Chapter 20

DNA Technology and Genomics

PowerPoint Lectures for
Biology, Seventh Edition
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- Overview: Understanding and Manipulating Genomes
- One of the greatest achievements of modern science
  - Has been the sequencing of the human genome, which was largely com pleted by 2003
- DNA sequencing accomplishments
  - Have all depended on advances in DNA technology, starting with the invention of methods for making recombinant DNA

- DNA technology has launched a revolution in the area of biotechnology
  - The manipulation of organisms or their genetic components to make useful products
- An example of DNA technology is the microarray
  - A measurement of gene expression of thousands of different genes

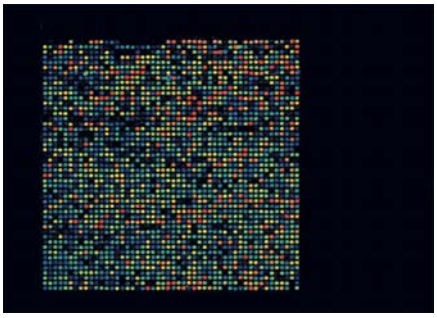


Figure 20.1

- Concept 20.1: DNA cloning permits production of multiple copies of a specific gene or other DNA segment
- To work directly with specific genes
  - Scientists have developed methods for preparing well-defined, gene-sized pieces of DNA in multiple identical copies, a process called gene cloning

DNA Cloning and Its Applications: *A Preview*

- Most methods for cloning pieces of DNA in the laboratory
  - Share certain general features, such as the use of bacteria and their plasmids

- Overview of gene cloning with a bacterial plasmid, showing various uses of cloned genes

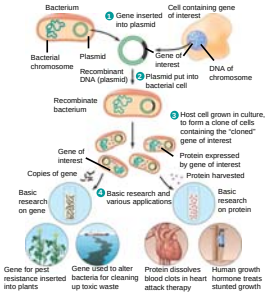


Figure 20.2

Using Restriction Enzymes to Make Recombinant DNA

- Bacterial restriction enzymes
  - Cut DNA molecules at a limited number of specific DNA sequences, called restriction sites

- A restriction enzyme will usually make many cuts in a DNA molecule
  - Yielding a set of restriction fragments
- The most useful restriction enzymes cut DNA in a staggered way
  - Producing fragments with "sticky ends" that can bond with complementary "sticky ends" of other fragments
- DNA ligase is an enzyme
  - That seals the bonds between restriction fragments

- Using a restriction enzyme and DNA ligase to make recombinant DNA

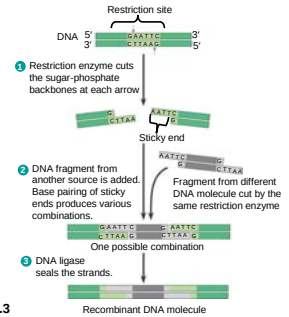


Figure 20.3

Cloning a Eukraryotic Gene in a Bacterial Plasmid

- In gene cloning, the original plasmid is called a cloning vector
  - Defined as a DNA molecule that can carry foreign DNA into a cell and replicate there

Producing Clones of Cells

APPLICATION Cloning is used to prepare many copies of a gene of interest for use in sequencing the gene, in producing its encoded protein, in gene therapy, or in basic research.

TECHNIQUE In this example, a human gene is inserted into a plasmid from *E. coli*. The plasmid contains the amp^R gene, which makes *E. coli* cells resistant to the antibiotic ampicillin. It also contains the *lacZ* gene, which encodes β-galactosidase. This enzyme hydrolyzes a molecular mimic of lactose (X-gal) to form a blue product. Only three plasmids and three human DNA fragments are shown, but millions of copies of the plasmid and a mixture of millions of different human DNA fragments would be present in the samples.

1. Isolate plasmid DNA and human DNA.
2. Cut both DNA samples with the same restriction enzyme
3. Mix the DNAs; they join by base pairing. The products are recombinant plasmids and many nonrecombinant plasmids.

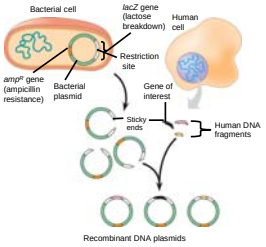
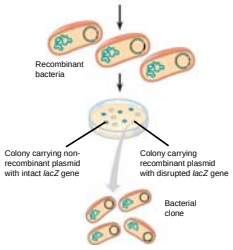


Figure 20.4

4 Introduce the DNA into bacterial cells that have a mutation in their own *lacZ* gene.

5 Plate the bacteria on agar containing ampicillin and X-gal. Incubate until colonies grow.



RESULTS Only a cell that took up a plasmid, which has the amp^R gene, will reproduce and form a colony. Colonies with nonrecombinant plasmids will be blue, because they can hydrolyze X-gal. Colonies with recombinant plasmids, in which *lacZ* is disrupted, will be white, because they cannot hydrolyze X-gal. By screening the white colonies with a nucleic acid probe (see Figure 20.5), researchers can identify clones of bacterial cells carrying the gene of interest.

