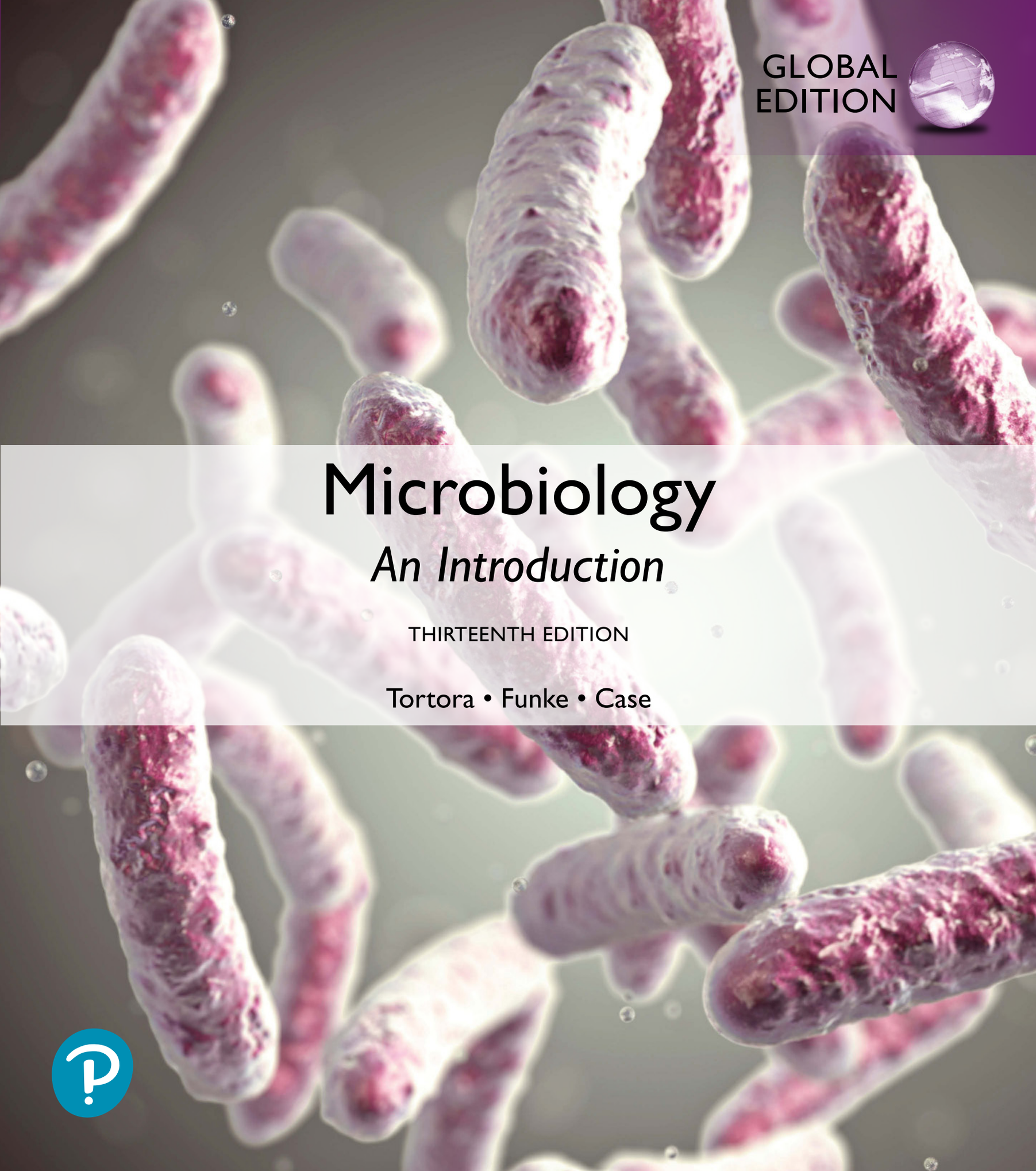


Microbiology

An Introduction

THIRTEENTH EDITION

Tortora • Funke • Case

Brief Contents

AMERICAN SOCIETY FOR MICROBIOLOGY

All chapter content is tagged to ASM Curriculum Guidelines for Undergraduate Microbiology

