mRNA molecules in a pituitary gland cell, so this method also produces a ²² _____ library; however, this library is smaller than a library produced by cutting up the entire genome, because it is limited to the genes actually ²³ _____ in a pituitary gland cell.

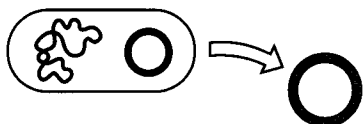
At this point you have isolated and cloned the HGH gene, but where is it? A genomic library can consist of thousands of bacterial colonies. The bacteria of one of the colonies contains the HGH gene, but which one? One way to look for the gene is to look for HGH, its ²⁴ _____ product. But usually you look for the gene itself, a search that is made easier by using a nucleic acid ²⁵ _____. If you know part of the amino acid sequence of HGH, you can work backward to figure out the probable nucleotide sequence of part of the HGH gene—AAGTGTAG, for example. Now you can produce an artificial RNA (or DNA) molecule with a complementary base sequence—²⁶ _____, in this case. This complementary molecule is labeled with a ²⁷ _____ isotope or a fluorescent dye, and is called a probe because it can be used to find the gene. To find the bacterial clone that holds the gene, DNA is obtained from each colony of bacteria and treated to separate the DNA strands. The probe is then mixed with the DNA strands, and it hydrogen-bonds only with the recombinant DNA with a complementary base sequence—the HGH gene. Once you have figured out which bacterial colony in the library contains the HGH gene, you can grow these bacteria in larger amounts.

If you know the nucleotide sequence of the desired gene, you can bypass much of the trouble of cloning and searching for the gene by simply synthesizing it in the laboratory. There are now gene machines that can put together artificial genes several hundred ²⁸ _____ in length. There are also automatic DNA sequencers capable of determining the sequences of large genes in a day or so. The genes are cut into fragments by restriction enzymes and machine analyzed. The sequences are then fed into computer data banks for interpretation.

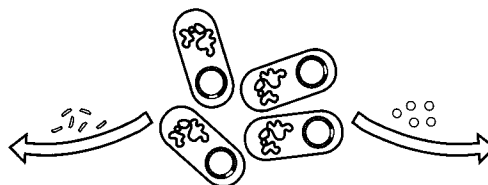
The final step in engineering bacteria to produce human growth hormone is to grow the bacteria in large quantities (usually done in large vats) and extract the protein. The bacteria will manufacture the protein on command if you have spliced the proper control sequences into your recombinant plasmids. Now it is only necessary to collect and purify the protein (and get approval from the Food and Drug Administration!) to start treating patients with recombinant DNA HGH.

Exercise 3 (Modules 12.3 – 12.8)Web/CD Activity 12A *Restriction Enzymes*Web/CD Activity 12B *Cloning a Gene in Bacteria*

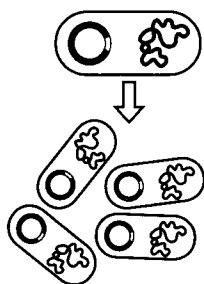
Review recombinant DNA techniques by matching each of the diagrams (or parts of diagrams) below with one of the following processes: **isolating plasmid from *E. coli***; **extracting DNA from a eukaryotic cell**; **obtaining copies of gene and protein from cloned bacteria**; **cutting DNA with restriction enzyme**; **joining plasmid and DNA fragment using DNA ligase**; **cloning recombinant DNA**; **using reverse transcriptase to make an artificial gene**; **using a nucleic acid probe to find a gene**; **inserting a plasmid into a bacterium via transformation**; and **mixing plasmids and DNA fragments with sticky ends**.



