Introduction – Chapter 12

- DNA technology
  - has rapidly revolutionized the field of forensics,
  - permits the use of gene cloning to produce medical and industrial products,
  - allows for the development of genetically modified organisms for agriculture,
  - permits the investigation of historical questions about human family and evolutionary relationships, and
  - is invaluable in many areas of biological research.

Figure 12.0_1

Chapter 12: Big Ideas

- Gene Cloning
- Genetically Modified Organisms
- DNA Profiling
- Genomics
Genes can be cloned in recombinant plasmids

- **Biotechnology** is the manipulation of organisms or their components to make useful products.
- For thousands of years, humans have
  - used microbes to make wine and cheese and
  - selectively bred stock, dogs, and other animals.
- **DNA technology** is the set of modern techniques used to study and manipulate genetic material.

- **Genetic engineering** involves manipulating genes for practical purposes.
  - **Gene cloning** leads to the production of multiple, identical copies of a gene-carrying piece of DNA.
  - **Recombinant DNA** is formed by joining nucleotide sequences from two different sources.
    - One source contains the gene that will be cloned.
    - Another source is a gene carrier, called a **vector**.
    - **Plasmids** (small, circular DNA molecules independent of the bacterial chromosome) are often used as vectors.

**Steps in cloning a gene**

1. Plasmid DNA is isolated.
2. DNA containing the gene of interest is isolated.
3. Plasmid DNA is treated with a restriction enzyme that cuts in one place, opening the circle.
4. DNA with the target gene is treated with the same enzyme and many fragments are produced.
5. Plasmid and target DNA are mixed and associate with each other.
12.1 Genes can be cloned in recombinant plasmids

6. Recombinant DNA molecules are produced when DNA ligase joins plasmid and target segments together.

7. The recombinant plasmid containing the target gene is taken up by a bacterial cell.

8. The bacterial cell reproduces to form a clone, a group of genetically identical cells descended from a single ancestral cell.
12.2 Enzymes are used to “cut and paste” DNA

- **Restriction enzymes** cut DNA at specific sequences.
  - Each enzyme binds to DNA at a different restriction site.
  - Many restriction enzymes make staggered cuts that produce **restriction fragments** with single-stranded ends called “sticky ends.”
  - Fragments with complementary sticky ends can associate with each other, forming recombinant DNA.
- DNA ligase joins DNA fragments together.
12.3 Cloned genes can be stored in genomic libraries

- A **genomic library** is a collection of all of the cloned DNA fragments from a target genome.
- Genomic libraries can be constructed with different types of vectors:
  - plasmid library: genomic DNA is carried by plasmids,
  - bacteriophage (phage) library: genomic DNA is incorporated into bacteriophage DNA,
  - bacterial artificial chromosome (BAC) library: specialized plasmids that can carry large DNA sequences.

![Diagram of cloning process]

12.4 Reverse transcriptase can help make genes for cloning

- **Complementary DNA (cDNA)** can be used to clone eukaryotic genes.
  - In this process, mRNA from a specific cell type is the template.
  - **Reverse transcriptase** produces a DNA strand from mRNA.
  - DNA polymerase produces the second DNA strand.
12.4 Reverse transcriptase can help make genes for cloning

- Advantages of cloning with cDNA include the ability to
  - study genes responsible for specialized characteristics of a particular cell type and
  - obtain gene sequences
    - that are smaller in size,
    - easier to handle, and
    - do not have introns.

12.5 Nucleic acid probes identify clones carrying specific genes

- **Nucleic acid probes** bind very selectively to cloned DNA.
  - Probes can be DNA or RNA sequences complementary to a portion of the gene of interest.
  - A probe binds to a gene of interest by base pairing.
  - Probes are labeled with a radioactive isotope or fluorescent tag for detection.
12.5 Nucleic acid probes identify clones carrying specific genes

- One way to screen a gene library is as follows:
  1. Bacterial clones are transferred to filter paper.
  2. Cells are broken apart and the DNA is separated into single strands.
  3. A probe solution is added and any bacterial colonies carrying the gene of interest will be tagged on the filter paper.
  4. The clone carrying the gene of interest is grown for further study.