Exploring the Microbiome

EXPLORING THE MICROBIOME

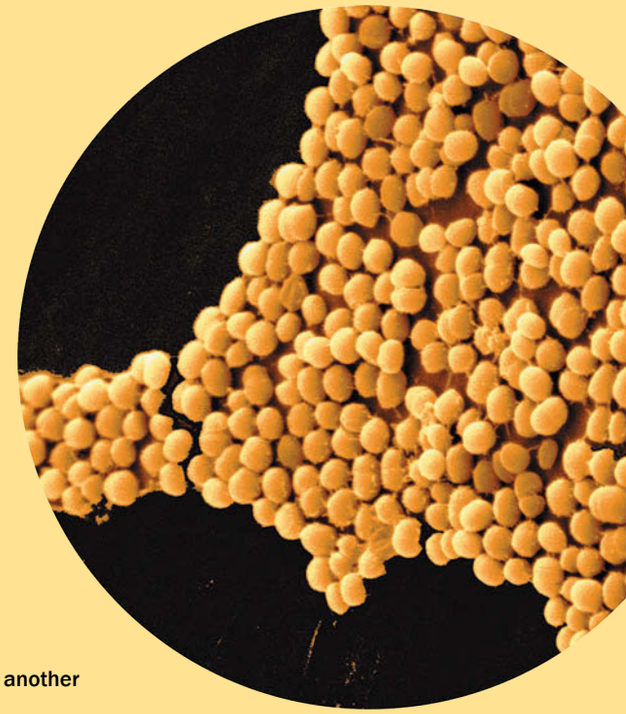
Crime Scene Investigation and Your Microbiome

Fingerprints, blood types, and DNA were once new to crime scene investigations (CSI). Each technique uses unique profiles from the human body to draw conclusions about a person's actions or whereabouts. Now the microbiome might be the next CSI tool.

Even after we wash our hands, certain bacteria persist. These microbes can also be transferred to objects in the home or office or to other people we live with. But which microbes commonly live on the body also varies greatly throughout the population as a whole—meaning that the microbiome can become a unique identifier in certain situations.

A research project called The Home Microbiome Project followed seven families and their pets over 6 weeks. Researchers discovered distinct microbial communities in each house. Couples and their young children shared most of their microbial community. When three of the families moved, it took less than a day for the new house to have the same microbial population as the old one.

In another study, it was shown that a person's "microbiome fingerprint" remains fairly consistent over time. All this research suggests that microbiome composition may be the basis for a reliable forensic tool. Microbiome profiles could be used to track whether a person lived somewhere, used a particular cell phone, or walked over a surface. Humans also exchange microbes during intercourse, so microbes on pubic hair might also provide evidence of sexual assault.



Microbiota, like this skin biofilm, may one day be another crime scene "fingerprint."

causes the formation of a tumorlike growth called a crown gall (Figure 9.19). A part of the Ti plasmid, called T-DNA, integrates into the genome of the infected plant. The T-DNA stimulates local cellular growth (the crown gall) and simultaneously causes the production of certain products used by the bacteria as a source of nutritional carbon and nitrogen.

For plant scientists, the attraction of the Ti plasmid is that it provides a vehicle for introducing rDNA into a plant (Figure 9.20). A scientist can insert foreign genes into the T-DNA, put the recombinant plasmid back into the *Agrobacterium* cell, and use the bacterium to insert the recombinant Ti plasmid into a plant cell. The plant cell with the foreign gene can then be used to generate a new plant. With luck, the new plant will express the foreign gene. Unfortunately, *Agrobacterium* does not naturally infect grasses, so it cannot be used to improve grains such as wheat, rice, or corn.

Noteworthy accomplishments of this approach are the introduction into plants of resistance to the herbicide glyphosate. Normally, the herbicide kills both weeds and useful plants by inhibiting an enzyme necessary for making certain essential amino acids. Some *Salmonella* bacteria happen to have this enzyme, but are resistant to the herbicide. When the DNA for this enzyme is introduced into a crop plant, the crop

Figure 9.19 Crown gall disease on a rose plant. The tumorlike growth is stimulated by a gene on the Ti plasmid that *Agrobacterium tumefaciens* inserted into a plant cell.

Q What are some of the agricultural applications of rDNA technology?