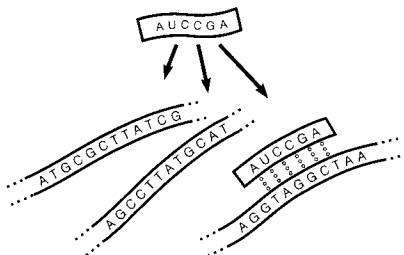
1. _____



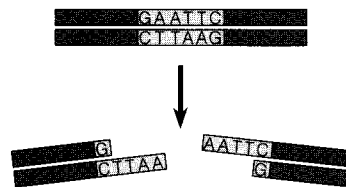
2. _____



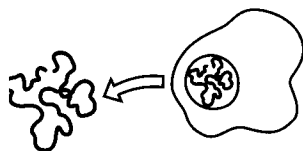
3. _____



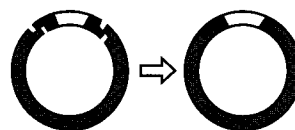
4. _____



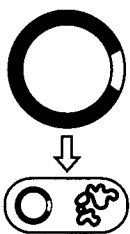
5. _____



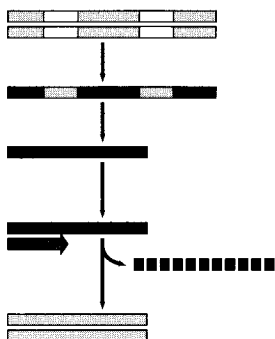
6. _____



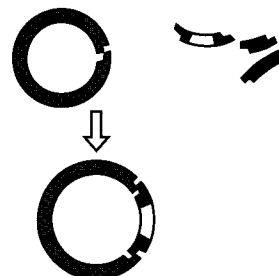
7. _____



8. _____



9. _____



10. _____

Exercise 4 (Modules 12.9 – 12.12)Web/CD Activity 12C *Gel Electrophoresis of DNA*Web/CD Activity 12D *Analyzing DNA Fragments Using Gel Electrophoresis*

Powerful molecular biology techniques now allow us to amplify, analyze, and compare genes. Review these methods by matching each phrase on the right with a term on the left.

- | | | |
|------------------------------------|-------|--|
| A. restriction fragment analysis | _____ | 1. Transferring DNA to paper so it can be exposed to a probe |
| B. carrier | _____ | 2. Used to cut up DNA for analysis |
| C. DNA microarray | _____ | 3. Piece of DNA cut up by restriction enzymes |
| D. recognition sequence | _____ | 4. Place where enzyme cleaves DNA |
| E. positive pole | _____ | 5. Used to find bands with particular DNA sequences |
| F. restriction fragment | _____ | 6. Type of cell often used in restriction fragment analysis |
| G. DNA polymerase | _____ | 7. Separates DNA fragments by size and electrical charge |
| H. DNA probe | _____ | 8. Restriction fragments move through this |
| I. blotting | _____ | 9. Restriction fragments are attracted to this |
| J. white blood cell | _____ | 10. Where specific restriction fragment collects in gel |
| K. band | _____ | 11. Chromosomal "landmark" that can be studied |
| L. restriction enzyme | _____ | 12. Comparing restriction fragment patterns |
| M. genetic marker | _____ | 13. Method for making many copies of a DNA molecule |
| N. gel electrophoresis | _____ | 14. Used to replicate DNA in a test tube for PCR method |
| O. polymerase chain reaction (PCR) | _____ | 15. Heterozygote who might possess a harmful allele |
| P. gel | _____ | 16. A "DNA chip"—tests for many expressed genes at once |

Exercise 5 (Module 12.13)

This module gives a number of facts and figures that will help put the human genome into perspective. It is not important to memorize the figures, but it is important for you to get an idea of how big and how small some of these things are. Choose the correct number to complete each of the statements. Choose from **10, 23, 46, 97, 1000, 2000, 35,000, 130 million, and 3 billion.**

1. Number of chromosomes in a diploid human cell: _____
2. Number of chromosomes in a haploid set: _____
3. Number of nucleotide pairs in a haploid set of human chromosomes: _____
4. Number of nucleotide pairs in an average human chromosome: _____
5. Estimated number of genes in a human cell: _____
6. Number of genes in *E. coli*: _____
7. Amount of DNA in a human cell divided by amount of DNA in *E. coli*: _____
8. Percentage of DNA in a human cell that is thought to be noncoding DNA: _____
9. Amount of human DNA in introns divided by amount in exons: _____

Exercise 6 (Module 12.13)

See if you can summarize this module on Barbara McClintock's work with "jumping genes" in *exactly* 25 words.

Exercise 7 (Module 12.14)

Web/CD Activity 12E *The human Genome Project: Human Chromosome 17*

The goal of the Human Genome Project (HGP) was nothing less than mapping the entire nucleotide sequence of all the DNA in a human cell. A map of the human genome promises great insights into human evolution, development, health, and disease. There are three major stages in mapping the human genome: genetic (linkage) mapping, physical mapping, and DNA sequencing. Match each of these stages with one or more of the descriptive phrases below.

