

CHAPTER 20

DNA TECHNOLOGY AND GENOMICS

I. Introduction

- A. The mapping and sequencing of the human genome has been made possible by advances in DNA technology.
- B. Progress began with the development of techniques for making **recombinant DNA**, in which genes from two different sources - often different species - are combined *in vitro* into the same molecule.
- C. These methods form part of **genetic engineering**, the direct manipulation of genes for practical purposes.
 - 1. Applications include the introduction of a desired gene into the DNA of a host that will produce the desired protein.
- D. DNA technology has launched a revolution in **biotechnology**, the manipulation of organisms or their components to make useful products.
 - 1. Practices that go back centuries, such as the use of microbes to make wine and cheese and the selective breeding of livestock, are examples of biotechnology.
 - 2. Biotechnology based on the manipulation of DNA *in vitro* differs from earlier practices by enabling scientists to modify specific genes and move them between organisms as distinct as bacteria, plants, and animals.
- E. DNA technology is now applied in areas ranging from agriculture to criminal law, but its most important achievements are in basic research.

II. DNA Cloning

- 1. To study a particular gene, scientists needed to develop methods to isolate only the small, well-defined, portion of a chromosome containing the gene.
 - 2. Techniques for **gene cloning** enable scientists to prepare multiple identical copies of gene-sized pieces of DNA.
- B. DNA technology makes it possible to clone genes for basic research and commercial applications: *an overview*
- C. Most methods for cloning pieces of DNA share certain general features.
 - 1. For example, a foreign gene is inserted into a bacterial plasmid and this recombinant DNA molecule is returned to a bacterial cell.
 - 2. Every time this cell reproduces, the recombinant plasmid is replicated as well and passed on to its descendants.
 - 3. Under suitable conditions, the bacterial clone will make the protein encoded by the foreign gene.
 - 4. One basic cloning technique begins with the insertion of a foreign gene into a bacterial plasmid.
- D. The potential uses of cloned genes fall into two general categories.
 - 1. First, the goal may be to produce a protein product.
 - a) *For example, bacteria carrying the gene for human growth hormone can produce large quantities of the hormone for treating stunted growth.*
 - 2. Alternatively, the goal may be to prepare many copies of the gene itself.

- a) *This may enable scientists to determine the gene's nucleotide sequence or provide an organism with a new metabolic capability by transferring a gene from another organism.*

E. Restriction enzymes are used to make recombinant DNA

- Gene cloning and genetic engineering were made possible by the discovery of **restriction enzymes** that cut DNA molecules at specific locations.
- In nature, bacteria use restriction enzymes to cut foreign DNA, such as from phages or other bacteria.
- Most restriction enzymes are very specific, recognizing short DNA nucleotide sequences and cutting at specific point in these sequences.
 - Bacteria protect their own DNA by methylation.*
- Each restriction enzyme cleaves a specific sequence of bases or **restriction site**.
 - These are often a symmetrical series of four to eight bases on both strands running in opposite directions.*
If the restriction site on one strand is 3'-CTTAAG-5', the complementary strand is 5'-GAATTC-3'.
- Because the target sequence usually occurs (by chance) many times on a long DNA molecule, an enzyme will make many cuts.
 - Copies of a DNA molecule will always yield the same set of **restriction fragments** when exposed to a specific enzyme.*
Restriction enzymes cut covalent phosphodiester bonds of both strands, often in a staggered way creating single-stranded ends, **sticky ends**.
 - These extensions will form hydrogen-bonded base pairs with complementary single-stranded stretches on other DNA molecules cut with the same restriction enzyme.*
These DNA fusions can be made permanent by **DNA ligase** which seals the strand by catalyzing the formation of phosphodiester bonds.
- Restriction enzymes and DNA ligase can be used to make recombinant DNA, DNA that has been spliced together from two different sources.

F. Genes can be cloned in recombinant DNA vectors: a closer look

- Recombinant plasmids are produced by splicing restriction fragments from foreign DNA into plasmids.
 - These can be returned relatively easily to bacteria.*
 - The original plasmid used to produce recombinant DNA is called a **cloning vector**, which is a DNA molecule that can carry foreign DNA into a cell and replicate there.*
- Then, as a bacterium carrying a recombinant plasmid reproduces, the plasmid replicates within it.
 - Bacteria are most commonly used as host cells for gene cloning because DNA can be easily isolated reintroduced into their cells.*
 - Bacteria cultures also grow quickly, rapidly replicating the foreign genes.*
- The process of cloning a human gene in a bacterial plasmid can be divided into five steps.
 - 1) Isolation of vector and gene-source DNA.
 - The source DNA comes from human tissue cells.*
 - The source of the plasmid is typically E. coli.*
 - This plasmid carries two useful genes, amp, conferring resistance to the antibiotic ampicillin and lacZ, encoding the enzyme beta-galactosidase which catalyzes the hydrolysis of sugar.*
 - The plasmid has a single recognition sequence, within the lacZ gene, for the restriction enzyme used.*
 - 2) Insertion of DNA into the vector.