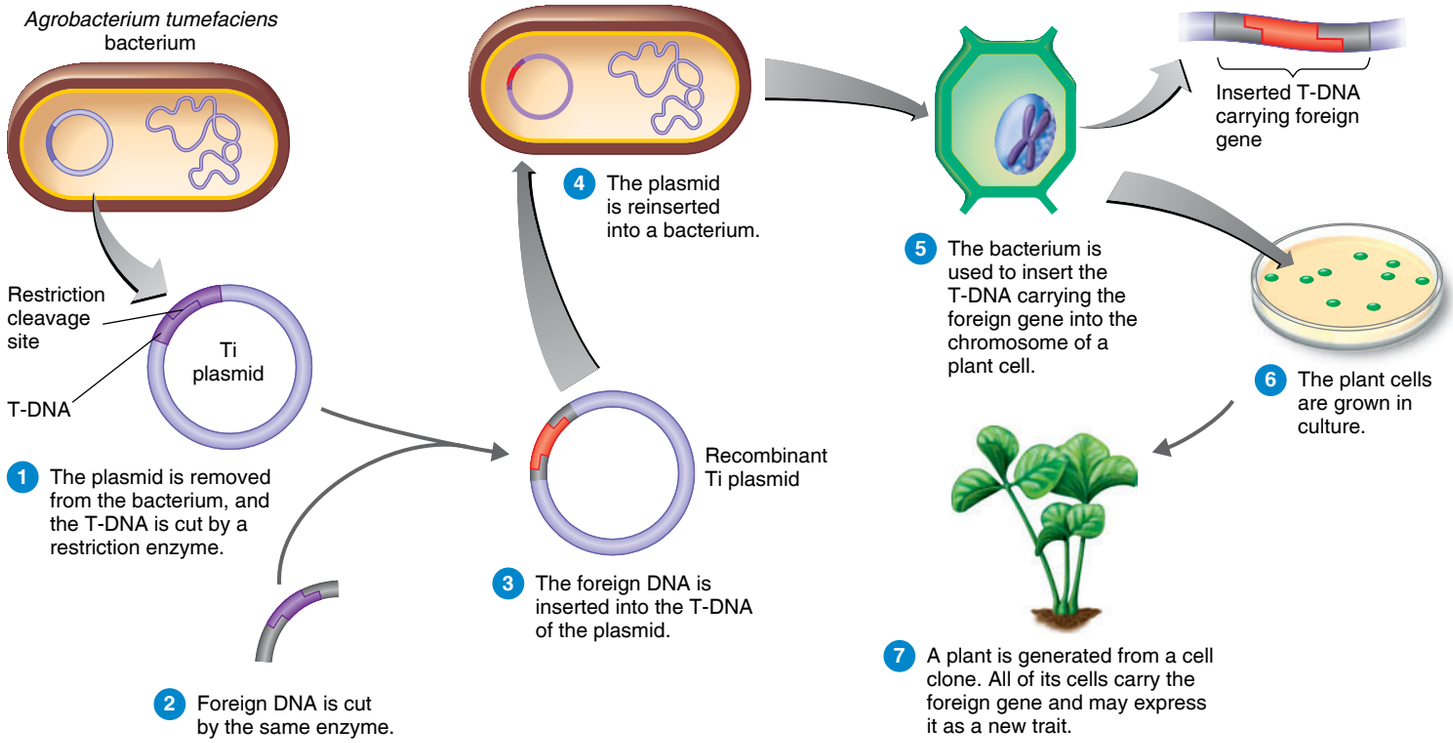


Figure 9.20 Using the Ti plasmid as a vector for genetic modification in plants.

Q Why is the Ti plasmid important to biotechnology?

becomes resistant to the herbicide, which then kills only the weeds. The Bt gene from *Bacillus thuringiensis* has been inserted into a variety of crop plants, including cotton and potatoes, so insects that eat the plants will be killed. Resistance to drought, viral infection, and several other environmental stresses has also been engineered into crop plants.

