CHAPTER 19: GENE TECHNOLOGY

CHAPTER SYNOPSIS

Science has reached the exciting, but potentially dangerous stage, at which we are learning to manipulate the materials of heredity. The first human genes isolated and inserted into bacteria turned these cells into miniature factories producing interferon. Many bacteria possess restriction endonucleases to protect themselves from invading viruses. Scientists use these enzymes to chop up strands of DNA at specific locations. Such specificity assures that a given enzyme will always break up a specific kind of DNA into exactly the same size and number of fragments. These fragments constitute a library of DNA sequence information. Restriction enzyme specificity also assures that all of the fragments possess identical, short sequences called "sticky ends." Each strand of a sticky end is complementary to the other strand and can be joined to the other ends when treated with a DNA ligase. Fragments, even those from different organisms, that have been cut with the same restriction enzyme can be joined enabling the insertion of foreign genes into a plant, animal, or bacterial genome.

Bacterial plasmids and viruses are the vehicles by which such genes are inserted into the host DNA, the crux of genetic engineering. There are four steps in this process: cleavage, producing recombinant DNA, cloning, and screening. Cleavage is accomplished using the restriction endonuclease that will produce the desired sticky ends. The fragments are then inserted into the desired vehicle. Unfortunately, very few vehicles actually receive DNA fragments and even fewer get the desired piece. At this point, vehicles not carrying fragments are eliminated, generally by prior association with an antibiotic resistance gene. Each colony of cells is cloned and allowed to multiply, thus replicating not only its own genome but the added fragment as well. The clones are then screened to determine which clonal line contains the desired fragment.

Polymerase chain reaction is another new molecular technique that amplifies DNA in an *in vitro* sample. Frequently the DNA in a sample (of blood for example) is so small that it cannot be analyzed directly. With PCR, the DNA is copied using a microprocessor-controlled thermoregulator. The DNA unzips as the temperature is increased. When it is lowered, polymerase enzymes catalyze the replication of DNA from special primers, making a new strand from each original strand. Thus the amount of DNA is doubled at each cycle – 2 strands to 4 strands to 8 strands to 16 strands and so forth. This method is substantially quicker than cloning the DNA strand via plasmids or viruses. DNA is readily identified using a technique called Southern blotting. Differences in DNA sequences are identified by RFLP analysis. DNA is readily sequenced using Sanger's chain termination method.

Biotechnology uses genetic engineering techniques to solve practical problems. The biological community is busy sequencing the entire human genome, certainly an enormous task. DNA fingerprinting has been used to identify and convict numerous criminals. Dozens of commercial applications exist to utilize this revolutionary technology. The most obvious, pharmaceuticals, encounters additional problems of separating the desired product from the rest of the cellular material. Attempts are being made to construct piggyback vaccines, placing genes coding for the exterior of a virulent virus within the harmless vaccinia virus. An individual's entire DNA profile can be stored on a single biochip the size of a postage stamp. Agricultural uses range from developing resistance to herbicides, viruses, and insects; to inserting genes for nitrogen fixation and improving growth and plant nutritional value. The first clone from adult tissue, a sheep named Dolly, has been born. Tissue-specific stem cells can be isolated and transplanted into damaged tissues to affect their repair.

Society must be informed about these biological processes to ensure our safety and economic well-being, as well as that of future generations. Lack of sufficient biological knowledge is the source of most of the public's concern about genetically engineered products. Many assume that BST in milk products may cause human growth problems, they lack the physiological knowledge that this protein is degraded in the stomach like all other

proteins. A great many people do not trust governmental safeguards and fear the inadvertent or intentional development of lethal viruses and bacteria. Although there is little scientific need for labeling genetically modified food products, the public has the right to insist upon it. If properly done, labeling should serve to educate consumers as well as inform them.

CHAPTER OBJECTIVES

- Understand the importance of plasmids and viruses to genetic engineering.
- Know the natural function of restriction endonucleases and how a normal bacterial cell protects its DNA from their activity.
- Understand how "sticky ends" are formed and their importance to gene technology.
- Describe how a chimeric genome is constructed.
- Explain the four steps of genetic engineering experiments.
- Explain how to screen for clones that contain a desired gene fragment.
- Understand the value of and the processes involved with the polymerase chain reaction (PCR).
- Know how DNA is sequenced via the Sanger method.
- Describe several commercial applications of gene technology.
- Know how a biochip is made, its applications, and its ethical implications.
- Understand how piggyback vaccines are constructed and why they are effective.
- Explain the process and importance of Wilmut's cloned sheep, Dolly.
- Understand what stem cells are and their potential medical value.
- Understand the ethical implications of genetic engineering and why education and responsible regulation are necessary.