- e) *By digesting both the plasmid and human DNA with the same restriction enzyme we can create thousands of human DNA fragments, one fragment with the gene that we want, and with compatible sticky ends on bacterial plasmids.*
 - f) *After mixing, the human fragments and cut plasmids form complementary pairs that are then joined by DNA ligase.*
 - g) *This creates a mixture of recombinant DNA molecules.*
- 3) Introduction of the cloning vector into cells.
 - h) *Bacterial cells take up the recombinant plasmids by transformation.*
 - i) *These bacteria are lacZ, unable to hydrolyze lactose.*
 - j) *This creates a diverse pool of bacteria, some bacteria that have taken up the desired recombinant plasmid DNA, other bacteria that have taken up other DNA, both recombinant and nonrecombinant.*
- 4) Cloning of cells (and foreign genes).
 - k) *We can plate out the transformed bacteria on a solid nutrient medium containing ampicillin and a sugar called X-gal.*
 - l) *Only bacteria that have the ampicillin-resistance plasmid will grow.*
 - m) *The X-gal in the medium is used to identify plasmids that carry foreign DNA.*
 Bacteria with plasmids lacking foreign DNA stain blue when beta-galactosidase hydrolyzes X-gal.
 Bacteria with plasmids containing foreign DNA are white because they lack beta-galactosidase.
- 5) Identifying cell clones with the right gene.
 - n) *In the final step, we will sort through the thousands of bacterial colonies with foreign DNA to find those containing our gene of interest.*
 - o) *One technique, **nucleic acid hybridization**, depends on base-pairing between our gene and a complementary sequence, a **nucleic acid probe**, on another nucleic acid molecule.*
 The sequence of our RNA or DNA probe depends on knowledge of at least part of the sequence of our gene.
 A radioactive or fluorescent tag labels the probe.
 The probe will hydrogen-bond specifically to complementary single strands of the desired gene.
 After **denaturation** (separating) the DNA strands in the plasmid, the probe will bind with its complementary sequence, tagging colonies with the targeted gene.
 - p) *Because of different details between prokaryotes and eukaryotes, inducing a cloned eukaryotic gene to function in a prokaryotic host can be difficult.*
 - q) *One way around this is to employ an **expression vector**, a cloning vector containing the requisite prokaryotic promoter upstream of the restriction site.*
 - r) *The bacterial host will then recognize the promoter and proceed to express the foreign gene that has been linked to it, including many eukaryotic proteins.*
4. The presence of introns, long non-coding regions, in eukaryotic genes creates problems for expressing these genes in bacteria.
 5. To express eukaryotic genes in bacteria, a fully processed mRNA acts as the template for the synthesis of a complementary strand using reverse transcriptase.
 6. This **complementary DNA (cDNA)**, with a promoter, can be attached to a vector for replication, transcription, and translation inside bacteria.
 7. Complementary is DNA in vitro using mRNA as a template and the enzyme reverse transcriptase.
 8. Molecular biologists can avoid incompatibility problems by using eukaryotic cells as host for cloning and expressing eukaryotic genes.
 9. Yeast cells, single-celled fungi, are as easy to grow as bacteria and have plasmids, rare for eukaryotes.
 10. Scientists have constructed **yeast artificial chromosomes (YACs)** - an origin site for replication, a centromere, and two telomeres -with foreign DNA.
 11. These chromosomes behave normally in mitosis and can carry more DNA than a plasmid.

- a) Another advantage of eukaryotic hosts is that they are capable of providing the posttranslational modifications that many proteins require.
 - b) This includes adding carbohydrates or lipids.
 - c) For some mammalian proteins, the host must be an animal or plant cell to perform the necessary modifications.
12. Many eukaryotic cells can take up DNA from their surroundings, but often not efficiently.
13. Several techniques facilitate entry of foreign DNA.
- a) In **electroporation**, brief electrical pulses create a temporary hole in the plasma membrane through which DNA can enter.
 - b) Alternatively, scientists can inject DNA into individual cells using microscopically thin needles.
 - c) In a technique used primarily for plants, DNA is attached to microscopic metal particles and fired into cells with a gun.
 - d) Once inside the cell, the DNA is incorporated into the cell's DNA by natural genetic recombination.

G. Cloned genes are stored in DNA libraries

1. In the “shotgun” cloning approach, a mixture of fragments from the entire genome is included in thousands of different recombinant plasmids.
2. A complete set of recombinant plasmid clones, each carrying copies of a particular segment from the initial genome, forms a **genomic library**.
 - a) The library can be saved and used as a source of other genes or for gene mapping.
3. In addition to plasmids, certain bacteriophages are also common cloning vectors for making libraries.
 - a) Fragments of foreign DNA can be spliced into a phage genome using a restriction enzyme and DNA ligase.
 - b) The recombinant phage DNA is packaged in a capsid in vitro and allowed to infect a bacterial cell.
 - c) Infected bacteria produce new phage particles, each with the foreign DNA.
4. A more limited kind of gene library can be developed from complementary DNA.
 - a) During the process of producing cDNA, all mRNAs are converted to cDNA strands.
 - b) This **cDNA library** represents that part of a cell's genome that was transcribed in the starting cells.
 - c) This is an advantage if a researcher wants to study the genes responsible for specialized functions of a particular kind of cell.
 - d) By making cDNA libraries from cells of the same type at different times in the life of an organism, one can trace changes in the patterns of gene expression.