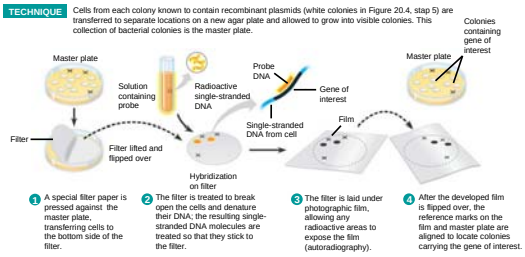
Identifying Clones Carrying a Gene of Interest

- A clone carrying the gene of interest
  - Can be identified with a radioactively labeled nucleic acid probe that has a sequence complementary to the gene, a process called nucleic acid hybridization

- Nucleic acid probe hybridization

APPLICATION Hybridization with a complementary nucleic acid probe detects a specific DNA within a mixture of DNA molecules. In this example, a collection of bacterial clones (colonies) are screened to identify those carrying a plasmid with a gene of interest.

TECHNIQUE Cells from each colony known to contain recombinant plasmids (white colonies in Figure 20.4, stap 5) are transferred to separate locations on a new agar plate and allowed to grow into visible colonies. This collection of bacterial colonies is the master plate.



1 A special filter paper is pressed against the master plate, transferring cells to the bottom side of the filter.

2 The filter is treated to break open the cells and denature their DNA; the resulting single-stranded DNA molecules are treated so that they stick to the filter.

3 The filter is laid under photographic film, allowing any radioactive areas to expose the film (autoradiography).

4 After the developed film is flipped over, the reference marks on the film and master plate are aligned to locate colonies carrying the gene of interest.

RESULTS Colonies of cells containing the gene of interest have been identified by nucleic acid hybridization. Cells from colonies tagged with the probe can be grown in large tanks of liquid growth medium. Large amounts of the DNA containing the gene of interest can be isolated from these cultures. By using probes with different nucleotide sequences, the collection of bacterial clones can be screened for different genes.

Figure 20.5

Storing Cloned Genes in DNA Libraries

- A genomic library made using bacteria
  - Is the collection of recombinant vector clones produced by cloning DNA fragments derived from an entire genome

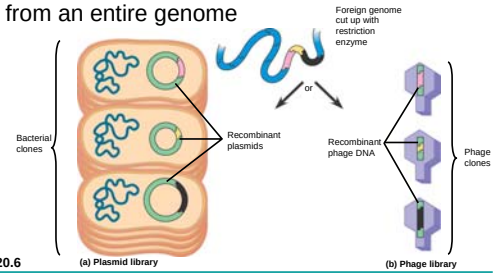


Figure 20.6

- A genomic library made using bacteriophages
  - Is stored as a collection of phage clones

- A complementary DNA (cDNA) library
  - Is made by cloning DNA made *in vitro* by reverse transcription of all the mRNA produced by a particular cell

Cloning and Expressing Eukaryotic Genes

- As an alternative to screening a DNA library for a particular nucleotide sequence
  - The clones can sometimes be screened for a desired gene based on detection of its encoded protein

Bacterial Expression Systems

- Several technical difficulties
  - Hinder the expression of cloned eukaryotic genes in bacterial host cells
- To overcome differences in promoters and other DNA control sequences
  - Scientists usually employ an expression vector, a cloning vector that contains a highly active prokaryotic promoter

Eukaryotic Cloning and Expression Systems

- The use of cultured eukaryotic cells as host cells and yeast artificial chromosomes (YACs) as vectors
  - Helps avoid gene expression problems

Amplifying DNA *in Vitro*: The Polymerase Chain Reaction (PCR)

- The polymerase chain reaction, PCR
  - Can produce many copies of a specific target segment of DNA
  - Uses primers that bracket the desired sequence
  - Uses a heat-resistant DNA polymerase

- The PCR procedure

APPLICATION With PCR, any specific segment—the target sequence—within a DNA sample can be copied many times (amplified) completely *in vitro*.

