5. Clones containing foreign DNA can be tested for the desired gene product.
6. A short piece of labeled DNA called a DNA probe can be used to identify clones carrying the desired gene.

Making a Gene Product (pp. 260–262)

1. *E. coli* is used to produce proteins by genetic engineering because it is easily grown and its genomics are well understood.
2. Efforts must be made to ensure that *E. coli*'s endotoxin does not contaminate a product intended for human use.
3. To recover the product, *E. coli* must be lysed or the gene must be linked to a gene that produces a naturally secreted protein.
4. Yeasts can be genetically engineered and are likely to continuously secrete a gene product.
5. Mammalian cells can be engineered to produce proteins such as hormones for medical use.
6. Plant cells can be engineered and used to produce plants with new properties.

APPLICATIONS OF GENETIC ENGINEERING (pp. 262–268)

1. Cloned DNA is used to produce products, study the cloned DNA, and alter the phenotype of an organism.

Therapeutic Applications (pp. 262–264)

1. Synthetic genes linked to the β-galactosidase gene *(lacZ)* in a plasmid vector were inserted into *E. coli*, allowing *E. coli* to produce and secrete the two polypeptides used to make human insulin.
2. Cells can be engineered to produce a pathogen's surface protein, which can be used as a subunit vaccine.
3. Animal viruses can be engineered to carry a gene for a pathogen's surface protein. When the virus is used as a vaccine, the host develops an immunity to the pathogen.
4. Gene therapy can be used to cure genetic diseases by replacing the defective or missing gene.

Scientific Applications (pp. 264–266)

1. Recombinant DNA techniques can be used to increase understanding of DNA, for genetic fingerprinting, and for gene therapy.
2. DNA sequencing machines are used to determine the nucleotide base sequence in a gene.
3. Southern blotting can be used to locate a gene in a cell.
4. Genetic screening uses Southern blotting to look for mutations responsible for inherited diseases in humans.
5. Southern blotting is used in DNA fingerprinting to identify bacterial or viral pathogens.
6. DNA probes can be used to quickly identify a pathogen in body tissue or food.

Agricultural Applications (pp. 266–268)

1. Cells from plants with desirable characteristics can be cloned to produce many identical cells. These cells can then be used to produce whole plants from which seeds can be harvested.
2. Plant cells can be engineered by using the Ti plasmid vector. The tumor-producing T genes are replaced with desired genes, and the recombinant DNA is inserted into *Agrobacterium*. The bacterium naturally transforms its plant hosts.
3. Genes for glyphosate resistance, BT toxin, and pectinase suppression have been engineered into crop plants.
4. *Rhizobium* has been engineered for enhanced nitrogen fixation.
5. *Pseudomonas* has been engineered to produce *Bacillus thuringiensis* toxin against insects.
6. Bovine growth hormone is being produced by *E. coli*.

SAFETY ISSUES AND THE ETHICS OF GENETIC ENGINEERING (pp. 268–269)

1. Strict safety standards are used to avoid the accidental release of genetically engineered microorganisms.
2. Some microbes used in genetic engineering have been altered so that they cannot survive outside the laboratory.
3. Microorganisms intended for use in the environment may be engineered to contain suicide genes so that the organisms do not persist in the environment.
4. Genetic technology raises ethical questions such as: Should employers and insurance companies have access to a person's genetic records? Will some people be targeted for either breeding or sterilization? Will genetic counseling be available to everyone?
5. Genetically-engineered crops must be safe for consumption and for release in the environment.
6. Genetic engineering techniques are being used to map the human genome through the Human Genome Project.
7. This will provide tools for diagnosis and possibly the repair of genetic diseases.

Study Questions

REVIEW

1. Differentiate recombinant DNA from genetic engineering.
2. Compare and contrast the following terms:
   a. cDNA and gene
   b. restriction fragment and gene
   c. DNA probe and gene
   d. DNA polymerase and DNA ligase
   e. recombinant DNA and cDNA
3. How is each of the following used in genetic engineering?
   a. plasmid
   b. viral genome
   c. antibiotic-resistance genes
   d. restriction enzyme

4. Differentiate between a gene library and synthetic DNA.
5. Differentiate among the following terms. Which one is "hit and miss"—that is, does not add a specific gene to a cell?
   a. protoplast fusion
   b. gene gun
   c. microinjection
   d. electroporation
6. Some commonly used restriction enzymes are listed in the following table. The cutting site is indicated by ↓. Indicate which enzymes produce sticky ends. Of what value are sticky ends in making recombinant DNA?