H. The polymerase chain reaction (PCR) clones DNA entirely in vitro

- a) DNA cloning is the best method for preparing large quantities of a particular gene or other DNA sequence.
 - b) When the source of DNA is scanty or impure, the **polymerase chain reaction (PCR)** is quicker and more selective.
 - c) This technique can quickly amplify any piece of DNA without using cells.
2. The DNA is incubated in a test tube with special DNA polymerase, a supply of nucleotides, and short pieces of single-stranded DNA as a primer.
 - a) PCR can make billions of copies of a targeted DNA segment in a few hours.
 - b) This is faster than cloning via recombinant bacteria.
 - c) In PCR, a three-step cycle—heating, cooling, and replication—brings about a chain reaction that produces an exponentially growing population of DNA molecules.
 - d) The key to easy PCR automation was the discovery of an unusual DNA polymerase, isolated from bacteria living in hot springs, which can withstand the heat needed to separate the DNA strands at the start of each cycle.
 3. PCR is very specific.

4. By their complementarity to sequences bracketing the targeted sequence, the primers determine the DNA sequence that is amplified.
 - a) *PCR can make many copies of a specific gene before cloning in cells, simplifying the task of finding a clone with that gene.*
 - b) *PCR is so specific and powerful that only minute amounts of DNA need be present in the starting material.*
5. Occasional errors during PCR replication impose limits to the number of good copies that can be made when large amounts of a gene are needed.
6. Devised in 1985, PCR has had a major impact on biological research and technology.
7. PCR has amplified DNA from a variety of sources:
 - a) *Fragments of ancient DNA from a 40,000-year-old frozen woolly mammoth.*
 - b) *DNA from tiny amount of blood or semen found at the scenes of violent crimes.*
 - c) *DNA from single embryonic cells for rapid prenatal diagnosis of genetic disorders.*
 - d) *DNA of viral genes from cells infected with difficult-to-detect viruses such as HIV.*

III. DNA Analysis and Genomics

1. Once we have prepared homogeneous samples of DNA, each containing a large number of identical segments, we can begin to ask some **far-ranging questions**.
 - a) *These include:*
 - Are there differences in a gene in different people?
 - Where and when is a gene expressed?
 - What is the location of a gene in the genome?
 2. How has a gene evolved as revealed in interspecific comparisons?
 3. To answer these questions, we will eventually need to know the nucleotide sequence of the gene and ultimately the sequences of entire genomes.
 4. Comparisons among whole sets of genes and their interactions is the field of **genomics**.
 5. One indirect method of rapidly analyzing and comparing genomes is **gel electrophoresis**.
 - a) *Gel electrophoresis separates macromolecules - nucleic acids or proteins - on the basis of their rate of movement through a gel in an electrical field.*
 - Rate of movement depends on size, electrical charge, and other physical properties of the macromolecules.
 6. For linear DNA molecules, separation depends mainly on size (length of fragment) with longer fragments migrating less along the gel.
- B. Restriction fragment analysis detects DNA differences that affect restriction sites
1. Restriction fragment analysis indirectly detects certain differences in DNA nucleotide sequences.

- a) *After treating long DNA molecules with a restriction enzyme, the fragments can be separated by size via gel electrophoresis.*
 - b) *This produces a series of bands that are characteristic of the starting molecule and that restriction enzyme.*
 - c) *The separated fragments can be recovered undamaged from gels, providing pure samples of individual fragments.*
 - d) *We can use restriction fragment analysis to compare two different DNA molecules representing, for example, different alleles.*
 - e) *Because the two alleles must differ slightly in DNA sequence, they may differ in one or more restriction sites.*
 - f) *If they do differ in restriction sites, each will produce different-sized fragments when digested by the same restriction enzyme.*
 - g) *In gel electrophoresis, the restriction fragments from the two alleles will produce different band patterns, allowing us to distinguish the two alleles.*
2. Restriction fragment analysis is sensitive enough to distinguish between two alleles of a gene that differ by only base pair in a restriction site.
 3. Gel electrophoresis combined with nucleic acid hybridization allows analyses to be conducted on the whole genome, not just cloned and purified genes.
 4. Although electrophoresis will yield too many bands to distinguish individually, we can use nucleic acid hybridization with a specific probe to label discrete bands that derive from our gene of interest.
 5. The radioactive label on the single-stranded probe can be detected by autoradiography, identifying the fragments that we are interested in.
 - a) *We can tie together several molecular techniques to compare DNA samples from three individuals.*
 - b) *We start by adding the restriction enzyme to each of the three samples to produce restriction fragments.*
 - c) *We then separate the fragments by gel electrophoresis.*
 - d) **Southern blotting** (Southern hybridization) allows us to transfer the DNA fragments from the gel to a sheet of nitrocellulose paper, still separated by size.
 - e) *This also denatures the DNA fragments.*
 - f) *Bathing this sheet in a solution containing our probe allows the probe to attach by base-pairing (hybridize) to the DNA sequence of interest and we can visualize bands containing the label with autoradiography.*
 6. For our three individuals, the results of these steps show that individual III has a different restriction pattern than individuals I or II.
 7. Southern blotting can be used to examine differences in *noncoding* DNA as well.
 8. Differences in DNA sequence on homologous chromosomes that produce different restriction fragment patterns are scattered abundantly throughout genomes, including the human genome.
 9. These **restriction fragment length polymorphisms (RFLPs)** can serve as a genetic marker for a particular location (locus) in the genome.
 - a) *A given RFLP marker frequently occurs in numerous variants in a population.*
 10. RFLPs are detected and analyzed by Southern blotting, frequently using the entire genome as the DNA starting material.
 - a) *These techniques will detect RFLPs in noncoding or coding DNA.*
 11. Because RFLP markers are inherited in a Mendelian fashion, they can serve as genetic markers for making linkage maps.
 - a) *The frequency with which two RFLP markers - or a RFLP marker and a certain allele for a gene - are inherited together is a measure of the closeness of the two loci on a chromosome.*

C. Entire genomes can be mapped at the DNA level

1. As early as 1980, Daniel Botstein and colleagues proposed that the DNA variations reflected in RFLPs could serve as the basis of an extremely detailed map of the entire human genome.
2. For some organisms, researchers have succeeded in bringing genome maps to the ultimate level of detail: the entire sequence of nucleotides in the DNA.