KEY TERMS

atrial peptide
biotechnology
chimera
cloning
complementary DNA (cDNA)
DNA ligase
DNA vaccine
genetic engineering
glyphosate
hybridize
interferon
methylase
nif genes
polymerase chain reaction (PCR)
probe
recombinant DNA
restriction endonuclease
restriction fragment length polymorphism (RFLP)
Southern blot
subunit vaccine
Ti plasmid
tissue plasminogen activator
transgenic
vector

CHAPTER OUTLINE

19.0 Introduction

I. NEW TECHNIQUES DEVELOPED TO MANIPULATE DNA

A. Techniques Can Be Applied to Alter an Organism's Genes fig 19.1

B. Have Great Impact on Future Lives

19.1 The ability to manipulate DNA has led to a new genetics

I. RESTRICTION ENDONUCLEASES

A. First Human Gene Inserted into Bacteria
   1. Interferon
      a. Increases resistance to viral infection
      b. Rare, purification of small quantities is very expensive
   2. Bacterial cells made to produce protein at high rate
      a. Gene for interferon placed in bacterial cell
      b. Cells grew, divided, produced interferon

B. The Advent of Genetic Engineering
   1. Cloning
      a. Produce genetically identical cells from single altered cell
      b. Cells in culture become factories for producing chemicals
         1) Interferon
         2) Human insulin
   2. Revolutionary process called genetic engineering
      a. Ability to cut up DNA into pieces and rearrange them
      b. Segments inserted via plasmids or infective viruses
      c. Recognize and cleave specific nucleotide sequences

C. Discovery of Restriction Endonucleases
   1. Bacteria are natural source of enzymes provide protection from viruses
   2. Bacteriophage viruses infect bacteria, multiply within and release progeny
   3. Bacteria have enzymes that chop up invading viruses
      a. Enzymes are restriction endonucleases
      b. Bacterial DNA not damaged because it is modified
      c. Recognize sequence, bind to DNA, and cleave strand
   4. Methylase enzymes recognize bacterial DNA
      a. Bind to same bacterial sites
      b. Add methyl groups to nucleotides
      c. Restriction endonucleases do not recognize methylated sites
      d. Bacterial DNA protected from fragmentation

D. How Restriction Endonucleases Cut DNA
   1. Endonucleases recognize sites
      a. Recognize a variety of four to six nucleotide sequences
      b. Segments are often palindromes
      c. Nucleotides at one end are complementary to those at other end
   2. Enzyme binds at and cleaves both strands of DNA at same time
      a. Restriction enzymes effectively cut DNA in half
      b. Site where DNA is cut has offset ends — fig 19.2
      c. Ends are complementary to each other

E. Why Restriction Endonucleases Are So Useful
   1. Each enzyme always cuts at same sequence
      a. Fragments always have same ends that are complementary to other ends
      b. Sets of nucleotides called “sticky ends”
   2. Ends can pair with each other
      a. Two fragments can be glued together by DNA ligase
      b. Any two fragments cleaved by same endonuclease can be joined together
      c. Fragments can be from entirely different organisms

II. USING RESTRICTION ENDONUCLEASES TO MANIPULATE GENES

A. Mythological Chimera Composed of Parts of Several Animals

B. Constructing pSC101
1. Cohen and Boyer: First artificial bacterial plasmid
2. Cut plasmid containing resistance transfer factor with *Eco*RI
   a. One fragment contained replication origin and tetracycline resistance gene
   b. Complementary ends joined forming pSC101 plasmid fig 19.3

C. Using pSC101 to Make Recombinant DNA
1. Same restriction enzymes used to cut frog genome
   a. Frog DNA pieces added to open pSC101 plasmids
   b. Added to bacteria, cells became resistant to tetracycline
   c. Also began to produce frog ribosomal RNA
   d. Concluded frog gene inserted into pSC101 plasmid
2. Recombinant DNA: DNA created in laboratory
   a. New genome that never existed in nature
   b. Unable to evolve by natural means

D. Other Vectors
1. Vector is the genome that carried foreign DNA into host cell
2. Current plasmids, like pUC18, induced to make copies of selves and their foreign genes
3. Yeast artificial chromosomes (YAKs) used to insert larger pieces of DNA
4. Bacterial viruses also used as vectors
5. Animal viruses used to insert bacterial genes into monkey cells
6. Animal genes inserted into plant cells

19.2 Genetic engineering involves easily understood procedures

I. THE FOUR STAGES OF A GENETIC ENGINEERING EXPERIMENT

A. Stage 1: DNA Cleavage
1. Via restriction endonucleases
2. Large number of specific fragments produced
3. Different set of fragments for each specific sequence
4. Fragments compared by electrophoresis fig 19.4