12.6 Recombinant cells and organisms can mass-produce gene products

- Recombinant cells and organisms constructed by DNA technologies are used to manufacture many useful products, chiefly proteins.
- Bacteria are often the best organisms for manufacturing a protein product because bacteria
  - have plasmids and phages available for use as gene-cloning vectors,
  - can be grown rapidly and cheaply,
  - can be engineered to produce large amounts of a particular protein, and
  - often secrete the proteins directly into their growth medium.
12.6 Recombinant cells and organisms can mass-produce gene products

- Yeast cells
  - are eukaryotes,
  - have long been used to make bread and beer,
  - can take up foreign DNA and integrate it into their genomes,
  - have plasmids that can be used as gene vectors, and
  - are often better than bacteria at synthesizing and secreting eukaryotic proteins.

- Mammalian cells must be used to produce proteins with chains of sugars. Examples include
  - human erythropoietin (EPO), which stimulates the production of red blood cells,
  - factor VIII to treat hemophilia, and
  - tissue plasminogen activator (TPA) used to treat heart attacks and strokes.

### Table 12.6

<table>
<thead>
<tr>
<th>Product</th>
<th>Mode of Use</th>
<th>Mode of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human insulin</td>
<td>E. coli</td>
<td>Treatment for diabetes</td>
</tr>
<tr>
<td>Human growth hormone (HGH)</td>
<td>E. coli</td>
<td>Treatment for growth defects</td>
</tr>
<tr>
<td>Epidermal growth factor (EGF)</td>
<td>E. coli</td>
<td>Treatment for burns, ulcers</td>
</tr>
<tr>
<td>Interleukin 2 (IL-2)</td>
<td>E. coli</td>
<td>Resistant treatment for cancer</td>
</tr>
<tr>
<td>Bone growth hormone (BGH)</td>
<td>E. coli</td>
<td>Increasing weight gain in cattle</td>
</tr>
<tr>
<td>Cellulase</td>
<td>E. coli</td>
<td>Breaking down cellulose for animal feeds</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>E. coli</td>
<td>Treatment for ovarian cancer</td>
</tr>
<tr>
<td>Interferon beta and gamma</td>
<td>S. cerevisiae</td>
<td>Resistant treatment for cancer and viral infections</td>
</tr>
<tr>
<td>Neutrophil B vaccine</td>
<td>S. cerevisiae</td>
<td>Repair of viral infections</td>
</tr>
<tr>
<td>Interferon alpha (IFN)</td>
<td>Mammalian cells</td>
<td>Treatment for arthritis</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Mammalian cells</td>
<td>Treatment for hemophilia</td>
</tr>
<tr>
<td>Tissue plasminogen activator (TPA)</td>
<td>Mammalian cells</td>
<td>Treatment for heart attacks and some strokes</td>
</tr>
</tbody>
</table>
12.6 Recombinant cells and organisms can mass-produce gene products

- Pharmaceutical researchers are currently exploring the mass production of gene products by
  - whole animals or
  - plants.
- Recombinant animals
  - are difficult and costly to produce and
  - must be cloned to produce more animals with the same traits.
12.7 CONNECTION: DNA technology has changed the pharmaceutical industry and medicine

- Products of DNA technology are already in use.
  - Therapeutic hormones produced by DNA technology include
    - insulin to treat diabetes and
    - human growth hormone to treat dwarfism.
  - DNA technology is used to
    - test for inherited diseases,
    - detect infectious agents such as HIV, and
    - produce vaccines, harmless variants (mutants) or derivatives of a pathogen that stimulate the immune system.
12.8 CONNECTION: Genetically modified organisms are transforming agriculture

- **Genetically modified (GM)** organisms contain one or more genes introduced by artificial means.
- **Transgenic organisms** contain at least one gene from another species.

The most common vector used to introduce new genes into plant cells is
- a plasmid from the soil bacterium *Agrobacterium tumefaciens* and
- called the **Ti plasmid**.

