Chapter 20

DNA Technology and Genomics

Key Concepts

20.1 DNA cloning permits production of multiple copies of a specific gene or other DNA segment

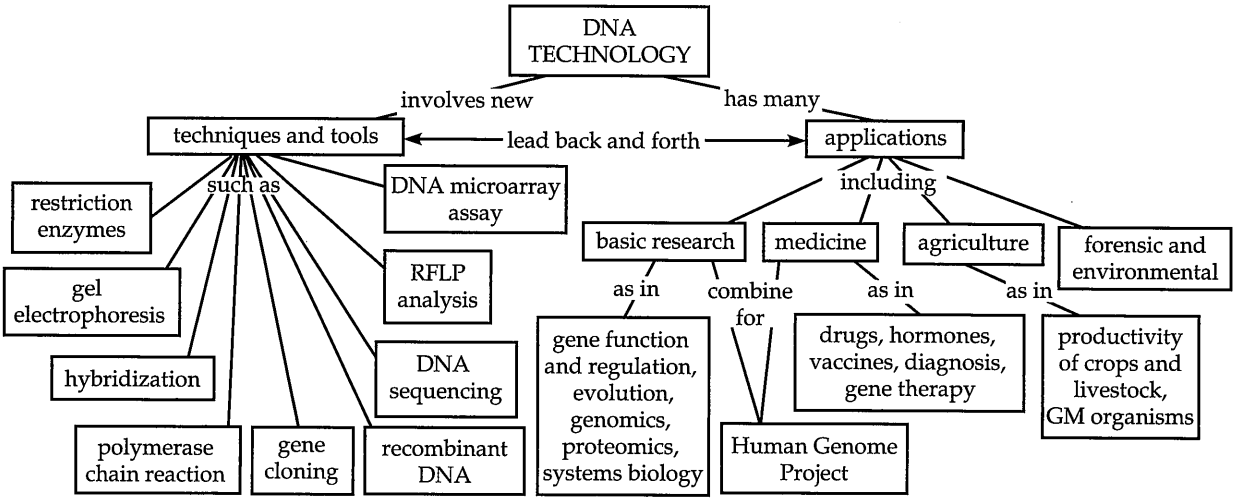
20.2 Restriction fragment analysis detects DNA differences that affect restriction sites

20.3 Entire genomes can be mapped at the DNA level

20.4 Genome sequences provide clues to important biological questions

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

Framework



Chapter Review

DNA technology began with techniques for making **recombinant DNA,** which combines nucleotide sequences from different organisms or species into the same DNA molecule. **Genetic engineering,** the direct manipulation of genetic material for practical purposes, has begun an industrial revolution in **biotechnology.** This use of living organisms or their components to manufacture desirable products dates back centuries, but advances in DNA technology have resulted in hundreds of new products. DNA technology has led to major advances in all fields of biology and in our knowledge of the humane genome.

20.1 DNA cloning permits production of multiple copies of a specific gene or other DNA segment

Techniques for **gene cloning** are used to prepare multiple identical copies of pieces of DNA.

DNA Cloning and Its Applications: **A Preview** One of the approaches used to clone pieces of DNA makes use of the plasmids of bacterial cells. Recombinant DNA may be made by inserting foreign DNA into plasmids. These plasmids are put back into bacterial cells where they will replicate as the *recombinant bacteria* reproduce to form **clones** of identical cells. Such cloned

DNA provides multiple copies of the gene and may also be used to produce protein coded for by the foreign DNA.

Using Restriction Enzymes to Make Recombinant DNA **Restriction enzymes** protect bacteria from the DNA of phages or other bacteria by cutting up foreign DNA in a process called *restriction*. Most restriction enzymes recognize short nucleotide sequences, called **restriction sites,** and cut at specific points within them. The cell protects its own DNA from restriction by methylating nucleotide bases within its own restriction sites.

A restriction site is usually symmetrical, the same sequence of four to eight nucleotides running in opposite directions on the two strands. The most useful restriction enzymes cut phosphodiester bonds in a staggered way, leaving **sticky ends** of short single-stranded sequences on both sides of the resulting **restriction fragment.**

DNA from different sources can be combined in the laboratory when the DNA is cut by the same restriction enzyme and the complementary bases on the sticky ends of the restriction fragments form hydrogen-bonded base pairs. **DNA ligase** is used to seal the strands together.

■ INTERACTIVE QUESTION 20.1

Which of these DNA sequences would most likely function as a restriction site for a restriction enzyme? Why?