Another example involves FlavrSavr™ tomatoes, which stay firm after harvest because the gene for polygalacturonase (PG), the enzyme that breaks down pectin, is suppressed. The suppression was accomplished by **antisense DNA** technology. First, a length of DNA complementary to the PG mRNA is synthesized. This antisense DNA is taken up by the cell and binds to the mRNA to inhibit translation. The DNA-RNA hybrid is broken down by the cell's enzymes, freeing the antisense DNA to disable another mRNA.

An example of a genetically modified bacterium now in agricultural use is *Pseudomonas fluorescens* that has been engineered to produce Bt toxin, normally produced by *Bacillus thuringiensis*. The genetically altered *Pseudomonas*, which produces much more toxin than *B. thuringiensis*, can be added to plant seeds and in time will enter the vascular system of the growing plant. Its toxin is ingested by the feeding insect larvae and kills them (but is harmless to humans and other warm-blooded animals).

Animal husbandry has also benefited from rDNA technology to develop disease-resistant food animals. Techniques for making cattle resistant to bovine spongiform encephalopathy and chickens and pigs resistant to avian influenza are currently being researched.

Table 9.3 lists several rDNA products used in agriculture and animal husbandry.

CHECK YOUR UNDERSTANDING

9-19 Of what value is the plant pathogen *Agrobacterium*?

Safety Issues and the Ethics of Using DNA Technology

LEARNING OBJECTIVE

9-20 List the advantages of, and problems associated with, the use of genetic modification techniques.

There will always be concern about the safety of any new technology, and genetic modification and biotechnology are certainly no exceptions. One reason for this concern is it's nearly impossible to prove that something is entirely safe under all conceivable conditions. People worry that the same techniques that can alter a microbe or plant to make them useful to humans could also inadvertently make them pathogenic to

TABLE 9.3 Some Agriculturally Important Products of rDNA Technology

Product	Comments
AGRICULTURAL PRODUCTS	
Button mushroom (*Agaricus bisporus*)	Gene for polyphenyl oxidase, which causes browning, is deleted.
Bt cotton and Bt corn	Plants have toxin-producing gene from *Bacillus thuringiensis*; toxin kills insects that eat plants.
Genetically modified tomatoes, raspberries	Antisense gene blocks pectin degradation, so fruits have longer shelf life.
Pseudomonas syringae, ice-minus bacterium	Lacks normal protein product that initiates undesirable ice formation on plants.
RoundUp (glyphosate)-resistant crops	Plants have bacterial gene; allows use of herbicide on weeds without damaging crops.
ANIMAL PRODUCTS	
Aedes aegypti	Male mosquito with a gene that causes larvae to die; used to control spread of Zika virus.
Atlantic salmon	Salmon grow faster with a gene from Chinook salmon and promoter from another fish (pout).
GloFish®	Brightly colored fluorescent aquarium fish with the color-protein genes from marine invertebrates.

humans or otherwise dangerous to living organisms or could create an ecological nightmare. Therefore, laboratories engaged in rDNA research must meet rigorous standards of control to avoid either accidental release of genetically modified organisms into the environment or exposure of humans to any risk of infection. To reduce risk further, microbiologists engaged in genetic modification often delete from the microbes' genomes certain genes that are essential for growth in environments outside the laboratory. Genetically modified organisms intended for use in the environment (in agriculture, for example) may be engineered to contain "suicide genes"—genes that eventually turn on to produce a toxin that kills the microbes, thus ensuring that they will not survive in the environment for very long after they have accomplished their task.

The safety issues in agricultural biotechnology are similar to those concerning chemical pesticides: toxicity to humans and to nonpest species. Although not shown to be harmful, genetically modified foods have not been popular with consumers. In 1999, researchers in Ohio noticed that humans may develop allergies to *Bacillus thuringiensis* (Bt) toxin after working in fields sprayed with the insecticide. And an Iowa study showed that the caterpillar stage of Monarch butterflies could be killed by ingesting windblown Bt-carrying pollen that landed on milkweed, the caterpillars' normal food. Crop plants can be genetically modified for herbicide resistance so that fields can be sprayed to eliminate weeds without killing the desired crop. However, if the modified plants pollinate related weed species, weeds could become resistant to herbicides, making it more difficult to control unwanted plants. An unanswered question is whether releasing genetically modified organisms will alter evolution as genes move to wild species.