**Figure 12.8A**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The gene is inserted into the plasmid.</td>
</tr>
<tr>
<td>2</td>
<td>The recombinant plasmid is introduced into a plant cell.</td>
</tr>
<tr>
<td>3</td>
<td>The plant cell grows into a plant.</td>
</tr>
<tr>
<td>4</td>
<td>A plant with the new trait</td>
</tr>
</tbody>
</table>

**DNA containing the gene for a desired trait**

**Restriction site**

**Agrobacterium tumefaciens**

**Recombinant Ti plasmid**

**Plant cell**

**DNA carrying the new gene**
12.8 CONNECTION: Genetically modified organisms are transforming agriculture

- GM plants are being produced that
  - are more resistant to herbicides and pests and
  - provide nutrients that help address malnutrition.
- GM animals are being produced with improved nutritional or other qualities.

12.9 Genetically modified organisms raise concerns about human and environmental health

- Scientists use safety measures to guard against production and release of new pathogens.
- Concerns related to GM organisms include the potential
  - introduction of allergens into the food supply and
  - spread of genes to closely related organisms.
- Regulatory agencies are trying to address the
  - safety of GM products,
  - labeling of GM produced foods, and
  - safe use of biotechnology.
Gene therapy aims to treat a disease by supplying a functional allele.

One possible procedure is the following:

1. Clone the functional allele and insert it in a retroviral vector.
2. Use the virus to deliver the gene to an affected cell type from the patient, such as a bone marrow cell.
3. Viral DNA and the functional allele will insert into the patient’s chromosome.
4. Return the cells to the patient for growth and division.

Gene therapy is an alteration of an afflicted individual’s genes and attempt to treat disease.

Gene therapy may be best used to treat disorders traceable to a single defective gene.
12.10 CONNECTION: Gene therapy may someday help treat a variety of diseases

- The first successful human gene therapy trial in 2000
  - tried to treat ten children with SCID (severe combined immune deficiency),
  - helped nine of these patients, but
  - caused leukemia in three of the patients, and
  - resulted in one death.

12.10 CONNECTION: Gene therapy may someday help treat a variety of diseases

- The use of gene therapy raises many questions.
  - How can we build in gene control mechanisms that make appropriate amounts of the product at the right time and place?
  - How can gene insertion be performed without harming other cell functions?
  - Will gene therapy lead to efforts to control the genetic makeup of human populations?
  - Should we try to eliminate genetic defects in our children and descendants when genetic variety is a necessary ingredient for the survival of a species?
12.11 The analysis of genetic markers can produce a DNA profile

- **DNA profiling** is the analysis of DNA fragments to determine whether they come from the same individual. DNA profiling
  - compares genetic markers from noncoding regions that show variation between individuals and
  - involves amplifying (copying) of markers for analysis.

12.12 The PCR method is used to amplify DNA sequences

- **Polymerase chain reaction (PCR)** is a method of amplifying a specific segment of a DNA molecule.
- PCR relies upon a pair of **primers** that are
  - short,
  - chemically synthesized, single-stranded DNA molecules, and
  - complementary to sequences at each end of the target sequence.
- PCR
  - is a three-step cycle that
  - doubles the amount of DNA in each turn of the cycle.
12.12 The PCR method is used to amplify DNA sequences

- The advantages of PCR include
  - the ability to amplify DNA from a small sample,
  - obtaining results rapidly, and
  - a reaction that is highly sensitive, copying only the target sequence.