··CAGCAG·· ··GTGCTG·· ··GAATTC··

··GTCGTC·· ··CACGAC·· ··CTTAAG··

Cloning a Eukaryotic Gene in a Bacterial Plasmid **Cloning vectors** are DNA molecules that can move foreign DNA into a cell and replicate there. Recombinant plasmids returned to bacterial cells will replicate the foreign DNA as the bacteria reproduce. The ease with which plasmids can be isolated from and returned to bacterial cells makes bacteria the most common host for gene cloning.

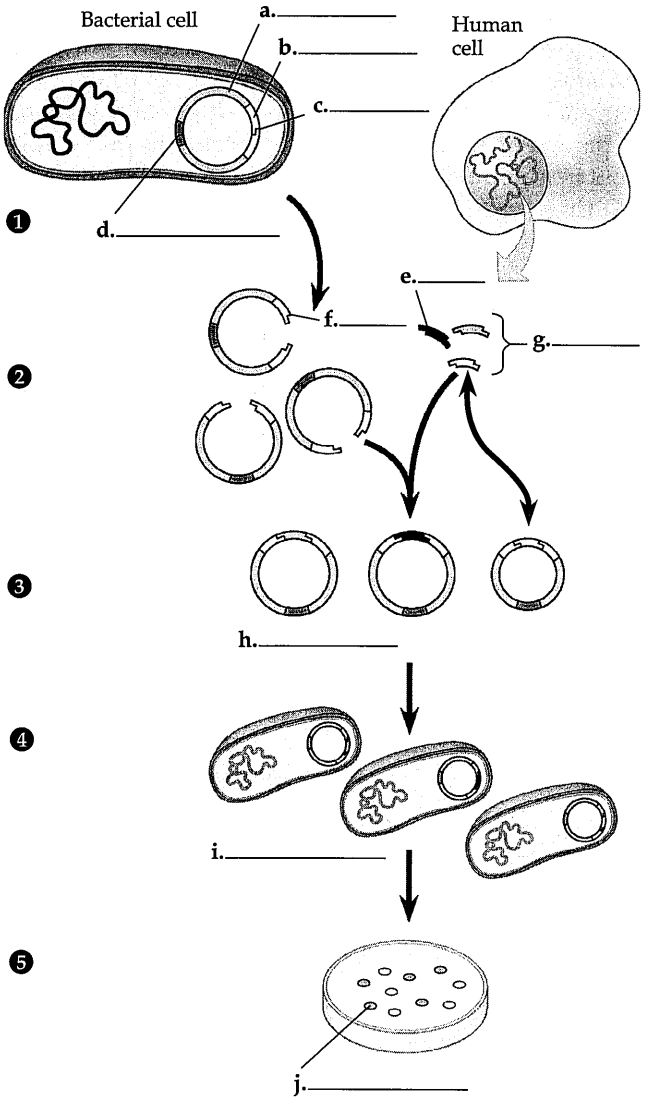
The plasmid method of gene cloning involves treating plasmids containing an antibiotic-resistant gene with a restriction enzyme that cuts the DNA ring at a single restriction site and disrupts a gene whose activity is easily determined, such as *lacZ*, the gene for β-galactosidase. The clipped plasmids are mixed with DNA containing the gene of interest, which has also been treated with the same restriction enzyme, producing many different fragments. The sticky ends form hydrogen bonds with each other, and DNA ligase seals the recombinant molecules.

The plasmids are introduced by transformation into bacterial cells that have a mutation in their *lacZ* gene, and thus are unable to produce β-galactosidase. Bacteria are plated onto a medium containing ampicillin and X-gal, a compound that is cleaved by β-galactosidase and yields a blue product. Colonies that are able to grow on the medium (because they contain a plasmid with the amp^R gene) and are not blue (because the foreign DNA inserted in the middle of the β-galactosidase gene) are carrying a recombinant plasmid.

The colonies are tested to find the ones that contain the gene of interest. One technique detects the gene itself by **nucleic acid hybridization,** using a **nucleic acid probe** that has complementary sequences to segments of the gene. The probe hybridizes with the DNA of the gene after **denaturation** of the cell's DNA by heat or chemicals produces single-stranded DNA. The probe is located by its radioactively labeled molecules or fluorescent tag. Once the desired clone is identified, it can be grown in liquid culture and the gene of interest isolated in large quantities. (Complete Interactive Question 20.2 to review plasmid cloning.)

■ INTERACTIVE QUESTION 20.2

This schematic diagram shows the steps in plasmid cloning of a gene. Identify components a–j. Briefly describe the five steps of the process. How are bacterial clones that have picked up the recombinant plasmid identified?