- a) *They have taken advantage of all the tools and techniques already discussed - restriction enzymes, DNA cloning, gel electrophoresis, labeled probes, and so forth.*
3. One ambitious research project made possible by DNA technology has been the **Human Genome Project**, begun in 1990.
- a) *This is an effort to map the entire human genome, ultimately by determining the complete nucleotide sequence of each human chromosome.*
- b) *An international, publicly funded consortium has proceeded in three phases: genetic (linkage) mapping, physical mapping, and DNA sequencing.*
4. In addition to mapping human DNA, the genomes of other organisms important to biological research are also being mapped.
- a) *These include E. coli, yeast, fruit fly, and mouse.*
- b) *In mapping a large genome, the first stage is to construct a linkage map of several thousand markers spaced throughout the chromosomes.*
- c) *The order of the markers and the relative distances between them on such a map are based on recombination frequencies.*
- d) *The markers can be genes or any other identifiable sequences in DNA, such as RFLPs or microsatellites.*
- e) *The human map with 5,000 genetic markers enabled researchers to locate other markers, including genes, by testing for genetic linkage with the known markers.*
- f) *The next step was converting the relative distances to some physical measure, usually the number of nucleotides along the DNA.*
- g) *For whole-genome mapping, a physical map is made by cutting the DNA of each chromosome into identifiable restriction fragments and then determining the original order of the fragments.*
- h) *The key is to make fragments that overlap and then use probes or automated nucleotide sequencing of the ends to find the overlaps.*
5. In **chromosome walking**, the researcher starts with a known DNA segment (cloned, mapped, and sequenced) and “walks” along the DNA from that locus, producing a map of overlapping fragments.
6. When working with large genomes, researchers carry out several rounds of DNA cutting, cloning, and physical mapping.
- a) *The first cloning vector is often a yeast artificial chromosome (YAC), which can carry inserted fragments up to a million base pairs long, or a **bacterial artificial chromosome (BAC)**, which can carry inserts of 100,000 to 500,000 base pairs.*
- b) *After the order of these long fragments has been determined (perhaps by chromosome walking), each fragment is cut into pieces, which are cloned and ordered in turn.*
- c) *The final sets of fragments, about 1,000 base pairs long, are cloned in plasmids or phage and then sequenced.*
- d) *The complete nucleotide sequence of a genome is the ultimate map.*
- e) *Starting with a pure preparation of many copies of a relatively short DNA fragment, the nucleotide sequence of the fragment can be determined by a sequencing machine.*
- f) *The usual sequencing technique combines DNA labeling, DNA synthesis with special chain-terminating nucleotides, and high resolution gel electrophoresis.*
- g) *A major thrust of the Human Genome Project has been the development of technology for faster sequencing and more sophisticated software for analyzing and assembling the partial sequences.*
7. One common method of sequencing DNA, the Sanger method, is similar to PCR.
8. However, inclusion of special dideoxynucleotides in the reaction mix ensures that rather than copying the whole template, fragments of various lengths will be synthesized.
- a) *These dideoxynucleotides, marked radioactively or fluorescently, terminate elongation when they are incorporated randomly into the growing strand because they lack a 3'-OH to attach the next nucleotide.*
9. The order of these fragments via gel electrophoresis can be interpreted as the nucleotide sequence.

- a) While the public consortium has followed a hierarchical, three-stage approach for sequencing an entire genome, J. Craig Venter decided in 1992 to try a whole-genome shotgun approach.
- b) This uses powerful computers to assemble sequences from random fragments, skipping the first two steps.
- c) The worth of his approach was demonstrated in 1995 when he and colleagues reported the complete sequence of a bacterium.
- d) His private company, Celera Genomics, finished the sequence of *Drosophila melanogaster* in 2000.
- e) In February, 2001, Celera and the public consortium separately announced sequencing over 90% of the human genome.
- f) Competition and an exchange of information and approaches between the two groups has hastened progress.
- g) By mid-2001, the genomes of about 50 species had been completely (or almost completely) sequenced.
- h) They include *E. coli* and a number of other bacteria and several archaea.
- i) Sequenced eukaryotes include a yeast, a nematode, and a plant *Arabidopsis thaliana*.
- j) There are still many gaps in the human sequence.
- k) Areas with repetitive DNA and certain parts of the chromosomes of multicellular organisms resist detailed mapping by the usual methods.
- l) On the other hand, the sequencing of the mouse genome (about 85% identical to the human genome) is being greatly aided by knowledge of the human sequence.
- m)