12.13 Gel electrophoresis sorts DNA molecules by size

- Gel electrophoresis can be used to separate DNA molecules based on size as follows:
  1. A DNA sample is placed at one end of a porous gel.
  2. Current is applied and DNA molecules move from the negative electrode toward the positive electrode.
  3. Shorter DNA fragments move through the gel matrix more quickly and travel farther through the gel.
  4. DNA fragments appear as bands, visualized through staining or detecting radioactivity or fluorescence.
  5. Each band is a collection of DNA molecules of the same length.
12.14 STR analysis is commonly used for DNA profiling

- **Repetitive DNA** consists of nucleotide sequences that are present in multiple copies in the genome.
- **Short tandem repeats (STRs)** are short nucleotide sequences that are repeated in tandem,
  - composed of different numbers of repeating units in individuals and
  - used in DNA profiling.
- **STR analysis**
  - compares the lengths of STR sequences at specific sites in the genome and
  - typically analyzes 13 different STR sites.
12.15 CONNECTION: DNA profiling has provided evidence in many forensic investigations

- DNA profiling is used to
  - determine guilt or innocence in a crime,
  - settle questions of paternity,
  - identify victims of accidents, and
  - probe the origin of nonhuman materials.
12.16 RFLPs can be used to detect differences in DNA sequences

- **A single nucleotide polymorphism (SNP)** is a variation at a single base pair within a genome.
- **Restriction fragment length polymorphism (RFLP)** is a change in the length of restriction fragments due to a SNP that alters a restriction site.
- RFLP analysis involves
  - producing DNA fragments by restriction enzymes and
  - sorting these fragments by gel electrophoresis.
Genomics is the scientific study of whole genomes

Genomics is the study of an organism’s complete set of genes and their interactions.

- Initial studies focused on prokaryotic genomes.
- Many eukaryotic genomes have since been investigated.

Genomic studies showed a 96% similarity in DNA sequences between chimpanzees and humans.

Functions of human disease-causing genes have been determined by comparing human genes to similar genes in yeast.
12.18 CONNECTION: The Human Genome Project revealed that most of the human genome does not consist of genes

- The goals of the Human Genome Project (HGP) included
  - determining the nucleotide sequence of all DNA in the human genome and
  - identifying the location and sequence of every human gene.

- Results of the Human Genome Project indicate that
  - humans have about 20,000 genes in 3.2 billion nucleotide pairs,
  - only 1.5% of the DNA codes for proteins, tRNAs, or rRNAs, and
  - the remaining 98.5% of the DNA is noncoding DNA including
    - telomeres, stretches of noncoding DNA at the ends of chromosomes, and
    - transposable elements, DNA segments that can move or be copied from one location to another within or between chromosomes.
12.19 The whole-genome shotgun method of sequencing a genome can provide a wealth of data quickly

- The Human Genome Project proceeded through three stages that provided progressively more detailed views of the human genome.
  1. A low-resolution linkage map was developed using RFLP analysis of 5,000 genetic markers.
  2. A physical map was constructed from nucleotide distances between the linkage-map markers.
  3. DNA sequences for the mapped fragments were determined.

- The whole-genome shotgun method - was proposed in 1992 by molecular biologist J. Craig Venter, who
  - used restriction enzymes to produce fragments that were cloned and sequenced in just one stage and
  - ran high-performance computer analyses to assemble the sequence by aligning overlapping regions.

- Today, this whole-genome shotgun approach is the method of choice for genomic researchers because it is
  - relatively fast and
  - inexpensive.

- However, limitations of the whole-genome shotgun method suggest that a hybrid approach using genome shotgunning and physical maps may prove to be the most useful.
12.20 Proteomics is the scientific study of the full set of proteins encoded by a genome

- Proteomics
  - is the study of the full protein sets encoded by genomes and
  - investigates protein functions and interactions.
- The human proteome includes about 100,000 proteins.
- Genomics and proteomics are helping biologists study life from an increasingly holistic approach.

12.21 EVOLUTION CONNECTION: Genomes hold clues to human evolution

- Human and chimp genomes differ by
  - 1.2% in single-base substitutions and
  - 2.7% in insertions and deletions of larger DNA sequences.
- Genes showing rapid evolution in humans include
  - genes for defense against malaria and tuberculosis,
  - a gene regulating brain size, and
  - the FOXP2 gene involved with speech and vocalization.
12.21 EVOLUTION CONNECTION: Genomes hold clues to human evolution

- Neanderthals
  - were close human relatives,
  - were a separate species,
  - also had the FOXP2 gene,
  - may have had pale skin and red hair, and
  - were lactose intolerant.