Storing Cloned Genes in DNA Libraries An organism's DNA can be cut into thousands of pieces with restriction enzymes and inserted into plasmids. The collection of the thousands of clones of bacteria containing recombinant plasmids derived from this "shotgun" approach is called a **genomic library.**

Bacteriophages are also used as vectors for creating genomic libraries. DNA fragments are spliced into phage DNA, which is packaged into capsids and used to infect bacteria. The production of new phage clones the foreign DNA.

A partial genomic library can be produced using the mRNA molecules isolated from a cell. Using reverse transcriptase from retroviruses, mRNA is used as a template to produce **complementary DNA (cDNA).** Restriction sites are added to the ends and the cDNA is inserted into vector DNA. Such a **cDNA library** contains only the genes that are expressed (transcribed) within the cell.

■ INTERACTIVE QUESTION 20.3

Compare genomic and cDNA libraries with regard to their advantages and disadvantages.

Cloning and Expressing Eukaryotic Genes Clones carrying a particular gene can also be identified based on the presence of its protein product, either by detecting enzyme activity or by antibody binding.

Differences between prokaryotic and eukaryotic mechanisms for gene expression can be overcome by using an **expression vector,** a cloning vector that has an active prokaryotic promoter just upstream from the eukaryotic gene insertion site. Using a cDNA gene removes the problem of long introns, especially since bacteria do not have RNA-splicing machinery.

Yeast cells are eukaryotic hosts used to clone eukaryotic genes; they are easy to grow and have plasmids that serve as vectors. **Yeast artificial chromosomes (YACs)** have also been constructed that carry foreign DNA and contain an origin for DNA replication, a centromere, and two telomeres. These vectors behave normally in mitosis and are able to clone large pieces of DNA.

Plant and animal cells in culture can serve as hosts and may be necessary when a protein must be modified following translation.

More efficient means for introducing DNA into eukaryotic cells include **electroporation,** in which an electric pulse briefly opens holes in the plasma membrane through which DNA can enter, injection into cells using microscopically thin needles, or using the bacterium *Agrobacterium* to introduce DNA into plant cells.

Amplifying DNA **in Vitro:** *The Polymerase Chain Reaction (PCR)* The **polymerase chain reaction (PCR)** can produce billions of copies of a section of DNA *in vitro* in only a few hours. DNA containing the region of interest is incubated with the four nucleotides, a special heat-resistant type of DNA polymerase, and specially synthesized primers that bind upstream from the target sequence. The solution is heated to separate the DNA strands then cooled so the

primers can anneal (hydrogen-bond) to complementary sequences. DNA polymerase adds nucleotides to the 3′ ends of the primers. The solution is heated again and the process repeated. The desired DNA segment does not need to be purified from the starting material, and very small samples can be used.

■ INTERACTIVE QUESTION 20.4

a. Why is PCR often used prior to cloning a gene in cells?

b. Why even bother cloning genes in cells, since PCR produces so many copies so fast?

20.2 Restriction fragment analysis detects DNA differences that affect restriction sites

Producing cloned segments of DNA allows scientists to study the expression of particular genes and to make comparisons between the genes of different cells, individuals, and species.

Gel Electrophoresis and Southern Blotting Many of the methods for analyzing and comparing DNA make use of **gel electrophoresis,** a technique that separates nucleic acids an0d proteins on the basis of their size and electrical charge. Due to the negative charge of their phosphate groups, DNA molecules migrate through the electric field produced in a thin slab of gel toward the positive electrode. Linear molecules of DNA move at a rate inversely proportional to their length, producing band patterns in the gel of fragments of decreasing size.

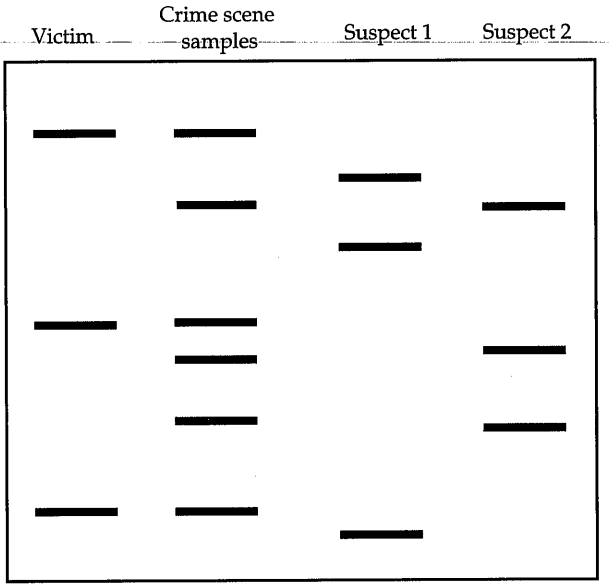
Cutting a long DNA molecule with a particular restriction enzyme and separating the resulting restriction fragments by gel electrophoresis produces a characteristic pattern of bands, a process called restriction fragment analysis. Pure samples of such bands can be recovered from the gel and retain their biological activity.