Enzyme	Bacterial Source	Recognition Sequence
*Bam*HI	*Bacillus amyloliquefaciens*	G↓G A T C C G C T A G↑G
*Eco*RI	*Escherichia coli*	G↓A A T T C C T T A A↑G
*Hae*III	*Haemophilus aegyptius*	G G↓C C C C↑G G
*Hind*III	*Haemophilus influenzae*	A↓A G C T T T T C G A↑A

7. Suppose you want multiple copies of a gene you have synthesized. How would you obtain the necessary copies by cloning? By PCR?
8. Describe a genetic engineering experiment in two or three sentences. Use the following terms: intron, exon, DNA, mRNA, cDNA, RNA polymerase, reverse transcriptase.
9. List at least two examples of the use of genetic engineering in medicine and in agriculture.
10. You are attempting to insert a gene for saltwater tolerance into a plant by using the Ti plasmid. In addition to the desired gene, you add a gene for tetracycline resistance (tet^R) to the plasmid. What is the purpose of the tet^R gene?

MULTIPLE CHOICE

1. Restriction enzymes were first discovered with the observation that
   a. DNA is restricted to the nucleus.
   b. phage DNA is destroyed in a host cell.
   c. foreign DNA is kept out of a cell.
   d. foreign DNA is restricted to the cytoplasm.
   e. all of the above
2. The DNA probe, 3′GGCTTA, will hybridize with DNA containing
   a. 5′CCGUUA.
   b. 5′CCGAAT.
   c. 5′GGCTTA.
   d. 3′CCGAAT.
   e. 3′GGCAAU.
3. Which of the following is the fourth basic step of genetic engineering?
   a. transformation
   b. ligation
   c. plasmid cleavage
   d. restriction-enzyme digestion of gene
   e. isolation of gene
4. The following steps are used to make cDNA. What is the second step?
   a. reverse transcription
   b. RNA processing to remove introns
   c. transcription
   d. translation
5. If you put a gene in a virus, the next step in genetic engineering would be
   a. insertion of a plasmid.
   b. transformation.
   c. transduction.
   d. PCR.
   e. Southern blotting.
6. You have a small gene that you want replicated by PCR. You add radioactively labeled nucleotides to the PCR thermalcycler. After three replication cycles, what percentage of the DNA single-strands are radioactively labeled?
   a. 0%
   b. 12.5%
   c. 50%
   d. 87.5%
   e. 100%

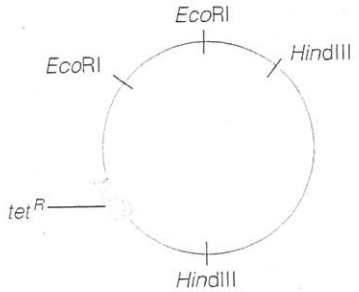
Match the following choices to the statements in questions 7 through 10.

   a. antisense
   b. clone
   c. library
   d. Southern blot
   e. vector

7. Pieces of human DNA stored in yeast cells.
8. A population of cells carrying a desired plasmid.
9. Self-replicating DNA for transmitting a gene from one organism to another.
10. A gene that hybridizes with mRNA.

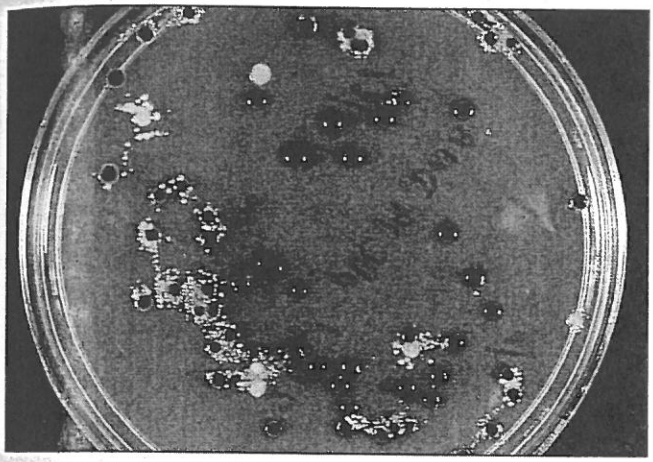
CRITICAL THINKING

1. Using the following map of plasmid pMICRO, give the number of restriction fragments that would result from digesting pMICRO with *Eco*RI, *Hind*III, and both enzymes together. Which enzyme makes the smallest fragment containing the tetracycline-resistance gene?