B. Stage 2: Production of Recombinant DNA
1. Fragments put into plasmids or virus
2. Cleaved with same endonuclease as host DNA

C. Stage 3: Cloning
1. Plasmid or virus serves as vector to introduce DNA into (usually) bacteria fig 19.5
2. Cell reproduces, making identical clones each containing the fragment
   a. Each clone maintained separately
   b. Whole set constitutes clone library of original source DNA

D. Stage 4: Screening
1. Identify clone line containing fragment of interest
2. Among most challenging of steps
3. 4-I: Preliminary screening of clones
   a. Eliminate bacteria not containing vector or proper DNA fragment

b. Eliminate clones without vectors
   1) Use vector with gene conferring antibiotic resistance fig 19.6a
   2) Gene *amp*^{r} confers resistance to ampicillin
   3) Culture clones on medium containing antibiotic
   4) Only bacteria resistant to antibiotic will grow on it
c. Eliminate bacteria with vector, but lacking chosen fragment
   1) Use vector with *lacZ'* gene for β-galactosidase
   2) Enables cell to metabolize X-gal sugar
   3) Metabolism of X-gal produces blue product
   4) Cells with vector and functional gene will turn blue fig 19.6b
d. Identify cells with vector and fragment
   1) Test clones for presence of X-gal metabolism
   2) DNA fragment within gene makes it inoperative
   3) Clones with fragment unable to metabolize sugar
   4) Desired cells remain colorless in presence of X-gal
e. Choose cells that grow on antibiotic, but don't turn blue

4. 4-II: Finding the gene of interest
   a. Clone library may contain thousands of DNA fragments
      1) Many clones will be identical
      2) May take hundreds of thousands of clones to assemble complete library
   b. Most general procedure utilizes hybridization fig 19.7
      1) Cloned genes form base pairs with probe DNA
      2) Part of gene nucleotide sequence must be known to produce probe
   c. Bacterial colonies with inserted gene are grown on agar
      1) Cells transferred to filter to produce replica of plate
      2) Filter treated with solution to denature bacterial DNA
      3) Solution also contains radioactive probe
      4) Probe hybridizes with complementary single-stranded bacterial DNA
      5) Filter exposed to photographic film (autoradiography)
      6) Only colonies with gene hybridize, become radioactive, and show up on film
      7) Identify colonies by comparing film to original bacterial plate

II. WORKING WITH GENE CLONES

A. Getting Enough DNA to Work With: The Polymerase Chain Reaction

1. Produce multiple identical copies of DNA
   a. Insert desired DNA into bacterium
   b. Millions of copies exist after multiple cell divisions
2. Polymerase chain reaction (PCR) is more direct approach fig 19.8
   a. Amplifies sequences
   b. Add sequences (endonuclease recognition sequences) as primers to cleaved DNA
3. Three steps in PCR process
   a. Step 1: Denaturation
      1) Primer with excess synthetic nucleotides mixed with DNA fragment
      2) Temperature of mixture increased to 98°C
      3) Fragment dissociates into single strands
   b. Step 2: Annealing of primers
      1) Solution cooled to 60°C
      2) Single strands of DNA reassociate into double strands
      3) Fragment base-pairs with complementary primer nucleotide
      4) Part of fragment still single stranded
   c. Step 3: Primer extension
      1) Heat stable DNA polymerase, Taq polymerase, added
      2) Supply of all four nucleotides also added

3) Polymerase copies rest of fragment as in DNA replication
4) Primer lengthened into complementary copy of single-stranded fragment
5) Two copies of original now exist

d. Repeating the cycle
1) Steps 1 through 3 repeated
2) More polymerase not needed since it is heat stable
3) Repeat heating and cooling in short cycles
4) Each cycle doubles amount of DNA
5) After twenty cycles, one fragment can become more than one million
6) In a few hours, 100 billion copies can be produced

4. PCR process now completely automated
5. Has revolutionized aspects of science and medicine
a. PCR allows investigation of minute samples of DNA
b. Allows identification of genetic defects in embryos using minute sample
c. Examine DNA of dead organisms, extinct species as long as any DNA intact

B. Identifying DNA: Southern Blotting fig 19.9
1. When a gene is identified it can be used as a probe to identify same or similar gene
2. Procedure called Southern blot
a. DNA from sample cleaved into fragments with restriction endonuclease
b. Fragments spread apart by gel electrophoresis
c. Make gel basic, double-stranded DNA denatured into single strands
d. Gel blotted with nitrocellulose, DNA transfers to sheet
e. Probe of purified single-stranded DNA from desired gene poured onto sheet
f. Only fragments with proper sequence hybridize with probe
g. Probe may be radioactively labeled with ^{32}P for clear identification
h. Shows as a band of radioactivity