Two DNA samples, such as alleles of a gene, will produce different patterns of bands when differences in their DNA sequences add or delete restriction sites. The addition of nucleic acid hybridization with a probe allows two or more unpurified samples of DNA (such as the entire genome) to be compared for the presence and band location of a particular DNA sequence. In a technique known as **Southern blotting,** DNA is treated with a restriction enzyme; the fragments are separated on a gel and then transferred to nitrocellulose paper by blotting; labeled probes of single-stranded DNA are added and hybridize with complementary DNA sequences; and the restriction fragment bands of interest are identified by autoradiography.

Restriction Fragment Length Differences as Genetic Markers Samples of noncoding DNA treated with restriction enzymes also produce different band patterns due to differences in nucleotide sequences in restriction sites. Such **restriction fragment length polymorphisms (RFLPs)** can serve as genetic markers on a chromosome and can be used in making linkage maps. A particular RFLP marker often occurs in many variants in a population. RFLPs are detected by Southern blotting, and the entire genome can be used as the DNA starting material.

■ INTERACTIVE QUESTION 20.5

A bloody crime has occurred. Police have collected blood samples from the victim, two suspects, and blood found at the scene. Briefly list the steps the lab went through to produce the following autoradiograph.



a.

b.

c.

d.

e.

Which suspect would you charge with the crime?

20.3 Entire genomes can be mapped at the DNA level

The international effort to map the human genome, called the **Human Genome Project,** was largely completed in 2003. It included three stages: genetic (linkage) mapping, physical mapping, and DNA sequencing. The project also included the mapping of important research organisms, such as *E. coli,* yeast, the nematode *C. elegans, Drosophila,* and *Mus musculus* (mouse).

Genetic (Linkage) Mapping: Relative Ordering of Markers Cytogenetic maps based on banding patterns and fluorescence *in situ* hybridization (FISH) provided the basis for mapping chromosomes. The first stage in mapping a large genome is to develop a **linkage map** of several thousand genetic markers, either genes or other identifiable sequences such as RFLPs or simple sequences repeats. Determining recombination frequencies with these known markers enabled researchers to order genes and locate other markers.

Physical Mapping: Ordering DNA Fragments In a **physical map,** the actual distances between markers are determined. The DNA of each chromosome is cut into restriction fragments, and their order on the chromosome is determined. First large fragments are cut, cloned, and ordered. Then those fragments are cut and their fragments ordered. Finally, fragments are cut, cloned, and ordered that are small enough to be sequenced.

Cloning vectors include yeast artificial chromosomes, which can carry long fragments, and **bacterial artificial chromosomes (BACs),** artificial bacterial chromosomes that carry shorter inserts.

DNA Sequencing Pure preparations of DNA fragments of about 800 base pairs are ultimately used to determine the nucleotide sequence of an entire genome. In the *dideoxyribonucleotide chain-termination method* of DNA sequencing, developed by F. Sanger, a sample of a denatured DNA fragment is incubated with a primer, DNA polymerase, the four deoxyribonucleotides, and the four modified nucleotides (dideoxyribonucleotides) that randomly block further synthesis when they are incorporated into a growing DNA strand. The sets of fluorescently tagged strands of varying lengths are separated through a polyacrylamide gel in a capillary tube. A fluorescence detector reads the sequence of their colors, which corresponds to the nucleotide sequence of the fragment.

Faster sequencing machines and better computer software were developed during the Human Genome Project. J. C. Venter, founder of the company Celera Genomics, developed a whole-genome shotgun approach that relies on powerful computer programs to order the large number of sequenced short sequences cut from a chromosome. In February 2001, Celera (as well as the public consortium) announced that more than 90% of the human genome had been sequenced. Although a few gaps remain, sequencing of the human genome is now basically complete.

■ INTERACTIVE QUESTION 20.6

Compare the strategies of the public consortium of the Human Genome Project and of Celera Genomics for mapping a genome.

20.4 Genome sequences provide clues to important biological questions

Genomics, the study of whole sets of genes and their interactions, is allowing researchers to address questions about gene expression, growth and development, and evolution.