- | | |
|-------|------------------------------|
| _____ | 1. Genetic (linkage) mapping |
| _____ | 2. Physical mapping |
| _____ | 3. DNA sequencing |
-
- A. Cloning DNA fragments and determining their original order in a chromosome
 - B. The most difficult part of the project
 - C. Using genetic crosses to map genes on a chromosome
 - D. Using probes to match fragments to markers mapped in stage 1
 - E. Automatic DNA sequencers essential to this stage of mapping
 - F. Mapping genetic markers using pedigree analysis
 - G. Determining nucleotide sequences of DNA fragments covering the entire genome
 - H. Making a low-resolution map useful for arranging later, more detailed maps.

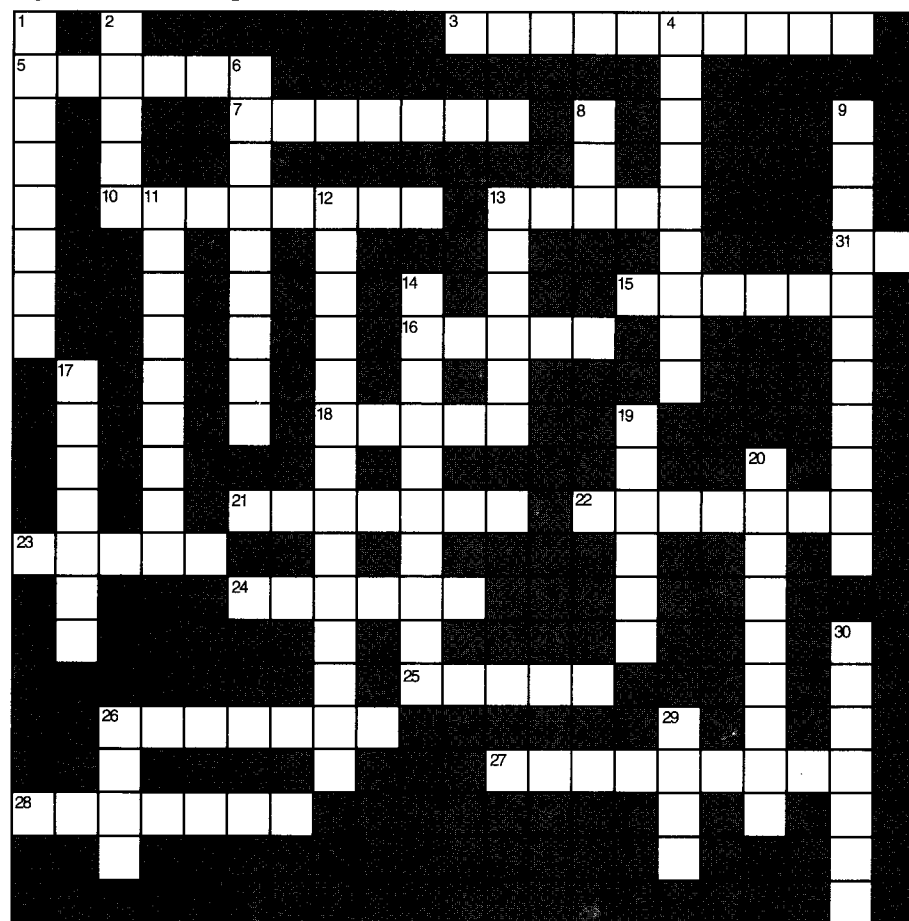
Exercise 8 (Modules 12.15 – 12.21)

Web/CD Activity 12F Connection: DNA Fingerprinting

Web/CD Activity 12G Connection: Application of DNA Technology

Web/CD Activity 12H Connection: DNA Technology and Golden Rice

DNA technology promises great benefits, but also presents some risks and raises ethical questions. Review some of the tools, uses, and dilemmas of DNA technology by completing this crossword puzzle.

**Across**

3. Microorganisms used in recombinant DNA experiments are genetically crippled so they cannot live outside the ____.

5. Except in the case of identical twins, the DNA sequence of every individual is ____.

7. A ____ is a harmless variant or derivative of a harmful bacterium or virus, used to prevent disease.

10. A harmless variant of the ____ virus might be able to confer immunity to several diseases at once.

13. There is some concern that transgenic plants may pass their new ____ to wild relatives.

15. In gene therapy, a gene is introduced that allows diseased or defective cells to make a needed protein, such as a growth factor or ____.

16. Many proteins important in medicine and agriculture are produced in the bacterium ____.

Down

1. Our society rejects the notion of ____—controlling the genetic makeup of human populations.

2. A ____ can be used as a vector to carry genes for gene therapy.

4. ____ nucleic acid prevents translation of mRNA coding for disease-causing proteins.