C. Distinguishing Differences in DNA: RFLP Analysis fig 19.10
1. Used to identify an individual that possesses specific gene as a marker
2. Utilize restriction fragment length polymorphism (RFLP) analysis
a. Point mutations, sequence mutations, transposons alter length of DNA
b. Also alter length of fragments produced via action of restriction endonucleases
c. DNA from different individuals rarely have same array of restriction sites
d. Population is polymorphic for restriction fragment patterns
3. Process of RFLP analysis
a. Cut DNA sample with particular restriction endonuclease
b. Separate fragments according to length with electrophoresis
c. Use radioactive probe to identify fragments
d. Obtain unique pattern of bands in gel
e. Called “DNA fingerprints”
1) Used in criminal forensic investigations
2) Used as markers to identify carriers of certain genetic disorders

D. Making an Intron-Free Copy of a Eukaryotic Gene
1. Eukaryotic genes encoded in exons separated by nontranslated introns
a. Transcribed gene to form primary transcript
b. Introns cut out during RNA processing to produce mature mRNA transcript
c. Better to transfer processed, not raw, DNA into bacteria
d. Bacteria lack enzymes to do RNA processing
2. Process fig 19.11
a. Isolate cytoplasmic mature mRNA for particular gene
b. Use reverse transcriptase enzyme to make DNA version of mature mRNA

c. Single strand DNA serves as template for synthesis of complementary strand
d. Produce double-stranded DNA lacking introns
e. Molecule called complementary DNA (cDNA)

E. Sequencing DNA: The Sanger Method
1. Most DNA sequencing done by "chain termination" technique
2. Process fig 19.12
a. Short, single-stranded primer added to end of unknown sequence single-strand DNA
b. Primer provides 3' end for DNA polymerase
c. Mix primed fragment, DNA polymerase, four deoxynucleotides (d-nucleotides)
d. Added to four synthesis tubes
1) Each tube contains a different dideoxynucleotide (dd-nucleotide)
2) Each dd-nucleotide lacks 2' and 3' –OH groups, are chain-terminating
e. Example: Tube contains ddATP
1) Synthesis stopped when ddATP added to DNA instead of dATP
2) Low concentration of ddATP compared to dATP
3) Synthesis not stopped at first A site, produces short fragments
4) Tube contains series of fragments of varying lengths
5) Fragments separated by size by electrophoresis
6) Radioactive label allows visualization of fragments on film
7) Newly made sequence read from film
8) Original DNA has complementary sequence

19.3 Biotechnology is producing a scientific revolution

I. DNA SEQUENCE TECHNOLOGY

A. Biotechnology: Application of Genetic Engineering to Practical Problems

B. Genome Sequencing
1. Propose sequencing of entire human genome
a. Construct detailed map of human genome
b. Controversial as it requires significant resources
2. Probing the human genome
a. Localize cloned gene location via radioactive probe
b. Construction of clonal libraries
1) Use large-size restriction fragments
2) Localize chromosomal site of gene using radioactive probes and hybridization
3) Mapping genes at astounding rate
4) Examples: Dyslexia, obesity, cholesterol-proof blood
c. Attempt treatment or cure with gene therapy
3. Complete genome sequencing of organisms with smaller genomes tbl 19.1
a. Generally one-half of genes have known functions
b. Brewer's yeast is first eukaryote totally sequenced
c. Many of 6,000 genes may be similar in structure to human genes
d. Also completed sequencing some larger organisms
1) Malaria parasite (30 Mb)
2) Nematode (100 Mb)
3) *Arabidopsis* plant (100 Mb) fig 19.13
4) *Drosophila* fruit fly (120 Mb)
5) Mouse (300 Mb)
4. Major effort to sequence entire human genome
a. Contains 3,000 Mb (million nucleotide base-pairs)
b. Rapid progress via shot-gun cloning

1) Entire genome fragmented, fragments sequenced by automated machines
2) Computers use overlaps to put fragments in order

c. All sequences complete

C. DNA Fingerprinting
1. First utilized in rape trial in 1987 fig 19.14
a. Consists of autoradiographs of parallel bars on X-ray film
b. Bar represents position of restriction endonuclease fragment
c. Include controls, patterns of two endonucleases on semen collected from victim
2. Comparison of samples
a. Suspect's two patterns match patterns of rapist, not those of victim
b. Semen of rapist and blood sample of suspect came from same person
3. Resulted in first conviction of suspect based on DNA evidence
4. Used as evidence in over 2,000 court cases since then fig 19.15
a. Some probes are common in populations, others are not
b. Use several probes to clearly establish identity
5. Has revolutionized forensic science
a. Can use minute samples of hair, blood, semen
b. Laboratory analyses must be done carefully, eliminate contamination
c. National standards being developed