TECHNIQUE The starting materials for PCR are double-stranded DNA containing the target nucleotide sequence to be copied, a heat-resistant DNA polymerase, all four nucleotides, and two short, single-stranded DNA molecules that serve as primers. One primer is complementary to one strand at one end of the target sequence; the second is complementary to the other strand at the other end of the sequence.

RESULTS During each PCR cycle, the target DNA sequence is doubled. By the end of the third cycle, one-fourth of the molecules correspond exactly to the target sequence, with both strands of the correct length (see white boxes above). After 20 or so cycles, the target sequence molecules outnumber all others by a billionfold or more.

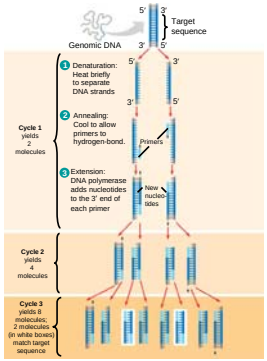


Figure 20.7

- Concept 20.2: Restriction fragment analysis detects DNA differences that affect restriction sites
- Restriction fragment analysis
  - Can rapidly provide useful comparative information about DNA sequences

Gel Electrophoresis and Southern Blotting

- Gel electrophoresis
  - Separates DNA restriction fragments of different lengths

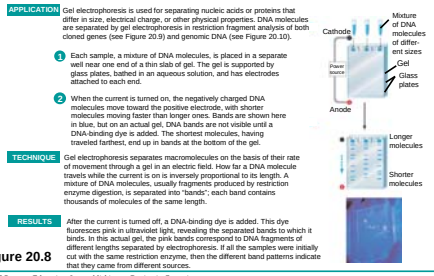


Figure 20.8

- Restriction fragment analysis
  - Is useful for comparing two different DNA molecules, such as two alleles for a gene

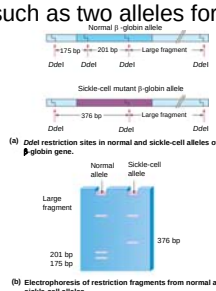


Figure 20.9a, b

- Specific DNA fragments can be identified by Southern blotting
  - Using labeled probes that hybridize to the DNA immobilized on a "blot" of the gel

- Southern blotting of DNA fragments

APPLICATION Researchers can detect specific nucleotide sequences within a DNA sample with this method. In particular, Southern blotting is useful for comparing the restriction fragments produced from different samples of genomic DNA.

TECHNIQUE In this example, we compare genomic DNA samples from three individuals: a homozygote for the normal β-globin allele (I), a homozygote for the mutant sickle-cell allele (II), and a heterozygote (III).

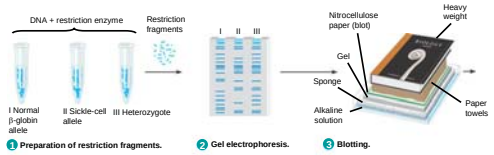
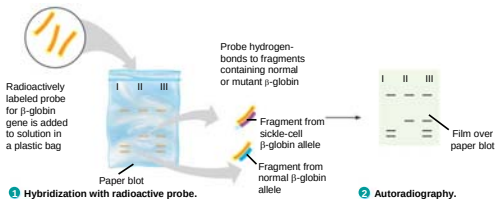


Figure 20.10



RESULTS Because the band patterns for the three samples are clearly different, this method can be used to identify heterozygous carriers of the sickle-cell allele (III), as well as those with the disease, who have two mutant alleles (II), and unaffected individuals, who have two normal alleles (I). The band patterns for samples I and II resemble those observed for the purified normal and mutant alleles, respectively, seen in Figure 20.9b. The band pattern for the sample from the heterozygote (III) is a combination of the patterns for the two homozygotes (I and II).

Restriction Fragment Length Differences as Genetic Markers

- Restriction fragment length polymorphisms (RFLPs)
  - Are differences in DNA sequences on homologous chromosomes that result in restriction fragments of different lengths

- Specific fragments
  - Can be detected and analyzed by Southern blotting
- The thousands of RFLPs present throughout eukaryotic DNA
  - Can serve as genetic markers

- Concept 20.3: Entire genomes can be mapped at the DNA level
- The Human Genome Project
  - Sequenced the human genome
- Scientists have also sequenced genomes of other organisms
  - Providing important insights of general biological significance

Genetic (Linkage) Mapping: Relative Ordering of Markers

- The initial stage in mapping a large genome
  - Is to construct a linkage map of several thousand genetic markers spaced throughout each of the chromosomes

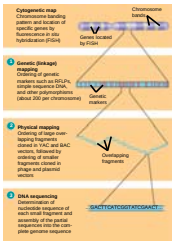


Figure 20.11

- The order of the markers and the relative distances between them on such a map
  - Are based on recombination frequencies

Physical Mapping: Ordering DNA Fragments

- A physical map
  - Is constructed by cutting a DNA molecule into many short fragments and arranging them in order by identifying overlaps
  - Gives the actual distance in base pairs between markers

DNA Sequencing

- Relatively short DNA fragments
  - Can be sequenced by the dideoxy chain-termination method

- Dideoxy chain-termination method for sequencing DNA

APPLICATION The sequence of nucleotides in any cloned DNA fragment up to about 800 base pairs in length can be determined rapidly with specialized machines that carry out sequencing reactions and separate the labeled reaction products by length.