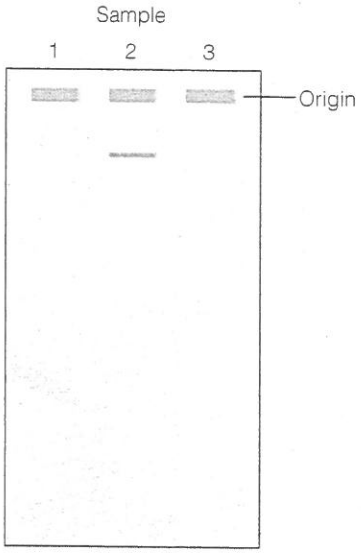
2. Design an experiment using vaccinia virus to make a vaccine against the AIDS virus (HIV).
3. Why did the use of DNA polymerase from the bacterium *Thermus aquaticus* allow researchers to add the necessary reagents to tubes in a preprogrammed heating block?

The following picture shows bacterial colonies growing on X-gal plus ampicillin in a blue-white screening test. Which colonies have the recombinant plasmid? The small satellite colonies do not have the plasmid. Why did they start growing on the medium 48 hours after the larger colonies?

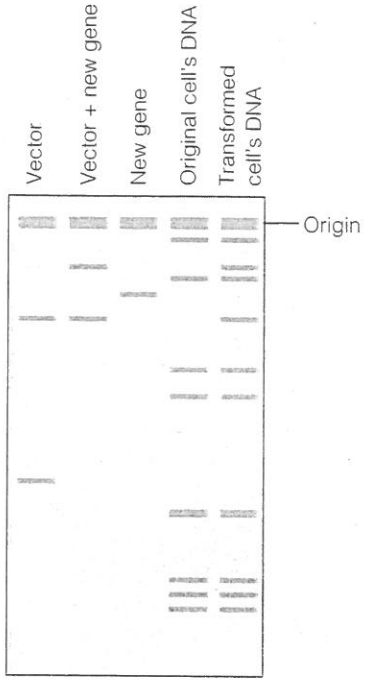


CLINICAL APPLICATIONS

1. PCR has been used to examine oysters for the presence of *Vibrio cholerae*. Oysters from different areas were homogenized, and DNA was extracted from the homogenates. The DNA was digested by the restriction enzyme *Hinc*II. A primer for the hemolysin gene of *V. cholerae* was used for the PCR reaction. After PCR, each sample was electrophoresed and stained with a probe for the hemolysin gene. Which of the oyster samples were (was) positive for *V. cholerae*? How can you tell? Why look for *V. cholerae* in oysters? What is the advantage of PCR over conventional biochemical tests to identify the bacteria?



2. Using the restriction enzyme *Eco*RI, the following gel electrophoresis patterns were obtained from digests of various DNA molecules from a transformation experiment. Can you conclude from these data that transformation occurred? Explain why or why not.



Learning with Technology

MP = The Microbiology Place website ST = Student Tutorial CD-ROM VU = VirtualUnknown CD-ROM

MP Don't forget to go to The Microbiology Place website (http://www.microbiologyplace.com) to take the practice tests, explore the interactive activity and case study, and check out the news articles and web links for this chapter.

ST Remember there is also a quiz for this chapter on the Microbiology Interactive Student Tutorial CD-ROM.

VU Enter the Virtual Lab, click the arrow next to the Session field, click Textbook Exercises, and select Chapter 9. Read the Case Study. Use this unknown for your work on the following problem:

You have been hired by a biotech firm to find new insecticides that are more environmentally friendly. You believe there are safe and effective ways to genetically engineer natural flora of pest insects to produce a recently discovered insecticidal protein. Always looking for potential hosts for the toxic gene, you have called your old classmates. One, an environmental microbiologist working for NASA, sends you this unknown as a candidate. Use the media and the tests in VirtualUnknown™ Microbiology to identify this unknown organism. Would you suggest that this microbe has economic potential for genetic engineering to control pest insects?