II. BIOCHIPS

A. A Genetic Microarray on a Slide
1. Glass square covered with millions of DNA strands
2. Uses same process that etches semiconductor circuits into silicon
3. Biochip assembly
a. Scanning beam moves over spots on biochip
b. Instructs addition of base pair onto growing DNA strand
c. Identity of nucleotide dependent on beam wavelength
d. Scanning continues until each strand is complete
e. One biochip contains hundreds of thousands of specific gene sequences
4. Use of biochip
a. Obtain sample of an individual's DNA
b. Flush fluid with that DNA over biochip
c. Any matching genes stick to biochip, detected by computer
5. Researchers already comparing reference sequence of human genome to individuals
a. Differences called single nucleotide polymorphisms (SNPs or snips)
b. SNPs may cause disease or identify hair color
c. Forming a huge database of SNPs
d. Difference between individuals cause by a few thousand SNPs

B. How Biochips Can Be Used to Screen for Cancer
1. Identification of cancer cells needed to select treatment
a. Without specific identity, treatment is general
b. May cause severe side effects
2. Compare mutations to identify types of cancer
a. Difference may be in only a single nucleotide
b. Such spot differences are examples of SNPs
3. Examine bone marrow of patients with two kinds of leukemia fig 19.16
a. Exposed to biochip with 6,817 known human genes
b. Two forms showed variations from normal
c. Two forms showed variation from each other
d. Each had its own characteristic SNPs

C. The Use of Gene Chips Will Soon Be Widespread
   1. May dominate medicine in the new millennium: Good and bad
   2. Researchers now compiling SNP database
      a. Screen for SNPs, compare to database to identify genes leading to genetic disease
      b. Diseases include cystic fibrosis and muscular dystrophy

D. Biochips Raise Critical Issues of Personal Privacy
   1. All 300,000 identified SNPs could fit on a single biochip
      a. Flush individual's DNA over chip to reveal entire SNP profile
      b. All genetic characteristics could be interpreted
   2. Controversy over how genes constitute a person's identity
      a. Strongly affected by genetic makeup
      b. Intelligence and personality traits are 80% heritable
   3. SNP profile reflects such variations
   4. Compare profiles/individuals to correlate DNA to personality and behavior

III. MEDICAL APPLICATIONS

A. Pharmaceuticals
   1. Most obvious commercial application of gene technology
      a. Bacteria can produce gene products in bulk
      b. Include human insulin, interferon, growth hormone, erythropoietin fig 19.17
   2. Produce medically important proteins
      a. Atrial peptides: Regulate blood pressure, kidney function
      b. Tissue plasminogen activator: Dissolves blood clots
   3. Must separate desired protein from bacterial proteins
      a. Time-consuming and expensive
      b. Produce RNA transcripts of cloned genes
      c. Make proteins directly in cell-free culture

B. Gene Therapy
   1. First attempts of transfer of human genes in 1990
      a. Obvious rationale if disease is caused by single defective gene
      b. Add working copy of gene to individual
   2. Clinical trials for several disorders including cystic fibrosis tbl 19.2
   3. Success in adding gene encoding adenosine deaminase to bone marrow

C. Piggyback Vaccines
   1. Produce subunit vaccines for herpes virus and hepatitis viruses fig 19.18
      a. Part of gene for protein-polysaccharide coat isolated
      b. Spliced to vaccinia virus DNA
      c. Live vaccinia added to cell culture with fragments
      d. Recombinant virus carries coat genes of other virus
      e. Infected animal produces antibodies to outer surface of virus
      f. Make antibodies against virus without exposure to it
   2. Clinical trials of new DNA vaccine in 1995
      a. Doesn't depend on antibodies
      b. Associated with cellular immune response
         1) Killer T cells in blood attack infected cells
         2) Infected cells have foreign proteins on outer surface
      c. First attempt used influenza virus gene encoding internal nucleoprotein
         1) Gene spliced onto plasmid, injected into mice
         2) Mice developed strong cellular immune response to influenza

IV. AGRICULTURAL APPLICATIONS

A. Manipulation of Genes in Key Crop Plants
   1. Initial difficulty in identifying suitable plant vector
   2. Plants lack numerous plasmids of bacteria
   3. Currently use Ti (tumor-inducing) plasmid of *Agrobacterium*
      a. Infects broad leaf plants like tomato, tobacco, soybean
      b. Attach other genes to this plasmid — fig 19.19
      c. Desire to develop resistance to disease, frost, other stress, nutritional balance, protein content, herbicide resistance
      d. Does not infect cereal plants like corn, rice, wheat
   4. Development of "Flavr Savr" tomato
      a. Inhibit genes that make cells produce ethylene
      b. Lack of ethylene delays ripening of fruit