D. Genome sequences provide clues to important biological questions

- a) Genomics, the study of genomes based on their DNA sequences, is yielding new insights into fundamental questions about genome organization, the control of gene expression, growth and development, and evolution.
 - b) Rather than inferring genotype from phenotype like classical geneticists, molecular geneticists try to determine the impact on the phenotype of details of the genotype.
2. DNA sequences, long lists of A's, T's, G's, and C's, are being collected in computer data banks that are available to researchers everywhere via the Internet.
 3. Special software can scan the sequences for the telltale signs of protein-coding genes, such as start and stop signals for transcription and translation, and those for RNA-splicing sites.
 4. From these expressed sequence tags (ESTs), researchers can collect a list of gene candidates.
 - a) The surprising—and humbling—result to date from the Human Genome Project is the small number of putative genes, 30,000 to 40,000.
 - b) This is far less than expected and only two to three times the number of genes in the fruit fly or nematodes.
 - c) Humans have enormous amounts of noncoding DNA, including repetitive DNA and unusually long introns.
 - d) By doing more mixing and matching of modular elements, humans—and vertebrates in general—reach more complexity than flies or worms.
 - e) The typical human gene probably specifies at least two or three different polypeptides by using different combinations of exons.
 - f) Along with this is additional polypeptide diversity via post-translational processing.
 - g) The human sequence suggests that our polypeptides tend to be more complicated than those of invertebrates.

While humans do not seem to have more types of domains, the domains are put together in many more combinations.
 5. About half of the human genes were already known before the Human Genome Project.

- a) *To determine what the others are and what they may do, scientists compare the sequences of new gene candidates with those of known genes.*
 - b) *In some cases, the sequence of a new gene candidate will be similar in part with that of known gene, suggesting similar function.*
 - c) *In other cases, the new sequences will be similar to a sequence encountered before, but of unknown function.*
 - d) *In still other cases, the sequence is entirely unlike anything ever seen before.*
 - e) *About 30% of the E. coli genes are new to us.*
 - f) *Comparisons of genome sequences confirm very strongly the evolutionary connections between even distantly related organisms and the relevance of research on simpler organisms to our understanding of human biology.*
 - g) *For example, yeast has a number of genes close enough to the human versions that they can substitute for them in a human cell.*
 - h) *Researchers may determine what a human disease gene does by studying its normal counterpart in yeast.*
 - i) *Bacterial sequences reveal unsuspected metabolic pathways that may have industrial or medical uses.*
6. Studies of genomes have also revealed how genes act together to produce a functioning organism through an unusually complex network of interactions among genes and their products.
 7. To determine which genes are transcribed under different situations, researchers isolate mRNA from particular cells and use the mRNA as templates to build a cDNA library.
 8. This cDNA can be compared to other collections of DNA by hybridization.
 - a) *This will reveal which genes are active at different developmental stages, in different tissues, or in tissues in different states of health.*
 - b) *Automation has allowed scientists to detect and measure the expression of thousands of genes at one time using **DNA microarray assays**.*
 - c) *Tiny amounts of a large number of single-stranded DNA fragments representing different genes are fixed on a glass slide in a tightly spaced array (grid).*
 - d) *The fragments are tested for hybridization with various samples of fluorescently labeled cDNA molecules.*
 9. Spots where any of the cDNA hybridizes fluoresce with an intensity indicating the relative amount of the mRNA that was in the tissue.
 10. Ultimately, information from microarray assays should provide us a grander view: how ensembles of genes interact to form a living organism.
 - a) *It already has confirmed the relationship between expression of genes for photosynthetic enzymes and tissue function in leaves versus roots of the plant Arabidopsis.*
 - b) *In other cases, DNA microarray assays are being used to compare cancerous versus noncancerous tissues.*
 - c) *This may lead to new diagnostic techniques and biochemically targeted treatments, as well as a fuller understanding of cancer.*
 11. Perhaps the most interesting genes discovered in genome sequencing and expression studies are those whose function is completely mysterious.
 12. One way to determine their function is to disable the gene and hope that the consequences provide clues to the gene's normal function.
 - a) *Using **in vitro mutagenesis**, specific changes are introduced into a cloned gene, altering or destroying its function.*
 - b) *When the mutated gene is returned to the cell, it may be possible to determine the function of the normal gene by examining the phenotype of the mutant.*
 13. In nonmammalian organisms, a simpler and faster method, **RNA interference (RNAi)**, has been applied to silence the expression of selected genes.

- a) *This method uses synthetic double-stranded RNA molecules matching the sequences of a particular gene to trigger breakdown of the gene's mRNA.*
 - b) *The mechanism underlying RNAi is still unknown.*
 - c) *Scientists have only recently achieved some success in using the method to silence genes in mammalian cells.*
 - d) *The next step after mapping and sequencing genomes is **proteomics**, the systematic study of full protein sets (proteomes) encoded by genomes.*
 - e) *One challenge is the sheer number of proteins in humans and our close relatives because of alternative RNA splicing and post-translational modifications.*
 - f) *Collecting all the proteins will be difficult because a cell's proteins differ with cell type and its state.*
 - g) *In addition, unlike DNA, proteins are extremely varied in structure and chemical and physical properties.*
 - h) *Because proteins are the molecules that actually carry out cell activities, we must study them to learn how cells and organisms function.*
14. Genomic and proteomics are giving biologists an increasingly global perspective on the study of life.
15. Eric Lander and Robert Weinberg predict that complete catalogs of genes and proteins will change the discipline of biology dramatically.
- a) *"For the first time in a century, reductionists [are yielding] ground to those trying to gain a holistic view of cells and tissues."*
16. Advances in **bioinformatics**, the application of computer science and mathematics to genetic and other biological information, will play a crucial role in dealing with the enormous mass of data.
17. These analyses will provide understanding of the spectrum of genetic variation in humans.
- a) *Because we are all probably descended from a small population living in Africa 150,000 to 200,000 years ago, the amount of DNA variation in humans is small.*
 - b) *Most of our diversity is in the form of **single nucleotide polymorphisms (SNPs)**, single base-pair variations. In humans, SNPs occur about once in 1,000 bases, meaning that any two humans are 99.9% identical.*
 - c) *The locations of the human SNP sites will provide useful markers for studying human evolution and for identifying disease genes and genes that influence our susceptibility to diseases, toxins or drugs.*