Identifying Protein-Coding Genes in DNA Sequences Computer data banks of DNA sequences are available to researchers on the Internet. Computer software can scan these sequences for signs of genes, such as start and stop signals; RNA-splicing sites; and *expressed sequence tags,* or *ESTs* (sequences similar to those of known genes).

The number of putative genes revealed by the analysis of the human genome is surprisingly small, perhaps only 25,000. Large amounts of noncoding DNA (in particular, repetitive DNA) and unusually long introns make up the majority of the human genome. Alternative splicing of exons, which creates different polypeptides from the same gene, and greater polypeptide diversity, as well as more complicated control of gene expression, may help explain how this relatively small number of genes can produce the complexity of humans (and vertebrates in general).

The sequences of new gene candidates are compared with sequences of known genes of that species and other species to look for similarities that might indicate the function of the new gene. Many of the putative genes identified so far have never been encountered before.

Determining Gene Function The function of unknown genes can be studied using ***in vitro*** **mutagenesis,** in which changes are made to a cloned gene, the

gene is returned to the cell, and changes in physiology or developmental patterns that result from the altered gene product are monitored. A new way to stop the expression of selected genes in cells is called **RNA interference (RNAi).** Synthetic double-stranded RNA molecules that match a gene sequence trigger the breakdown or block translation of that gene's mRNA. RNAi was used in one study to silence 86% of the genes of nematode embryos, one at a time.

Studying Expression of Interacting Groups of Genes In order to study patterns of gene expression, researchers isolate the mRNA made in different cells, create a cDNA library using reverse transcriptase, and then use the cDNA as probes to explore collections of genomic DNA. Using this cDNA in **DNA microarray assays,** scientists can test all the genes expressed in a tissue for hybridization with short, single-stranded DNA fragments from thousands of genes arrayed on a grid (called a DNA chip). The intensity with which the hybridized spots fluoresce indicates the relative amount of mRNA that was in the tissue. Gene expression in different tissues and at different stages of development can be compared.

Comparing Genomes of Different Species Genomes of about 150 species had been sequenced by the spring of 2004, the majority of these from prokaryotes. Analyses of genome sequences are used to explore evolutionary relationships between both distantly and closely related groups, as well as to answer questions about gene functions.

■ INTERACTIVE QUESTION 20.7

a. Give an example of how comparisons with the yeast genome have helped illuminate the human genome.

b. When sequencing the genomes of two closely related species, why is it easier to sequence the smaller genome first?

Future Directions of Genomics **Proteomics** is the challenging identification and study of entire protein sets coded for by a genome. In humans, the number of proteins greatly exceeds the number of genes. To understand how cells and organisms function requires the study of how proteins interact.

With lists compiled of the parts, researchers are now looking at the functional integration of these parts in biological systems. This systems biology approach is attempting to define gene circuits and protein interaction networks, using computer science and mathematics to process and integrate huge amounts of data.

The human species is comparatively young, and its genetic variation is small. Human DNA sequences are about 99.9% identical. Most variation seems to be one-base-pair variations called **single nucleotide polymorphisms (SNPs),** occurring at about 3 million sites in the human genome. Identifying these sites will provide genetic markers for studying human evolution and differences between human populations, as well as for locating health-related genes.

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

Medical Applications DNA technology is identifying genes responsible for genetic diseases; it is hoped this will lead to new ways to diagnose, treat, and even prevent those disorders. DNA microarray assays allow comparisons of gene expression in healthy and diseased tissues.

PCR and labeled DNA probes are being used to identify pathogens and to diagnose infectious diseases and genetic disorders. Even if a gene has not yet been cloned, a disease allele may be diagnosed when closely linked with an RFLP marker.

Gene therapy may provide the means for correcting genetic disorders in individuals by replacing or supplementing defective genes. New genes would be introduced into somatic cells of the affected tissue. For the correction to be permanent, the cells must be types that actively reproduce within the body, such as bone marrow cells. To date, a few trials have used retroviral vectors to carry a normal allele into bone marrow cells, but results have been mixed.

■ INTERACTIVE QUESTION 20.8

a. Why is it easier to perform a test for Huntington's disease now that the gene has been cloned?

b. What are some of the practical and ethical considerations in human gene therapy?

Pharmaceutical Products Many pharmaceutical proteins are produced using DNA technology. Engineering host cells to secrete a protein as it is made simplifies its purification.