B. Herbicide Resistance
   1. Broadleaf plants engineered to be resistant to glyphosate
      a. Active ingredient in Roundup herbicide — fig 19.20
      b. Inhibits enzyme EPSP synthetase, produces aromatic amino acids
      c. Humans get these amino acids from diet, unaffected by glyophosate
      d. Ti plasmid used to insert extra copies of EPSP synthetase gene
      e. Plants overproduce enzyme, overcome glyphosate suppression
   2. New bacterial gene unaffected by glyophosate inserted into plants
   3. Advantages of Roundup resistance
      a. Crops would not need to be weeded
      b. Wide variety of weeds killed and desired crop spared
      c. Glyphosate readily degradable

C. Nitrogen Fixation
   1. Insert legume nitrogen-fixing genes into non-leguminous plants
      a. *nif* genes found in bacteria associated with root nodules
      b. Bacteria convert atmospheric N_2 into NH_4
   2. Plants lacking such bacteria must obtain nitrogen from soil
      a. Farmland depleted of nitrogen unless fertilizer applied
      b. Provide crops with ability to produce own fertilizer
   3. Problems since genes do not function properly in eukaryotic cells

D. Insect Resistance
   1. Insects presently controlled via chemical insecticides
   2. Engineer plants for resistance to insects
      a. *Bacillus thuringiensis* insecticidal protein genes
      b. Ingested by tomato hornworm, converted to toxin
      c. Harmless to animals with different stomach enzymes
      d. Genes introduced into tomato, tobacco via Ti plasmid
      e. Transgenic plants safe from attack by insects that eat them
   3. Other examples
      a. Genetically altered potato kills Colorado potato beetle
      b. Cotton resistant to bollworms
      c. Corn resists European corn borer
   4. Isolation insect-killing enzyme from a fungus
      a. Cholesterol oxidase disrupts insect gut membranes
      b. Fungal Bollgard gene inserted into a variety of crops
      c. Kills variety of insects including cotton boll weevil and Colorado potato beetle

5. Introduce insecticidal protein into root bacteria
   a. *B. thuringiensis* does not normally inhabit roots
   b. Insert gene into root-colonizing bacteria like *Pseudomonas*

E. The Real Promise of Plant Genetic Engineering
1. Cultivation of genetically modified crops is common
   a. Genetically modified soybeans with herbicide resistance
   b. Directly benefits farmer, not consumer
2. Now developing genetically modified plants for consumer
3. Inhabitants of developing countries deficient in iron and vitamin A
4. Rice is poor source of dietary iron fig 19.21
   a. Rice endosperm proteins low in iron
   b. Transfer ferritin gene from bean to rice
   c. Rice contains phytate, inhibits intestinal absorption of iron
   d. Gene for phytate-destroying enzyme transferred from fungus to rice
   e. Rice low in sulfur, needed for iron absorption
   f. Transfer gene for sulfur-rich protein from wild rice
5. Rice is poor source of vitamin A
   a. Production of vitamin A stops at beta-carotene stage
   b. Final four steps to vitamin A can't be done in rice
   c. Genes for these steps transferred from daffodil flower
6. Developing procedures for plants to act as vaccine-producing factories
   a. Gene for vaccine against dog parvovirus put into petunias
   b. Inserted into area with nectar producing genes
   c. Drug extracted from honey produced by bees

F. Farm Animals
1. Somatotropin growth hormone (BST) synthetically produced
   a. Added to dairy cow's diet to increase milk yield fig 19.22
   b. Potential to increase weight of cattle and pigs
   c. Human tests to increase size of hormonal dwarfs
2. Public resistance to BST in milk
   a. Generalized fears of gene technology
   b. BST is a protein, digested in stomach
3. Development of transgenic animals faster than several generations of selective breeding

V. CLONING

A. Breeding Transgenic Animals Is Slow
1. Recombination reverses painstaking work of genetic engineer
2. Ideal is to "xerox" exact clones of the transgenic strain
3. First successful cloning of vertebrate in 1997
4. Stands to revolutionize agricultural science

B. Speman's "Fantastical Experiment"
1. First idea to clone animals in 1938
2. Proposal to remove nucleus from egg, replaced it with nucleus from another cell
   a. Technology appropriate to make attempt in 1952, attempted by Briggs and King
   b. Partial success in 1970 by Gurdon
      1) Inserted nuclei from advanced toad embryos
      2) Eggs developed into tadpoles, died before adulthood

C. The Path to Success
   1. Continued nuclear transplant experiments proved unsuccessful
   2. First successful cloning of sheep in 1984 used nucleus from cell of early embryo
   3. Succeeded at replicating result with other animals
      a. All required use of nucleus from early embryo
      b. Later stages assumed to be "committed" too differentiated to work
   4. Later knowledge proved this commitment idea wrong
      a. Cell division doesn't occur until conditions are proper
      b. Egg and donated nucleus needed to be at same stage
   5. First attempts starved cells to synchronize them at G_1 checkpoint