6. We might be able to eliminate genetic defects in children and their descendants, but some people wonder whether we should interfere with ____ in this way.

8. A gene ____ can fire pieces of DNA into cells.

9. A DNA ____ is a pattern of bands used to link an individual with evidence.

11. Only ____ cells can correctly manufacture glycoproteins.

12. DNA technology has been used to create many useful ____ products.

Across

18. ____ of genetic information might take the form of genetic discrimination or invasion of privacy.
21. Gene ____ is alteration of human genes to remedy a genetic disease.
22. Bacteria often are the best organisms to engineer for production of a ____ product.
23. A plasmid from a soil bacterium is used to get genes into ____ cells.
24. Several crop plants, such as ____, have been modified with a bacterial gene for herbicide resistance.
25. A ____ is often better than a bacterium at producing eukaryotic proteins.
26. There are difficult ____ questions relating to modification of the human genome.
27. A number of transgenic plants have been engineered with genes for ____ resistance.
28. It is easier to modify genes in human germ cells or ____ than in somatic cells.
31. A ____ organism has acquired a gene by artificial means.

Testing Your Knowledge**Multiple Choice**

1. The primary difference between bacterial sex and sexual reproduction in plants and animals is that
 - a. bacterial sex involves more than two individuals.
 - b. bacterial sex does not involve genetic recombination.
 - c. bacteria exchange RNA, not DNA.
 - d. bacterial sex does not produce offspring.
 - e. eggs and sperm are different, but bacterial gametes are all alike.
2. Sometimes a bacteriophage transfers a gene from one bacterium to another. This process is called
 - a. transduction.
 - b. conjugation.
 - c. cloning.
 - d. DNA splicing.
 - e. transformation.
3. There are thought to be about ____ genes in a human cell.
 - a. 23
 - b. 46
 - c. 2000
 - d. 35,000
 - e. 3 billion

Down

13. Results of the Human ____ Project are revolutionizing medicine.
14. Biotechnology is scrutinized for possible risks by government ____ agencies such as the EPA and FDA.
17. Human growth hormone and human ____ were the first pharmaceutical products made using DNA technology.
19. Bone ____ cells are prime targets of gene therapy.
20. Yeast cells have been engineered to make a vaccine against ____ B.
26. Researchers produce transgenic animals by injecting DNA into fertilized ____.
29. A sheep with a human gene can produce a desired protein in its ____.
30. DNA microarrays will assist in diagnosis of ____.

4. The ability of a male *E. coli* to carry out conjugation is usually due to a piece of DNA called
 - a. a probe.
 - b. an R plasmid.
 - c. recombinant DNA.
 - d. an F factor.
 - e. a Ti plasmid.
5. A genetic marker is
 - a. a place where a restriction enzyme cuts DNA.
 - b. a chart that traces the family history of a genetic trait.
 - c. a nucleotide sequence near a particular gene.
 - d. a radioactive probe used to find a gene.
 - e. an enzyme used to cut DNA.
6. In recombinant DNA experiments, ____ is used to cut pieces of DNA, and ____ joins these segments to form recombinant DNA.
 - a. a restriction enzyme . . . DNA ligase
 - b. a transposon . . . a restriction enzyme
 - c. a plasmid . . . DNA ligase
 - d. DNA ligase . . . a restriction enzyme
 - e. a transposon . . . a plasmid
7. A genomic library is
 - a. where you look to find out how to make recombinant DNA.
 - b. a listing of the known nucleotide sequences for a particular species.
 - c. all the genes contained in one kind of cell.
 - d. a collection of cloned DNA pieces from an organism's genome.
 - e. a place where one can obtain DNA samples from various species.