TECHNIQUE This method synthesizes a nested set of DNA strands complementary to the original DNA fragment. Each strand starts with the same primer and ends with a dideoxyribonucleotide (ddNTP), a modified nucleotide. Incorporation of a ddNTP terminates a growing DNA strand because it lacks a 3′—OH group, the site for attachment of the next nucleotide (see Figure 16.12). In the set of strands synthesized, each nucleotide position along the original sequence is represented by strands ending at that point with the complementary ddNT. Because each type of ddNTP is tagged with a distinct fluorescent label, the identity of the ending nucleotides of the new strands, and ultimately the entire original sequence, can be determined.

RESULTS The color of the fluorescent tag on each strand indicates the identity of the nucleotide at its end. The results can be printed out as a spectrogram, and the sequence, which is complementary to the template strand, can then be read from bottom to top. (Notice that the sequence here begins after the primer.)

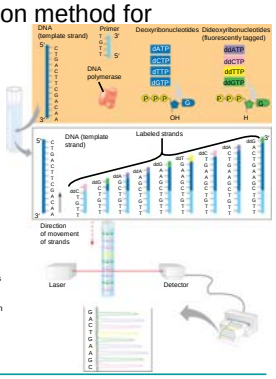


Figure 20.12

- Linkage mapping, physical mapping, and DNA sequencing
  - Represent the overarching strategy of the Human Genome Project
- An alternative approach to sequencing whole genomes starts with the sequencing of random DNA fragments
  - Powerful computer programs would then assemble the resulting very large number of overlapping short sequences into a single continuous sequence

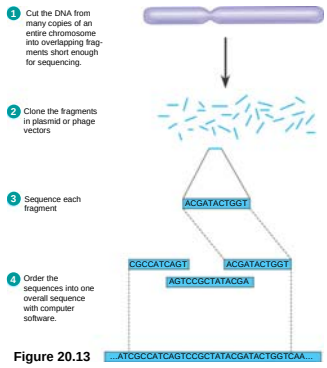


Figure 20.13

- Concept 20.4: Genome sequences provide clues to important biological questions
- In genomics
  - Scientists study whole sets of genes and their interactions

Identifying Protein-Coding Genes in DNA Sequences

- Computer analysis of genome sequences
  - Helps researchers identify sequences that are likely to encode proteins

- Current estimates are that the human genome contains about 25,000 genes
  - But the number of human proteins is much larger

Table 20.1 Genome Sizes and Estimated Numbers of Genes*

Organism	Haploid Genome Size (Mb)	Number of Genes	Genes per Mb
Haemophilus influenzae (bacterium)	1.8	1,700	940
Escherichia coli (bacterium)	4.6	4,400	950
Saccharomyces cerevisiae (yeast)	12	5,800	480
Caenorhabditis elegans (nematode)	97	19,000	200
Arabidopsis thaliana (plant)	118	25,500	215
Drosophila melanogaster (fruit fly)	180	13,700	76
Oryza sativa (rice)	430	60,000	140
Danio rerio (zebrafish)	1,700	22,000	13
Mus musculus (house mouse)	2,600	25,000	11
Homo sapiens (human)	2,900	25,000	10
Fritillaria assyriaca (plant)	120,000	ND	ND

*Strictly defined, "genome" refers to the haploid genome of an organism. Some values given here are likely to be revised as genome analysis continues. Mb = million base pairs. ND = not determined.