These developing technologies also raise a variety of ethical issues. Genetic testing for diseases is becoming routine. Who should have access to this information? Should employers have the right to know the results of such tests? How can we be assured that such information will not be used to discriminate against certain groups? Should individuals be told they will get an incurable disease? If so, when?

Genetic counseling, which provides advice and counseling to prospective parents with family histories of genetic disease, is becoming more important in considerations about whether to have children.

There are probably just as many harmful applications of a new technology as there are helpful ones. It is particularly easy to imagine DNA technology being used to develop new and powerful biological weapons. In addition, because such research efforts are performed under top-secret conditions, it is virtually impossible for the general public to learn of them.

Perhaps more than most new technologies, molecular genetics holds the promise of affecting human life in previously unimaginable ways. It is important that society and individuals be given every opportunity to understand the potential impact of these new developments.

CLINICAL FOCUS Norovirus—Who Is Responsible for the Outbreak?

As you read through this box, you will encounter a series of questions that microbiologists ask themselves as they trace a disease outbreak. Whether the microbiologist is called as an expert witness in court will depend on whether a lawsuit is filed. Try to answer each question before going on to the next one.

1. On May 7, Nadia Koehler, a microbiologist at a county health department, is notified of a gastroenteritis outbreak among 115 people. The case is defined as vomiting and diarrhea and fever, cramps, or nausea.
   What information does Nadia need?
2. Nadia needs to find out where the ill people have been in the past 48 hours. After several interviews, Nadia finds out that the ill people include 23 school employees, 55 publishing company employees, 9 employees of a social service organization, and 28 other people (see Figure A).
   Now what does Nadia need to know?
3. Next, Nadia finds out what these 115 people have in common. In her investigation, Nadia discovers that on May 2, the school staff had been served a party-sized sandwich catered by a national franchise restaurant. On May 3, the publishing company and social service staff luncheons were catered by the same restaurant. The remaining 28 people ate sandwiches at the same restaurant, at varying times between these two days.
   What does Nadia do next?
4. Nadia analyzes exposures to 16 food items; the results show that eating lettuce is significantly associated with illness.
   What is Nadia's next step?
5. Nadia then requests a reverse-transcription PCR (RT-PCR) using a norovirus primer to be done on stool samples (Figure B).
   What did Nadia conclude?
6. RT-PCR confirmed norovirus infection. Nadia's next request is for a sequence analysis to be performed on 21 stool specimens. The results demonstrated 100% sequence homology for the 21 specimens.
   What should Nadia do next?
7. Nadia learns that a food handler employed by the restaurant had experienced vomiting and diarrhea on May 1. The food handler believes he had acquired the illness from his child. The child's illness was traced to an ill cousin who had been exposed to norovirus at a child-care center. The food handler's vomiting ended by the early morning of May 2, and he returned to work at the restaurant later that morning.
   What should Nadia look for now?
8. Now Nadia compares the virus strains from the food handler to the ones from the ill customers. She requests a sequence analysis on viruses from the food handler and eight ill customers. They are identical to the strains identified in step 6.
   Where does Nadia look next?
9. Nadia looks for any areas in the restaurant that still may be contaminated by the norovirus. She finds out that the lettuce was sliced each morning by the food handler who had been sick. Nadia's inspection reveals that the food preparation sink is also used for handwashing. The sink was not sanitized before and after the lettuce was washed. The health department closes the restaurant until it can be cleaned with the proper sanitizers.