8. A nucleic acid probe might be used to
 - a. insert genes into a host cell.
 - b. make DNA for gene cloning.
 - c. splice pieces of DNA.
 - d. cut pieces of DNA down to manageable size.
 - e. find a nucleotide sequence.
9. It is sometimes necessary to genetically engineer mammalian cells to produce proteins because they
 - a. can produce larger quantities of protein than bacteria.
 - b. can read eukaryotic genes, and bacteria cannot.
 - c. can add sugars to make glycoproteins, and bacteria cannot.
 - d. are easier to grow than bacteria.
 - e. can be induced to secrete proteins into their environment.
10. Scientists have produced a smallpox virus that contains genes from several other disease-causing microorganisms. They hope to use the virus
 - a. in a vaccine against several diseases.
 - b. as a compact genomic library.
 - c. to perfect a germ-warfare weapon with no antidote.
 - d. as a gene vector for human gene therapy.
 - e. as a tool for diagnosing various infectious diseases.
11. Electrophoresis is used to
 - a. separate fragments of DNA.
 - b. clone genes.
 - c. cut DNA into fragments.
 - d. match a gene with its function.
 - e. amplify small DNA samples to obtain enough for analysis.
4. Describe some uses of recombinant DNA technology in agriculture.
5. Describe some uses of recombinant DNA technology in medicine.
6. What are some potential risks of genetically engineered organisms being accidentally or purposefully released into the environment? What kinds of safety measures guard against accidental release? Are you concerned about these risks? Why or why not?
7. Discuss some of the ethical questions raised by recombinant DNA technology. What is the most difficult ethical question concerning human gene therapy?
8. Describe how restriction fragment analysis is done: How is DNA cut into fragments? How are the fragments separated? What does the DNA fingerprint look like, and why? Why do different people have different DNA fingerprints?
9. As we learn more about the human genome, how might individual DNA data be misused?

Applying Your Knowledge

Multiple Choice

1. A geneticist found that a particular nucleotide sequence was found on different chromosomes in different mouse skin cells. This suggested that
 - a. transformation was occurring in some skin cells.
 - b. transposons were moving around.
 - c. the cells were engaging in conjugation.
 - d. plasmids were transferring genes from cell to cell.
 - e. the mice were transgenic animals.
2. A microbiologist analyzed the DNA of *E. coli* before and after conjugation. She found that
 - a. both cells lost some genes and gained others.
 - b. both cells gained genes but lost none of their original genes.
 - c. one cell lost genes, and the other gained genes.
 - d. one cell gained genes, and the genes of the other were unchanged.
 - e. the genes of both cells remained unchanged.

Essay

1. How might transposons harm an organism? How might they contribute to the evolution of a species?
2. Explain how DNA segments can be cut and spliced together to produce recombinant DNA. How do the segments “find” each other and stick together? How is recombinant DNA then cloned to produce multiple copies of the gene?
3. Explain why bacteria and yeast are often used as “factories” for gene products.

3. Which of the following do “sticky ends” and molecular probes have in common?
 - a. They both are used as gene vectors in genetic engineering.
 - b. They both involve complementary base pairing.
 - c. They both are parts of RNA molecules.
 - d. They both are produced by the action of restriction enzymes.
 - e. They both are important aspects of bacterial sex.
4. Because eukaryotic genes contain introns, they cannot be translated by bacteria, which lack RNA splicing machinery. If you want to engineer a bacterium to produce a eukaryotic protein, you can synthesize a gene without introns (if you know the nucleotide sequence) or
 - a. alter the bacteria used so that they can splice RNA.
 - b. use a molecular probe to find a gene without introns.
 - c. work backward from mRNA to a piece of DNA without introns.
 - d. use a phage to insert the desired gene into a bacterium.
 - e. use a restriction enzyme to remove introns from the gene.
5. When recombinant plasmids are added to *E. coli*, not all the bacteria take them in. This makes it difficult to spot the bacteria that actually contain the plasmids. For this reason, R plasmids are often used as gene vectors in recombinant DNA experiments. Using R plasmids would allow experimenters to find the bacteria with recombinant DNA easily by
 - a. using a molecular probe.
 - b. killing off the bacteria without the R plasmids.
 - c. feeding them a special diet.
 - d. exposing the bacteria to restriction enzymes.
 - e. watching what happens when the bacteria reproduce.
6. Scientists wished to create an organism capable of breaking down several kinds of toxic wastes, so they combined the genes of several bacteria to create a single “superbacterium.” They probably did not need to use which of the following in creating the superbacterium?
 - a. nucleic acid probes
 - b. F factors
 - c. plasmids
 - d. restriction enzymes
 - e. DNA ligase
7. A crop scientist spliced genes for disease resistance into Ti plasmids and then treated tomato plants with the plasmids. Some parts of some plants resisted the disease, but most of the plants eventually died. The researcher could increase his chances for success by
 - a. treating single cells and cloning whole plants from the cells.
 - b. using molecular probes to figure out where to put the genes.
 - c. using R plasmids rather than Ti plasmids to introduce the genes.
 - d. inserting the genes into the cells of the tomato plants with a needle.
 - e. employing bacteriophages as vectors to get the genes into the plants.
8. A molecular biologist used a virus to introduce a gene coding for a certain enzyme into mouse cells. Most of the mouse cells were able to make the enzyme, but most of them lost the ability to make some other protein (different ones in different cells), and many died. Which of the following best explains these results?
 - a. The viruses caused the mouse cells to become diseased.
 - b. The viruses transferred genes from one mouse cell to another.
 - c. The viruses inserted the enzyme gene into mouse cell genes.
 - d. The viruses activated transposons, which disrupted other genes.
 - e. The enzyme acted as a restriction enzyme, cutting up mouse DNA.
9. DNA fingerprints were used to determine whether Sam could be the father of Becky’s baby. Which of the following would show that Sam is not the father? If ____ genetic fingerprint showed some bands not in ____ genetic fingerprint.
 - a. Sam’s . . . the baby’s
 - b. Becky’s . . . the baby’s
 - c. the baby’s . . . Sam’s
 - d. the baby’s . . . Becky’s
 - e. the baby’s . . . Sam’s or Becky’s
10. Archaeologists unearthed a human skull with a small dried fragment of the scalp still attached. They extracted a tiny amount of DNA from the scalp tissue. How could they obtain sufficient DNA for an analysis of the ancient man’s genes?
 - a. subject the DNA to electrophoresis
 - b. use a molecular probe
 - c. subject the specimen to amniocentesis
 - d. use the polymerase chain reaction
 - e. subject the DNA to restriction enzymes