IV. Practical Applications of DNA Technology

A. DNA technology is reshaping medicine and the pharmaceutical industry

1. Modern biotechnology is making enormous contributions to both the diagnosis of diseases and in the development of pharmaceutical products.
 - a) *The identification of genes whose mutations are responsible for genetic diseases could lead to ways to diagnose, treat, or even prevent these conditions.*
 - b) *Susceptibility to many "nongenetic" diseases, from arthritis to AIDS, is influenced by a person's genes.*
 - c) *Diseases of all sorts involve changes in gene expression.*
 - d) *DNA technology can identify these changes and lead to the development of targets for prevention or therapy.*
2. PCR and labeled probes can track down the pathogens responsible for infectious diseases.
 - a) *For example, PCR can amplify and thus detect HIV DNA in blood and tissue samples, detecting an otherwise elusive infection.*
3. Medical scientists can use DNA technology to identify individuals with genetic diseases before the onset of symptoms, even before birth.
 - a) *It is also possible to identify symptomless carriers.*
 - b) *Genes have been cloned for many human diseases, including hemophilia, cystic fibrosis, and Duchenne muscular dystrophy.*
4. Hybridization analysis makes it possible to detect abnormal allelic forms of genes, even in cases in which the gene has not yet been cloned.

- a) *The presence of an abnormal allele can be diagnosed with reasonable accuracy if a closely linked RFLP marker has been found.*
 - b) *The closeness of the marker to the gene makes crossing over between them unlikely and the marker and gene will almost always stay together in inheritance.*
5. Techniques for gene manipulation hold great potential for treating disease by **gene therapy**.
- a) *This alters an afflicted individual's genes.*
 - b) *A normal allele is inserted into somatic cells of a tissue affected by a genetic disorder.*
 - c) *For gene therapy of somatic cells to be permanent, the cells that receive the normal allele must be ones that multiply throughout the patient's life.*
6. Bone marrow cells, which include the *stem cells* that give rise to blood and immune system cells, are prime candidates for gene therapy.
- a) *A normal allele could be inserted by a viral vector into some bone marrow cells removed from the patient.*
 - b) *If the procedure succeeds, the returned modified cells will multiply throughout the patient's life and express the normal gene, providing missing proteins.*
7. Despite "hype" in the news media over the past decade, there has been very little scientifically strong evidence of effective gene therapy.
- a) *Even when genes are successfully and safely transferred and expressed in their new host, their activity typically diminishes after a short period.*
8. Most current gene therapy trials are directed not at correcting genetic defects, but to fight major killers such as heart disease and cancer.
- a) *The most promising trials are those in which a limited activity period is not only sufficient but desirable.*
 - b) *Some success has been reported in stimulated new heart blood vessels in pigs after gene therapy.*
9. Gene therapy poses many technical questions.
- a) *These include regulation of the activity of the transferred gene to produce the appropriate amount of the gene product at the right time and place.*
 - b) *In addition, the insertion of the therapeutic gene must not harm some other necessary cell function.*
10. Gene therapy raises some difficult ethical and social questions.
- a) *Some critics suggest that tampering with human genes, even for those with life-threatening diseases, is wrong.*
 - b) *They argue that this will lead to the practice of eugenics, a deliberate effort to control the genetic makeup of human populations.*
11. The most difficult ethical question is whether we should treat human germ-line cells to correct the defect in future generations.
- a) *In laboratory mice, transferring foreign genes into egg cells is now a routine procedure.*
 - b) *Once technical problems relating to similar genetic engineering in humans are solved, we will have to face the question of whether it is advisable, under any circumstances, to alter the genomes of human germ lines or embryos.*
 - c) *Should we interfere with evolution in this way?*
12. From a biological perspective, the elimination of unwanted alleles from the gene pool could backfire.
- a) *Genetic variation is a necessary ingredient for the survival of a species as environmental conditions change with time.*
 - b) *Genes that are damaging under some conditions could be advantageous under other conditions, for example the sickle-cell allele.*
13. DNA technology has been used to create many useful pharmaceuticals, mostly proteins.
14. By transferring the gene for a protein into a host that is easily grown in culture, one can produce large quantities of normally rare proteins.
- a) *By including highly active promoters (and other control elements) into vector DNA, the host cell can be induced to make large amounts of the product of a gene into the vector.*
 - b) *In addition, host cells can be engineered to secrete a protein, simplifying the task of purification.*
15. One of the first practical applications of gene splicing was the production of mammalian hormones and other mammalian regulatory proteins in bacteria.