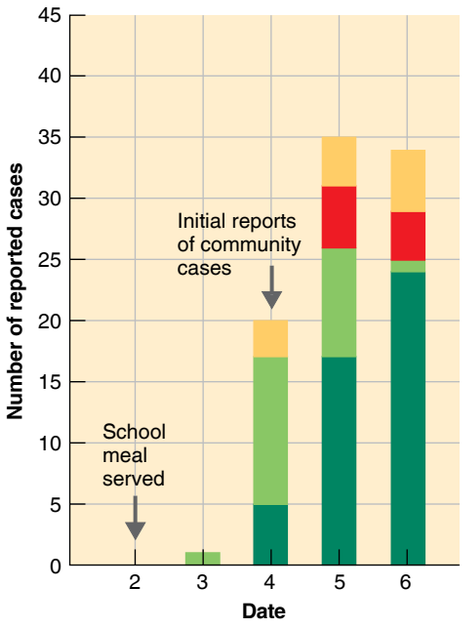


Figure A **Number of cases reported.**

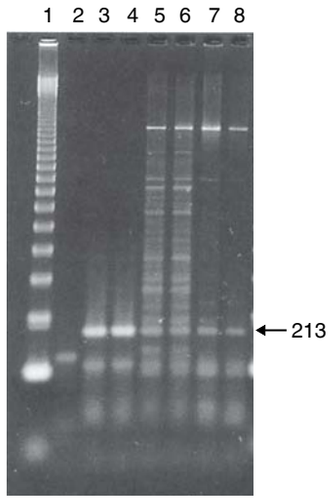


Figure B **Results of PCR of patient samples. Lane 1, 123-bp size ladders. Lane 2, negative RT-PCR control; Lanes 3–8, patient samples. Norovirus is identified by the 213-bp band of DNA.**

Noroviruses are the most common cause of outbreaks of acute gastroenteritis worldwide. Annually, norovirus causes 20 million cases of gastroenteritis. During 2015, 316 norovirus outbreaks in the United States were reported.

Source: Adapted from CDC, Foodborne Outbreak Online Database (FOOD).

Like the invention of the microscope, the development of DNA techniques is causing profound changes in science, agriculture, and human health care. With this technology not quite 50 years old, it is difficult to predict exactly what changes will occur. However, it is likely that within another 30 years, many of the treatments and diagnostic methods discussed in this book will have been replaced by far more powerful techniques based on the unprecedented ability to manipulate DNA precisely.

CHECK YOUR UNDERSTANDING

9-20 Identify two advantages and two problems associated with genetically modified organisms.

Study Outline

Go to @MasteringMicrobiology for **Interactive Microbiology,** *In the Clinic videos, MicroFlix, MicroBoosters, 3D animations, practice quizzes, and more.*

Introduction to Biotechnology (pp. 269–271)

1. Biotechnology is the use of microorganisms, cells, or cell components to make a product.

Recombinant DNA Technology (p. 269)

2. Closely related organisms can exchange genes in natural recombination.
3. Genes can be transferred among unrelated species via laboratory manipulation, called rDNA technology.
4. Recombinant DNA is DNA that has been artificially manipulated to combine genes from two different sources.

An Overview of Recombinant DNA Procedures (pp. 269–271)

5. A desired gene is inserted into a DNA vector, such as a plasmid or a viral genome.
6. The vector inserts the DNA into a new cell, which is grown to form a clone.
7. Large quantities of the gene product can be harvested from the clone.

Tools of Biotechnology (pp. 271–274)

Selection (p. 271)

1. Microbes with desirable traits are selected for culturing by artificial selection.

Mutation (p. 271)

2. Mutagens are used to cause mutations that might result in a microbe with desirable traits.
3. Site-directed mutagenesis is used to change a specific codon in a gene.

Restriction Enzymes (pp. 271–272)

4. Prepackaged kits are available for rDNA techniques.
5. A restriction enzyme recognizes and cuts only one particular nucleotide sequence in DNA.
6. Some restriction enzymes produce sticky ends, short stretches of single-stranded DNA at the ends of the DNA fragments.
7. Fragments of DNA produced by the same restriction enzyme will spontaneously join by base pairing. DNA ligase can covalently link the DNA backbones.

Vectors (pp. 272–273)

8. Vectors are DNA used to transfer other DNA between cells.
9. A plasmid containing a new gene can be inserted into a cell by transformation.
10. A virus containing a new gene can insert the gene into a cell.

Polymerase Chain Reaction (pp. 273–274)

11. The polymerase chain reaction (PCR) is used to make multiple copies of a desired piece of DNA enzymatically.
12. PCR can be used to increase the amounts of DNA in samples to detectable levels. This may allow sequencing of genes, the diagnosis of genetic diseases, or the detection of viruses.

Techniques of Genetic Modification (pp. 274–280)

Inserting Foreign DNA into Cells (pp. 275–276)

1. Cells can take up naked DNA by transformation. Chemical treatments are used to make cells that are not naturally competent take up DNA.
2. Pores made in protoplasts and animal cells by electric current in the process of electroporation can provide entrance for new pieces of DNA.
3. Protoplast fusion is the joining of cells whose cell walls have been removed.
4. Foreign DNA can be introduced into plant cells by shooting DNA-coated particles into the cells or by using a thin micropipette.