D. Wilmut's Lamb
   1. Wilmut transplanted nucleus from adult differentiated cell into egg
      a. Removed cells ("Dolly") from udder of six-year-old sheep fig 19.23
      b. Mammary cells grown in tissue culture
      c. Some frozen for future fingerprinting to prove identical genetic content
   2. Reduced nutrient content of mammary cell medium
   3. Eggs removed from ewe, nuclei removed with micropipette
   4. Mammary cells and egg cells surgically combined
      a. Mammary cells inserted inside covering of egg cell
      b. Electric shock causes plasma membranes of cells to become leaky
      c. Contents of mammary cell passes into egg cell
      d. Shock also causes cell to start division cycle
   5. After six days, 30 eggs reached hollow-ball blastula stage
      a. 29 transplanted into surrogate mother sheep
      b. Five months later one gave birth to a lamb in 1997
      c. First successful cloning from differentiated animal cell

E. The Future of Cloning
   1. First attempt is quite inefficient, established feasibility of process
   2. Future research must improve efficiency
      a. 800 mouse cells produced 10 clones
      b. These further produced 7 clones of clones
   3. Can have major impact on medicine as well as agriculture
      a. Animals with human genes to produce human hormones
      b. Sheep genetically engineered to produce alpha-1 antitrypsin in milk
      c. If cloned, can provide source of drug used to treat cystic fibrosis
   4. Question rationale for human cloning

VI. STEM CELLS

A. Stem Cells May Replace Damaged or Lost Tissue
   1. Embryonic cells first isolated in 1998
   2. Cells produced in early development of zygote
      a. Ball of a few dozen identical cells
      b. Each cell capable of producing healthy individual
      c. Used to produce multiple clones in cattle breeding
   3. Cells capable of developing into any kind of tissue fig 19.24
      a. Mouse stem cells grown to form healthy heart tissue
      b. Successfully integrated with living mouse heart tissue
      c. May be used to replace damaged spinal cord tissue
   4. Source of cells is discarded or aborted embryos, raises ethical issues

B. Tissue-Specific Stem Cells
   1. Developing new techniques to combat ethical issues
   2. While developing, embryonic stem cells take different developmental paths
      a. Once destined to be nerve cell, cannot become anything else
      b. Each major tissue destined to become one kind of tissue-specific stem cell
   3. Uses adult cells rather than embryonic stem cells

C. Transplanted Tissue-Specific Stem Cells Cure MS in Mice
   1. In 1999, tissue-specific stem cells restored lost brain tissue
      a. Injected neural stem cells into brains of newborn mice
      b. Mice had MS-like disease, lacked cells to produce myelin
      c. Injected cells migrated through brain, developed into missing cells
      d. Many treated mice fully recovered
   2. Human blood stem cells used to replace bone marrow in cancer patients

D. Transplanted Stem Cells Reverse Juvenile Diabetes in Mice
   1. Type-I juvenile diabetes individual lacks insulin-producing pancreas cells
      a. Person's immune cells attack and destroy these pancreas cells
      b. Insufficient insulin produced, must be injected daily
      c. Adding islet cells to pancreas doesn't work, new cells destroyed by immune system
   2. In 2000, Peck added stem cells to mouse pancreas
      a. Collected epithelial cells from pancreatic duct
      b. Cells grown in culture
      c. Ultimately produced insulin in response to sugar
      d. Culture contained stem cells from which islet cells developed
      e. Cells injected into diabetic mice – diabetes reversed
   3. Mice sacrificed, pancreas observed to contain normal islet cells
   4. Yet unknown if effect transitory or permanent
   5. Experimental trails with humans
      a. Cell source is donated organs from dead adults
      b. No ethical issues exist

VII. ETHICS AND REGULATION

A. Concerns Regarding Tampering with Genetic Material
   1. Accidental production of a cancer-transmitting bacterium
   2. Intentional development of a killer virus
   3. Dangerous complications of genetically engineered products administered to plants or animals in future generations
   4. Ecological impact of "improved" crops
   5. Potential of creating "genetically superior" organisms, including humans

B. How Do We Measure the Potential Risks of Genetically Modified Crops?
   1. Protesters attack experimental plots of genetically modified (GM) crops
   2. Is eating a genetically modified food dangerous?
      a. Some gene modifications make crops easier to grow
         1) Example: Roundup-resistant soybeans
         2) Beans raised without intensive cultivation to destroy weeds
         3) Soybean not nutritionally different
      b. Some modifications improve nutritional character of food
         1) Consumers may be allergic to product of introduced gene
         2) Example: Brazil nut gene in soybean caused reactions, use discontinued
         3) Methionine gene now used is from sunflower
      c. Risk on food supply is slight