- a) *These include human insulin and growth factor (HFG).*
 - b) *Human insulin, produced by bacteria, is superior for the control of diabetes than the older treatment of pig or cattle insulin.*
 - c) *Human growth hormone benefits children with hypopituitarism, a form of dwarfism.*
 - d) *Tissue plasminogen activator (TPA) helps dissolve blood clots and reduce the risk of future heart attacks.*
However, like many such drugs, it is expensive.
16. New pharmaceutical products are responsible for novel ways of fighting diseases that do not respond to traditional drug treatments.
- a) *One approach is to use genetically engineered proteins that either block or mimic surface receptors on cell membranes.*
 - b) *For example, one experimental drug mimics a receptor protein that HIV bonds to when entering white blood cells, but HIV binds to the drug instead and fails to enter the blood cells.*
17. Virtually the only way to fight viral diseases is by vaccination.
- a) *A **vaccine** is a harmless variant or derivative of a pathogen that stimulates the immune system.*
 - b) *Traditional vaccines are either particles of virulent viruses that have been inactivated by chemical or physical means or active virus particles of a nonpathogenic strain.*
 - c) *Both are similar enough to the active pathogen to trigger an immune response.*
18. Recombinant DNA techniques can generate large amounts of a specific protein molecule normally found on the pathogen's surface.
- a) *If this protein triggers an immune response against the intact pathogen, then it can be used as a vaccine.*
 - b) *Alternatively, genetic engineering can modify the genome of the pathogen to attenuate it.*
 - c) *These attenuated microbes are often more effective than a protein vaccine because it usually triggers a greater response by the immune system.*
 - d) *Pathogens attenuated by gene-splicing techniques may be safer than the natural mutants traditionally used.*
 - e)

B. DNA technology offers forensic, environmental, and agricultural applications

1. In violent crimes, blood, semen, or traces of other tissues may be left at the scene or on the clothes or other possessions of the victim or assailant.
2. If enough tissue is available, forensic laboratories can determine blood type or tissue type by using antibodies for specific cell surface proteins.
 - a) *However, these tests require relatively large amounts of fresh tissue.*
 - b) *Also, this approach can only exclude a suspect.*
3. DNA testing can identify the guilty individual with a much higher degree of certainty, because the DNA sequence of every person is unique (except for identical twins).
 - a) *RFLP analysis by Southern blotting can detect similarities and differences in DNA samples and requires only tiny amount of blood or other tissue.*
 - b) *Radioactive probes mark electrophoresis bands that contain certain RFLP markers.*
 - c) *Even as few as five markers from an individual can be used to create a **DNA fingerprint**.*
 - d) *The probability that two people (that are not identical twins) have the same DNA fingerprint is very small.*
4. DNA fingerprints can be used forensically to present evidence to juries in murder trials.
 - a) *This autoradiograph of RFLP bands of samples from a murder victim, the defendant, and the defendant's clothes is consistent with the conclusion that the blood on the clothes is from the victim, not the defendant.*
5. The forensic use of DNA fingerprinting extends beyond violent crimes.
 - a) *For instance, DNA fingerprinting can be used to settle conclusively a question of paternity.*
 - b) *These techniques can also be used to identify the remains of individuals killed in natural or man-made disasters.*
6. Variations in the lengths of satellite DNA are increasingly used as markers in DNA fingerprinting.

- a) *The most useful satellites are microsatellites, which are roughly 10 to 100 base pairs long.*
 - b) *They have repeating units of only a few base pairs and are highly variable from person to person.*
 - c) *Individuals may vary in the numbers of repeats, **simple tandem repeats (STRs)**, at a locus.*
 - d) *Restriction fragments with STRs vary in size among individuals because of differences in STR lengths.*
 - e) *PCR is often used to amplify selectively particular STRs or other markers before electrophoresis, especially if the DNA is poor or in minute quantities.*
7. The DNA fingerprint of an individual would be truly unique if it were feasible to perform restriction fragment analysis on the entire genome.
- a) *In practice, forensic DNA tests focus on only about five tiny regions of the genome.*
 - b) *The probability that two people will have identical DNA fingerprints in these highly variable regions is typically between one in 100,000 and one in a billion.*
 - c) *The exact figure depends on the number of markers and the frequency of those markers in the population.*
 - d) *Despite problems that might arise from insufficient statistical data, human error, or flawed evidence, DNA fingerprinting is now accepted as compelling evidence.*
8. Increasingly, genetic engineering is being applied to environmental work.
9. Scientists are engineering the metabolism of microorganisms to help cope with some environmental problems.
- a) *For example genetically engineered microbes that can extract heavy metals from their environments and incorporate the metals into recoverable compounds may become important both in mining materials and cleaning up highly toxic mining wastes.*
 - b) *In addition to the normal microbes that participate in sewage treatment, new microbes that can degrade other harmful compounds are being engineered.*
10. For many years scientists have been using DNA technology to improve agricultural productivity.
- a) *DNA technology is now routinely used to make vaccines and growth hormones for farm animals.*
 - b) **Transgenic organisms** with genes from another species have been developed to exploit the attributes of the new genes (for example, faster growth, larger muscles).
 - c) *Other transgenic organisms are pharmaceutical “factories” - a producer of large amounts of an otherwise rare substance for medical use.*
11. The human proteins produced by farm animals may or may not be structurally identical to natural human proteins.
- a) *Therefore, they have to be tested very carefully to ensure that they will not cause allergic reactions or other adverse effects in patients receiving them.*
 - b) *In addition, the health and welfare of transgenic farm animals are important issues, as they often suffer from lower fertility or increased susceptibility to disease.*
12. To develop a transgenic organism, scientists remove ova from a female and fertilize them *in vitro*.
- a) *The desired genes from another organism are cloned and then inserted into the nuclei of the eggs. Some cells will integrate the foreign DNA into their genomes and are able to express its protein.*
 - b) *The engineered eggs are then surgically implanted in a surrogate mother.*
 - c) *If development is successful, the result is a transgenic animal, containing genes from a “third” parent, even from another species.*
13. Agricultural scientists have engineered a number of crop plants with genes for desirable traits.
- a) *These include delayed ripening and resistance to spoilage and disease.*
 - b) *Because a single transgenic plant cell can be grown in culture to generate an adult plant, plants are easier to engineer than most animals.*
14. The **Ti plasmid**, from the soil bacterium *Agrobacterium tumefaciens*, is often used to introduce new genes into plant cells.
- a) *The Ti plasmid normally integrates a segment of its DNA into its host plant and induces tumors.*
15. Foreign genes can be inserted into the Ti plasmid (a version that does not cause disease) using recombinant DNA techniques.