Obtaining DNA (pp. 276–278)

5. Genomic libraries can be made by cutting up an entire genome with restriction enzymes and inserting the fragments into bacterial plasmids or phages.
6. Complementary DNA (cDNA) made from mRNA by reverse transcription can be cloned in genomic libraries.
7. Synthetic DNA can be made in vitro by a DNA synthesis machine.

Selecting a Clone (pp. 278–279)

8. Antibiotic-resistance markers on plasmid vectors are used to identify cells containing the engineered vector by direct selection.
9. In blue-white screening, the vector contains the genes for *amp* and β-galactosidase.
10. The desired gene is inserted into the β-galactosidase gene site, destroying the gene.
11. Clones containing the recombinant vector will be resistant to ampicillin and unable to hydrolyze X-gal (white colonies).
12. Clones containing foreign DNA can be tested for the desired gene product.
13. 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. 279–280)

14. *E. coli* is used to produce proteins using rDNA because *E. coli* is easily grown and its genomics are well understood.
15. Efforts must be made to ensure that *E. coli*'s endotoxin does not contaminate a product intended for human use.
16. To recover the product, *E. coli* must be lysed, or the gene must be linked to a gene that produces a naturally secreted protein.
17. Yeasts can be genetically modified and are likely to secrete a gene product continuously.
18. Genetically modified mammalian cells can be grown to produce proteins such as hormones for medical use.
19. Genetically modified plant cells can be grown and used to produce plants with new properties.

Applications of DNA Technology (pp. 280–288)

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

Therapeutic Applications (pp. 281–282)

2. 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.
3. Cells and viruses can be modified to produce a pathogen's surface protein, which can be used as a vaccine.
4. DNA vaccines consist of rDNA cloned in bacteria.
5. Gene therapy can be used to cure genetic diseases by replacing the defective or missing gene.
6. RNAi may be useful to prevent expression of abnormal proteins.

Genome Projects (pp. 282–283)

7. Nucleotide sequences of genomes from more than 1000 organisms, including humans, have been completed.
8. This leads to determining the proteins produced in a cell.

Scientific Applications (pp. 283–286)

9. DNA can be used to increase understanding of DNA, for genetic fingerprinting, and for gene therapy.
10. DNA sequencing machines are used to determine the nucleotide base sequence of restriction fragments in shotgun sequencing.
11. Bioinformatics is the use of computer applications to study genetic data; proteomics is the study of a cell's proteins.
12. Southern blotting can be used to locate a gene in a cell.
13. DNA probes can be used to quickly identify a pathogen in body tissue or food.
14. Forensic microbiologists use DNA fingerprinting to identify the source of bacterial or viral pathogens.
15. Bacteria may be used to make nano-sized materials for nanotechnology machines.

Agricultural Applications (pp. 286–288)

16. 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.
17. Plant cells can be modified by using the Ti plasmid vector. The tumor-producing T genes are replaced with desired genes, and the rDNA is inserted into *Agrobacterium*. The bacterium naturally transforms its plant hosts.
18. Antisense DNA can prevent expression of unwanted proteins.

Safety Issues and the Ethics of Using DNA Technology (pp. 288–291)

1. Strict safety standards are used to avoid the accidental release of genetically modified microorganisms.
2. Some microbes used in rDNA cloning have been altered so that they cannot survive outside the laboratory.
3. Microorganisms intended for use in the environment may be modified to contain suicide genes so that the organisms do not persist in the environment.
4. Genetic testing raises a number of ethical questions: Should employers have access to a person's genetic records? Will genetic information be used to discriminate against people? Will genetic counseling be available to everyone?
5. Genetically modified crops must be safe for consumption and for release in the environment.

Study Questions

For answers to Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Compare and contrast the following terms:
   a. *cDNA* and *gene*
   b. *RFLP* and *gene*
   c. *DNA probe* and *gene*
   d. *DNA polymerase* and *DNA ligase*
   e. *rDNA* and *cDNA*
   f. *genome* and *proteome*
2. Differentiate 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