3. Are GM crops harmful to the environment?
   a. GM corn pollen could kill butterflies eating it
      1) Will kill any insects in near vicinity of GM corn
      2) Eliminates need to spray insecticides which also kill butterflies
   b. Development of GM resistance slower than resistance developed to pesticides
   c. Transfer of introduced genes to plant relatives unlikely
      1) Potential relatives in crop fields unlikely
      2) Transfer of genes to other organisms unlikely

C. Should We Label Genetically Modified Foods?
   1. Risk is low, but consumers should be notified
      a. Little need for labeling since risk is low
      b. Public still has right to know when food product is altered
   2. Public caution based on fear of future danger/damage
   3. Labels need to be informative, not merely declarative
      a. Not simple "GM FOOD" label analogous to "POISON" label
      b. Short explanation of kind of processing and its purpose/advantage

INSTRUCTIONAL STRATEGY

PRESENTATION ASSISTANCE:

Here we have what's happening in biology today; where the money is to be made!

Discuss the mechanisms of gene technology before discussing its implications. Students have a hard time with the nature of the "sticky ends" resulting from treatment with restriction enzymes. To complicate matters, some blunt cutting enzymes have been discovered as well. It might be helpful to present them with several DNA sequences and show how different restriction enzymes would fragment the sequence. You can then show how identical "sticky ends" can be joined together. Four common restriction endonucleases are listed below; the ↓ indicates the endonuclease cleavage site.

*Eco*III	A↓AGCTT
Bg/II	A↓GATCT
*EcoR*I	G↓AATTC
*Nun*II	GG↓CGCC

Genetic engineering would be significantly more difficult without plasmid and viral vectors. Plasmids were presented in the last chapter, viruses were discussed to some extent in the chapter before that. Recall in either case, how the vector is naturally able to insert genetic material into a complete genome, the plasmid into bacteria, the virus into eukaryotes causing some forms of cancer. Science is merely adapting a natural phenomenon to its own benefit.

Screening is not only the most difficult part of genetic engineering to do, it is the hardest part to understand. Include the presence of the antibiotic resistance gene at the onset of your discussion. Explain its function at the screening step. The many technical terms associated with gene technology can be confusing; most are associated with genetic engineering in that they are means for identifying the cell with the correct stuff.

Probes have been developed for a number of tumor cell lines and Huntington's disease. The latter is 95% to 98% accurate in determining whether the gene is present. Thus persons with the disease in their family background can be tested long before the onset of the disease itself (most individuals refuse testing or are tested and don't want to be told the results). Knowledge of test results may impact personal lifestyle and plans for having children as well as insurance and health policies.

One merely needs to pick up the science section of the weekly newspaper, or a lay science magazine to see examples of gene technology in action. As a result, it is important to discuss the implications of such research and the necessary scientific and governmental regulations. This is one of the stronger reasons to have some knowledge of biology, to be able to make informed decisions, and to determine if the decisions made by those in power are indeed in the best interest of the populace. Someone will need to make difficult

decisions in the not-so-distant future. Just because science can perform certain technological feats doesn't mean that it should be allowed to do so. Conversely, just because some gene technology is potentially dangerous, doesn't mean that all related technology should be brought to a halt. It's your students who will be making the political decisions for the future of the world.

Cloning may not produce identical mammals as everyone expects. The maternal environment is vitally important to the development of the individual. Studies of fraternal twins versus siblings have shown that the former are much more alike than the latter, likely a result of maturing together within the same womb. Although some mammals are genetically very alike, humans are not. Considering the prenatal environment, the early nurturing environment and experience, it is highly unlikely that two completely identical, functional human clones will exist in the near future.

VISUAL RESOURCES:

Palindromes are words that exhibit two-fold rotational symmetry (bob, kook, deed). The phrase "a toyota" is a palindrome as is "a man, a plan, a canal, panama." Search the web for thousands of examples, but start here: http://www.cs.rdg.ac.uk/archive/evihcra/ku.ca.gdr.sc.www//:ptth/. Hopefully you will notice that the url itself is a palindrome!

Watch the comedy film "*Multiplicity*" starring Michael Keaton. He clones himself to help get work done, and still be able to spend time with his family. It is entertaining and has accurate undertones like the clones share memory until point of cloning, then they have their own, unshared memories. Also, the first clone clones himself with less pleasing results, this clone then clones himself. The resulting clone is quite dimwitted.

The scifi film "*Gattaca*" touches on future (or maybe not so future!) gene technology and the ethical implications of genetic control. Substantial information is available at the movie website http://www.sciflicks.com/gattaca/.