New approaches to disease treatments include genetically engineered proteins that either block or mimic membrane receptor proteins. Recombinant DNA techniques can been used to make vaccines that consist of surface molecules on pathogens, or to modify the genome of a pathogen so as to attenuate it (make it nonpathogenic) so it can be used as a vaccine.

Forensic Evidence RFLP analysis can be used in criminal cases to compare the **DNA fingerprint,** or specific pattern of RFLP bands, of a victim, suspect, and crime sample. Variations in the number of tandem repeated base sequences *(simple tandem repeats,* or *STRs)* found in various loci are now commonly used in DNA fingerprint analysis. Forensic tests are able to provide a high statistical probability that matching DNA fingerprints come from the same individual.

Environmental Cleanup Genetically engineered microorganisms that are able to extract heavy metals, such as copper, lead, and nickel, may become important in mining and cleaning up mining waste. Engineering organisms to degrade chlorinated hydrocarbons and other toxic compounds is an area of active research. Environmental disasters such as oil spills and waste dumps are other areas for which detoxifying microbes are being developed.

Agricultural Applications Vaccines and growth hormones for use in farm animals are being produced with DNA technology.

Transgenic animals containing genes from other organisms are being developed for potential agricultural use. In addition to increasing productivity, another use is as "pharm" animals engineered to produce large quantities of a pharmaceutical product, often by secretion in the animal's milk. Transgenic animals are produced by injecting a foreign gene into egg cells fertilized *in vitro.* The eggs are then transplanted into surrogate mothers.

The ability to regenerate plants from single cells growing in tissue culture has made plant cells easier to genetically manipulate than animal cells. The most commonly used vector is the **Ti plasmid** from the bacterium *Agrobacterium tumefaciens,* which integrates a segment of its DNA into plant chromosomes. Using DNA technology, foreign genes are inserted into Ti plasmids whose disease-causing properties have been eliminated, and the recombinant plasmids are introduced into plant cells growing in culture. When these cells regenerate whole plants, the foreign gene is included in the plant genome.

Many crops have been engineered with bacterial genes for herbicide resistance. Crop plants are being engineered to be resistant to infectious pathogens and insects. Research efforts are also focusing on producing more nutritional transgenic crops, as in the "golden" rice enriched with beta-carotene.

The pharmaceutical industry is working on pharm plants that can produce human proteins for medical use and viral proteins for vaccines.

Safety and Ethical Questions Raised by DNA Technology Safety regulations have focused on potential hazards of engineered microbes. Most public concern, however, now centers on agricultural **genetically modified (GM) organisms,** which contain artificially acquired genes from the same or different species. One environmental risk of transgenic plants is that their herbicide- or insect-resistant genes might pass to wild plants, creating "superweeds." Some fear that GM foodstuffs may be hazardous to human health. An international Biosafety Protocol requires exporters to label GM organisms in bulk food shipments so that importing countries can decide on potential environmental or health risks. In the United States, several federal agencies evaluate and regulate new products and procedures.

Ethical questions about human genetic information include who should have access to information about a person's genome and how that information should be used. Potential ethical, environmental, and health issues must be considered in the development of these powerful genetic techniques and remarkable products of biotechnology.

Word Roots

liga- = bound, tied (*DNA ligase:* a linking enzyme essential for DNA replication)
electro- = electricity (*electroporation:* a technique to introduce recombinant DNA into cells by applying a brief electrical pulse to a solution containing cells)
muta- = change; **-genesis** = origin, birth (*in vitro mutagenesis:* a technique to discover the function of a gene by introducing specific changes into the sequence of a cloned gene, reinserting the mutated gene into a cell, and studying the phenotype of the mutant)
poly- = many; **morph-** = form (*Single nucleotide polymorphisms:* one-base-pair variations in the genome sequence)

Structure Your Knowledge

1. Fill in the table below on the basic tools of gene manipulation used in DNA technology.

Technique or Tool	Brief Description	Some Uses in DNA Technology
Restriction enzymes	**a.**	
Gel electrophoresis	**b.**	
cDNA	**c.**	
Labeled probe	**d.**	
Southern blotting	**e.**	
DNA sequencing	**f.**	
PCR	**g.**	
RFLP analysis	**h.**	
DNA microarray assay	**i.**	