Table 20.1

- Comparison of the sequences of “new” genes
  - With those of known genes in other species may help identify new genes

Determining Gene Function

- For a gene of unknown function
  - Experimental inactivation of the gene and observation of the resulting phenotypic effects can provide clues to its function

Studying Expression of Interacting Groups of Genes

- DNA microarray assays allow researchers to compare patterns of gene expression
  - In different tissues, at different times, or under different conditions

- DNA microarray assay of gene expression levels

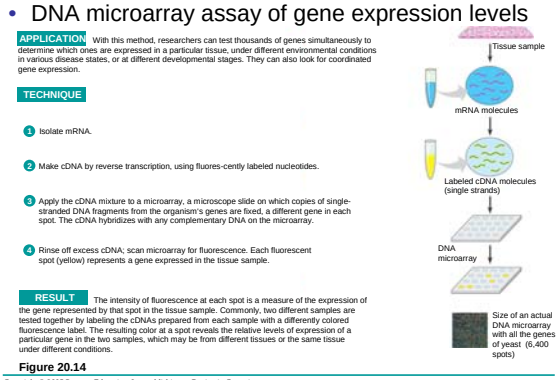


Figure 20.14

Comparing Genomes of Different Species

- Comparative studies of genomes from related and widely divergent species
  - Are providing valuable information in many fields of biology

Future Directions in Genomics

- Genomics
  - Is the study of entire genomes
- Proteomics
  - Is the systematic study of all the proteins encoded by a genome
- Single nucleotide polymorphisms (SNPs)
  - Provide useful markers for studying human genetic variation

- Concept 20.5: The practical applications of DNA technology affect our lives in many ways
- Numerous fields are benefiting from DNA technology and genetic engineering

Medical Applications

- One obvious benefit of DNA technology
  - Is the identification of human genes whose mutation plays a role in genetic diseases

Diagnosis of Diseases

- Medical scientists can now diagnose hundreds of human genetic disorders
  - By using PCR and primers corresponding to cloned disease genes, then sequencing the amplified product to look for the disease-causing mutation

- Even when a disease gene has not yet been cloned
  - The presence of an abnormal allele can be diagnosed with reasonable accuracy if a closely linked RFLP marker has been found

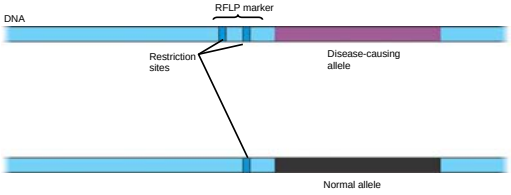


Figure 20.15

Human Gene Therapy

- Gene therapy
  - Is the alteration of an afflicted individual's genes
  - Holds great potential for treating disorders traceable to a single defective gene
  - Uses various vectors for delivery of genes into cells

- Gene therapy using a retroviral vector

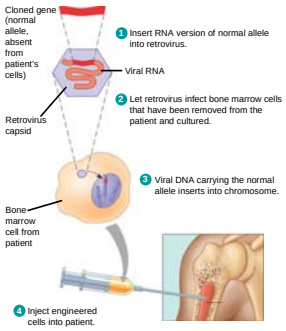


Figure 20.16

Pharmaceutical Products

- Applications of DNA technology include
  - Large-scale production of human hormones and other proteins with therapeutic uses
  - Production of safer vaccines

Forensic Evidence

- DNA "fingerprints" obtained by analysis of tissue or body fluids found at crime scenes
  - Can provide definitive evidence that a suspect is guilty or not

- A DNA fingerprint
  - Is a specific pattern of bands of RFLP markers on a gel

Figure 20.17

- DNA fingerprinting
  - Can also be used in establishing paternity

Environmental Cleanup

- Genetic engineering can be used to modify the metabolism of microorganisms
  - So that they can be used to extract minerals from the environment or degrade various types of potentially toxic waste materials

Agricultural Applications

- DNA technology
  - Is being used to improve agricultural productivity and food quality

Animal Husbandry and "Pharm" Animals

- Transgenic animals
  - Contain genes from other organisms

  - Have been engineered to be pharmaceutical "factories"

Figure 20.18

Genetic Engineering in Plants

- Agricultural scientists
  - Have already endowed a number of crop plants with genes for desirable traits

- The Ti plasmid
  - Is the most commonly used vector for introducing new genes into plant cells

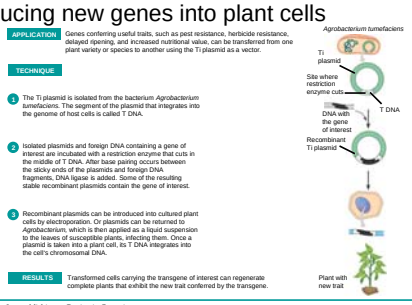


Figure 20.19

Safety and Ethical Questions Raised by DNA Technology

- The potential benefits of genetic engineering
  - Must be carefully weighed against the potential hazards of creating products or developing procedures that are harmful to humans or the environment

- Today, most public concern about possible hazards
  - Centers on genetically modified (GM) organisms used as food