11. There is about 1000 times as much DNA in a human cell as in an *E. coli* cell but only about 20 times as many genes. Why?
 - a. A human cell has much more noncoding DNA.
 - b. The DNA is much more tightly coiled in a human cell.
 - c. Most of the genes in a human cell are turned off.
 - d. *E. coli* genes are less efficient than human genes.
 - e. Human cells are much smaller than *E. coli* cells.

Essay

1. A mutant strain of *E. coli* bacteria will not grow unless they are supplied with the amino acid lysine. Another strain will not grow without a different amino acid, proline. When *E. coli* of the two strains are mixed, a few bacteria appear in the culture that are able to grow without either of the amino acids. Name and briefly describe three possible mechanisms that might account for this change.
2. Describe how you would go about genetically engineering a bacterium to produce human epidermal growth factor (EGF), a protein used in treating burns. Use the following: DNA ligase, *E. coli*, plasmids, genetic code chart, restriction enzyme, machine for synthesizing a gene, description of the amino acid sequence of EGF, and glassware and equipment for growing and handling bacteria and extracting protein.
3. In a "shotgun" experiment, all the DNA in a cell is cut up and cloned, producing a genomic library of DNA fragments, each fragment in a different bacterial colony. Why do you think some researchers question the safety of the shotgun approach?
4. A researcher is searching for the bacterial clone containing a particular cloned gene. She knows that part of the nucleotide sequence of the gene is ATGGCTATC. Explain how she might find the bacteria that contain the gene.
5. A microbiologist developed a strain of *E. coli* that were easily killed by sunlight and whose diet required two unusual amino acids not normally found outside the laboratory. Why would such a bacterium be useful in recombinant DNA work?
6. Manuel, a political activist, "disappeared" during the reign of a dictator. After the dictator was overthrown, human remains thought to be those of Manuel were found buried in a prison compound. A sample of DNA was extracted from the remains and subjected to restriction fragment analysis, along with DNA samples from Manuel's parents. The patterns of restriction fragments are shown below. Could the remains be those of Manuel, the missing activist? Explain your answer.

Human remains							
Manuel's father							
Manuel's mother							
7. A certain genetic disorder results from the lack of a blood enzyme that is secreted by bone marrow cells. A second disease occurs when nerve cells are unable to produce a particular enzyme. Which of these disorders would be a better candidate for gene therapy and why?

Extending Your Knowledge

1. Have you used any products produced by means of DNA technology? Think about drugs (HGH, insulin), foods made from GM organisms, vaccines, and hospital diagnostic tests.
2. Hardly a day goes by without a story about DNA technology appearing in the newspaper. For the next few days, look for examples of DNA technology in the news. You may see articles about new drugs, cancer treatment, genetic diseases, transgenic crop plants, mapping of the human genome, clarification of evolutionary relationships, and even crimes solved using recombinant DNA techniques. Do you understand these articles better after studying recombinant DNA technology? What did the authors omit that you would like to know?