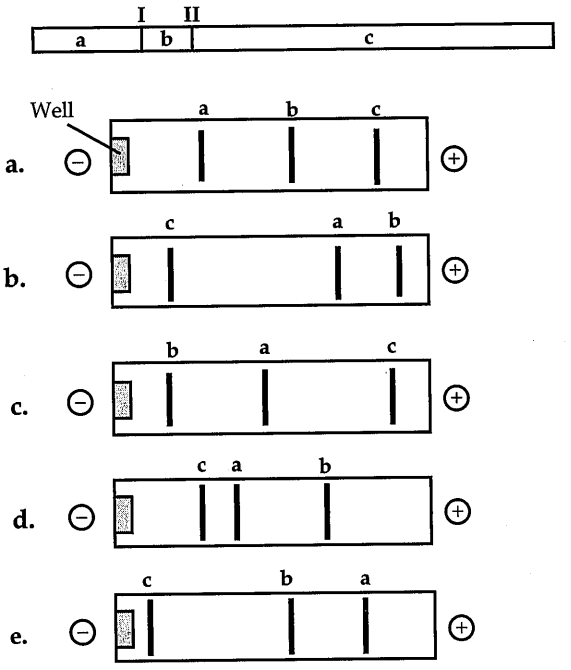
2. Describe several examples of the many possible applications for DNA technology in agriculture and in medicine.

Test Your Knowledge

MULTIPLE CHOICE: *Choose the one best answer.*

1. The role of restriction enzymes in DNA technology is to
   - **a.** provide a vector for the transfer of recombinant DNA.
   - **b.** produce cDNA from mRNA.
   - **c.** produce a cut (usually staggered) at specific restriction sites on DNA.
   - **d.** reseal "sticky ends" after base pairing of complementary bases.
   - **e.** denature DNA into single strands that can hybridize with complementary sequences.

2. The segment of DNA shown at right has restriction sites I and II, which create restriction fragments a, b, and c. Which of the following gels produced by electrophoresis would represent the separation and identity of these fragments?



3. Yeast has become important in genetic engineering because it
   - **a.** has RNA splicing machinery.
   - **b.** has plasmids that can be genetically engineered.
   - **c.** allows the study of eukaryotic gene regulation and expression.
   - **d.** grows readily and rapidly in the laboratory.
   - **e.** does all of the above.

4. Which of the following DNA sequences would most likely be a restriction site?
   a. AACCGG
TTGGCC
   b. GGTTGG
CCAACC
   c. AAGG
TTCC
   d. AATTCCGG
TTAAGGCC
   e. GAATTC
CTTAAG

5. What is genomics?
   a. the public consortium effort to sequence the human genome
   b. the Celera shotgun approach to sequencing the human genome
   c. the sequencing and systematic study of whole genomes
   d. the use of gene therapy in the treatment of human diseases
   e. the use of nucleotide sequences to determine the function of all proteins encoded by a genome

6. Petroleum-lysing bacteria are being engineered for the treatment of oil spills. What is the most realistic danger of these bacteria to the environment?
   a. mutations leading to the production of a strain pathogenic to humans
   b. extinction of natural microbes due to the competitive advantage of the "petro-bacterium"
   c. destruction of natural oil deposits
   d. poisoning of the food chain
   e. contamination of the water

7. You are attempting to introduce a gene that imparts larval moth resistance to bean plants. Which of the following vectors are you most likely to use?
   a. phage DNA
   b. *E. coli* plasmid
   c. Ti plasmid
   d. yeast plasmid
   e. bacterial artificial chromosome

8. An attenuated virus
   a. is a virus that is nonpathogenic.
   b. is an elongated viral particle.
   c. can transfer recombinant DNA to other viruses.
   d. will not produce an immune response.
   e. is made with cDNA.

9. Which of the following is a difficulty in getting prokaryotic cells to express eukaryotic genes?
   a. The signals that control gene expression are different and prokaryotic promoter regions must be added to the vector.
   b. The genetic code differs because prokaryotes substitute the base uracil for thymine.
   c. Prokaryotic cells cannot transcribe introns because their genes do not have them.
   d. The ribosomes of prokaryotes are not large enough to handle long eukaryotic genes.
   e. The RNA splicing enzymes of bacteria work differently from those of eukaryotes.

10. Complementary DNA does not create as complete a library of genes as the shotgun approach because
    a. it has eliminated introns from the genes.
    b. a cell produces mRNA for only a small portion of its genes.
    c. the shotgun approach produces more restriction fragments.
    d. cDNA is not as easily integrated into plasmids.
    e. restriction enzymes are not used to create cDNA.

11. Which of the following is *not* true of restriction sites?
    a. Modification by methylation of bases within them prevents restriction of bacterial DNA.
    b. They are usually symmetrical sequences of four to eight nucleotides.
    c. They signal the attachment of RNA polymerase.
    d. Each restriction site is cut by a specific restriction enzyme.
    e. Cutting a restriction site in the middle of a functional and identifiable gene is used to screen clones that have taken up foreign DNA.