- a) *The recombinant plasmid can be put back into Agrobacterium, which then infects plant cells, or introduced directly into plant cells.*
16. The Ti plasmid can only be used as a vector to transfer genes to dicots (plants with two seed leaves).
- a) *Monocots, including corn and wheat, cannot be infected by Agrobacterium (or the Ti plasmid).*
- b) *Other techniques, including electroporation and DNA guns, are used to introduce DNA into these plants.*
17. Genetic engineering is quickly replacing traditional plant-breeding programs.
- a) *In the past few years, roughly half of the soybeans and corn in America have been grown from genetically modified seeds.*
- b) *These plants may receive genes for resistance to weed-killing herbicides or to infectious microbes and pest insects.*
18. Scientists are using gene transfer to improve the nutritional value of crop plants.
- a) *For example, a transgenic rice plant has been developed that produces yellow grains containing beta-carotene.*
 Humans use beta-carotene to make vitamin A.
 Currently, 70% of children under the age of 5 in Southeast Asia are deficient in vitamin A, leading to vision impairment and increased disease rates.
19. An important potential use of DNA technology focuses on nitrogen fixation.
- a) *Nitrogen fixation occurs when certain bacteria in the soil or in plant roots convert atmospheric nitrogen to nitrogen compounds that plants can use.*
- b) *Plants use these to build nitrogen-containing compounds, such as amino acids.*
- c) *In areas with nitrogen-deficient soils, expensive fertilizers must be added for crops to grow.*
 Nitrogen fertilizers also contribute to water pollution.
- d) *DNA technology offers ways to increase bacterial nitrogen fixation and eventually, perhaps, to engineer crop plants to fix nitrogen themselves.*
20. DNA technology has led to new alliances between the pharmaceutical industry and agriculture.
- a) *Plants can be engineered to produce human proteins for medical use and viral proteins for use as vaccines.*
- b) *Several such “pharm” products are in clinical trials, including vaccines for hepatitis B and an antibody that blocks the bacteria that cause tooth decay.*
- c) *The advantage of “pharm” plants is that large amounts of these proteins might be made more economically by plants than by cultured cells.*

C. DNA technology raises important safety and ethical questions

- The power of DNA technology has led to worries about potential dangers.
 - For example, recombinant DNA technology may create hazardous new pathogens.*
- In response, scientists developed a set of guidelines that have become formal government regulations in the United States and some other countries.
- Strict laboratory procedures are designed to protect researchers from infection by engineered microbes and to prevent their accidental release.
- Some strains of microorganisms used in recombinant DNA experiments are genetically crippled to ensure that they cannot survive outside the laboratory.
- Finally, certain obviously dangerous experiments have been banned.
- Today, most public concern centers on **genetically modified (GM) organisms** used in agriculture.

- a) *"GM organisms" have acquired one or more genes (perhaps from another species) by artificial means.*
 - b) *Genetically modified animals are still not part of our food supply, but GM crop plants are.*
 - c) *In Europe, safety concerns have led to pending new legislation regarding GM crops and bans on the import of all GM foodstuffs.*
 - d) *In the United States and other countries where the GM revolution had proceeded more quietly, the labeling of GM foods is now being debated.*
 - e) *This is required by exporters in a Biosafety Protocol.*
7. Advocates of a cautious approach fear that GM crops might somehow be hazardous to human health or cause ecological harm.
- a) *In particular, transgenic plants may pass their new genes to close relatives in nearby wild areas through pollen transfer.*
 - b) *Transference of genes for resistance to herbicides, diseases, or insect pests may lead to the development of wild "superweeds" that would be difficult to control.*
8. To date there is little good data either for or against any special health or environmental risks posed by genetically modified crops.
9. Today, governments and regulatory agencies are grappling with how to facilitate the use of biotechnology in agriculture, industry, and medicine while ensuring that new products and procedures are safe.
- a) *In the United States, all projects are evaluated for potential risks by various regulatory agencies, including the Environmental Protection Agency, the National Institutes of Health, and the Department of Agriculture.*
 - b) *These agencies are under increasing pressures from some consumer groups.*
10. As with all new technologies, developments in DNA technology have ethical overtones.
- a) *Who should have the right to examine someone else's genes?*
 - b) *How should that information be used?*
 - c) *Should a person's genome be a factor in suitability for a job or eligibility for life insurance?*