Use the following choices to answer questions 12–14.

   a. restriction enzyme
   b. reverse transcriptase
   c. ligase
   d. DNA polymerase
   e. RNA polymerase

12. Which is the first enzyme used in the production of cDNA?

13. Which enzyme is used in the polymerase chain reaction?

14. Which is the first enzyme used in the production of DNA fragments for DNA fingerprinting?

15. A plasmid has two antibiotic resistance genes, one for ampicillin and one for tetracycline. It is treated with a restriction enzyme that cuts in the middle of the ampicillin gene. DNA fragments containing a human globin gene were cut with the same enzyme. The plasmids and fragments are mixed, treated with ligase, and used to transform bacterial cells. Clones that have taken up the recombinant DNA are the ones that
    a. are blue and can grow on plates with both antibiotics.
    b. can grow on plates with ampicillin but not with tetracycline.
    c. can grow on plates with tetracycline but not with ampicillin.
    d. cannot grow with any antibiotics.
    e. can grow on plates with tetracycline and are blue.

16. STRs (simple tandem repeats) are a valuable tool for
    a. DNA microarray assays.
    b. infecting plant cells with recombinant DNA.
    c. acting as probes in Southern blots.
    d. DNA fingerprinting.
    e. PCR to produce multiple copies of a DNA segment.

17. You have affixed the chromosomes from a cell onto a microscope slide. Which of the following would *not* make a good radioactively labeled probe to help map a particular gene to one of those chromosomes? (Assume DNA of chromosomes and probes is single stranded.)
    a. cDNA made from the mRNA transcribed from the gene
    b. a portion of the amino acid sequence of that protein
    c. mRNA transcribed from the gene
    d. a piece of the restriction fragment on which the gene is located
    e. a sequence of nucleotide bases determined from the genetic code needed to produce a known sequence of amino acids found in the protein product of the gene

18. If the first three nucleotides in a six-nucleotide restriction site are CTG, what would the next three nucleotides most likely be?
    a. AGG
    b. GTC
    c. CTG
    d. CAG
    e. GAC

19. Computer software is used to identify putative genes in the nucleotide sequences in data banks. Which of the following is the software probably *not* scanning for?
    a. promoters
    b. RNA-splicing sites
    c. STRs (simple tandem repeats)
    d. ESTs (expressed sequence tags)
    e. stop signals for transcription

20. The human genome appears to have only one-third more genes than the simple nematode, *C. elegans*. Which of the following best explains how the more complex humans can have relatively few genes?
    a. The unusually long introns in human genes are involved in regulation of gene expression.
    b. More than one polypeptide can be produced from a gene by alternative splicing.
    c. Human genes code for many more types of domains.
    d. The human genome has a high proportion of noncoding DNA.
    e. The large number of SNPs (single nucleotide polymorphisms) in the human genome provides for a great deal of genetic variability.

21. What is a "pharm" animal?
    a. a transgenic animal that produces large quantities of a pharmaceutical product
    b. an animal used by the pharmaceutical industry to test new medical treatments
    c. a cloned animal that was produced from an adult cell nucleus inserted into an ovum
    d. a genetically engineered animal that produces more meat or milk
    e. a genetically modified organism whose production is permitted in the United States but not in the European Union

22. Which of the following processes or procedures does *not* involve any nucleic acid hybridization?
    a. separation of fragments by gel electrophoresis
    b. Southern blotting
    c. polymerase chain reaction
    d. DNA fingerprinting
    e. DNA microarray assay

23. Which of the following genomes has been completely (or almost completely) sequenced?
    a. nematode *(C. elegans)*
    b. human
    c. *E. coli* and yeast *(Saccharomyces cerevisiae)*
    d. fruit fly *(Drosophila melanogaster)*
    e. all of the above

24. Which of the following techniques can be used to determine the function of a newly identified gene?
    a. comparisons with genes of known functions that have similar sequences and whose products have similar domains
    b. *in vitro* mutagenesis or RNA interference
    c. DNA microarray assay
    d. both a and b
    e. a, b, and c

25. This restriction fragment contains a gene whose recessive allele is lethal. The normal allele has restriction sites for the restriction enzyme *PST*I at sites I and II. The recessive allele lacks restriction site I. An individual who had a sister with the lethal trait is being tested to determine if he is a carrier of that allele. Indicate which of these band patterns would be produced on a gel if he is a carrier (*heterozygous* for the gene)?