Chapter 13
An Introduction to Genetic Technology
Historical look at genetic technology

- 18,000 B.C.E. – breeding of deer, antelope, sheep
- 4000 B.C.E. – use of bacteria to make alcohol, bread, cheese, yogurt
13.1 What Are Clones?

- **Clones**
  - Genetically identical molecules, cells, or organisms all derived from a single ancestor

- **Cloning**
  - The production of identical copies of molecules, cells, or organisms
Development of methods for cloning higher plants and animals represents a significant advance in genetic technology

- Improving crops
- Improved breeds of domestic animals

Acceptance of GMOs differs among nations and cultures.
Animal Cloning Methods

- **Embryo splitting**
  - After *in vitro* fertilization, early embryonic cells are divided and grown into clones

- **Nuclear transfer (cell fusion)**
  - Enucleated eggs are fused with embryonic or adult cells and grown into clones
  - Dolly the sheep

- **Nuclear injection**
  - A nucleus from a cell is injected into an egg
Nuclear Fusion

- A donor cell is taken from a sheep's udder.
- An egg cell is taken from an adult female sheep.
- The nucleus of the egg cell is removed.
- The two cells are fused using an electrical current.
- The fused cell containing the donor nucleus divides to form an embryo.
- The embryo is transferred to the uterus of a foster mother.
- The embryo develops into Dolly.
Dolly the Sheep

- Dolly was cloned by fusion of a nucleus from an adult cell with an enucleated egg.
Egg and its surrounding cumulus cells are removed from the ovary.

Cumulus cells separated from egg

Nucleus removed by microsurgery

Cumulus cell nucleus injected into egg

Egg implanted into surrogate mother

Twenty-one days later, a cloned mouse is born.

(a) Nuclear Injection
13.2 Cloning Genes
Is a Multistep Process

- Recombinant DNA technology and the cloning of DNA molecules has revolutionized laboratory research, healthcare, and the food we eat.

- Recombinant DNA technology: a series of techniques in which DNA fragments are linked to self-replicating vectors to create recombinant DNA molecules, which are replicated in host cells.
DNA Cloning Requires Three Things

- A way to cut DNA at specific sites (restriction enzymes)
- A carrier molecule (vector) to hold DNA for cloning and for transfer to a host cell
- A host cell where the DNA can be copied
- After making a large number of identical DNA sequences, it can be used for research, or in clinical and commercial applications.
Restriction Enzymes

- Bacterial enzymes that help protect bacteria from viral infections

- **Restriction enzymes** - cut DNA at specific locations – function like scissors

- Ex: EcoR1 recognizes and cuts GAATTC
  
  
  
  
CTTAAG

- Sequence is a **palindrome**
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Recognition and cleavage sequence</th>
<th>Cleavage pattern</th>
<th>Source organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eco RI</td>
<td>GAATTC CTTAAG</td>
<td>G AATTC CTTAA G</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>Hin dIII</td>
<td>AAGCTT TTCGAA</td>
<td>A AGCTT TTCGA A</td>
<td><em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td>Bam HI</td>
<td>GGATCC CCTAGG</td>
<td>G GATCC CCTAG G</td>
<td><em>Bacillus amyloliquefaciens</em></td>
</tr>
<tr>
<td>Sau 3A</td>
<td>GATC CTAG</td>
<td>GATC CTAG</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Hae III</td>
<td>GGCC CCGG</td>
<td>GG CC GG</td>
<td><em>Haemophilus aegypticus</em></td>
</tr>
</tbody>
</table>

Fig. 13-5, p. 296
Constructing a plasmid with a specific piece or “target DNA”

Target DNA with Gene of interest; Ends cut with a Restriction enzyme

Vector DNA; Ends cut with same Restriction enzyme

Recombinant plasmid

Bacterial cell

Like Fig. 13-7, p. 297
Colonies of Bacteria on Petri Plates

- Each colony is a clone, descended from a single cell
Steps in the Process of Cloning

1. Target DNA is cut with a restriction enzyme

2. Target DNA is mixed with vector molecules cut by the same enzyme
   - DNA ligase joins recombinant DNA molecules

3. Vector with target DNA (recombinant plasmid) is transferred into bacterial cell
   Recombinant plasmids replicate and produce many clones of the target DNA molecule

4. Colonies carrying target DNA molecules are identified, collected, and grown
   - Host cells are broken open and recombinant plasmids are extracted
An international conference was held at Asilomar, California, to consider the possible dangers of recombinant DNA technology

- In 1976, guidelines were set in place for experiments using recombinant bacteria
- New guidelines were published in 1982
- No experiments are currently prohibited
13.3 Cloned Libraries

- A collection of cloned DNA sequences from one source is a library
  - Genomic library - all of the sequences from one organism
  - Chromosomal library - all of the sequences from a single chromosome
  - Expressed sequence library - all of the genes expressed in a specific cell type

- Libraries are resources for gene studies

- We will not include topic 13.4 “Finding a specific clone in a library” in this course.
13.5 A Revolution in Cloning: The Polymerase Chain Reaction

- **Polymerase chain reaction (PCR)**
- PCR copies a DNA molecule without restriction enzymes, vectors, or host cells
  - Faster and easier than conventional cloning
Steps in a typical PCR cycle

PCR Mix- Target DNA, primers, polymerase, DNA bases

1. DNA is heated to break the hydrogen bonds between the strands of a DNA molecule

2. Short nucleotide sequences (primers) and bind to complementary regions on single-stranded DNA

3. Taq polymerase synthesizes complementary strands of both templates, beginning at the primers
The Polymerase Chain Reaction (PCR)

1. PCR starts with a fragment of double-stranded DNA.

2. The DNA is heated to 90°–94°C to unwind it. The single strands will become templates.

3. The reaction mixture contains primers designed to base-pair with complementary sequences at the end of the DNA to be copied.

4. The mixture is cooled. Lowering the temperature promotes base-pairing between primers and the DNA strands to be copied.

5. DNA polymerases recognize the primers and assemble complementary sequences to make new strands. This doubles the number of identical DNA fragments.

6. The mixture is heated again. Raising the temperature makes all of the double-stranded DNA fragments unwind.

7. The mixture is cooled. Lower temperature promotes base-pairing between more primers added to the mixture and the single strands.

8. DNA polymerase again doubles the number of identical fragments. *Repeating the sequence of reactions doubles the number of DNA fragments each time.* Billions of fragments are rapidly synthesized, as in the rows of PCR systems below that are copying human DNA.
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DNA polymerase again doubles the number of identical fragments. Repeating the sequence of reactions doubles the number of DNA fragments each time.
PCR Doubles the Amount of DNA With Each Cycle

**Table 13.1** DNA Sequence Amplification by PCR

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Number of Copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>10</td>
<td>1,024</td>
</tr>
<tr>
<td>15</td>
<td>32,768</td>
</tr>
<tr>
<td>20</td>
<td>1,048,576</td>
</tr>
<tr>
<td>25</td>
<td>33,544,432</td>
</tr>
<tr>
<td>30</td>
<td>1,073,741,820</td>
</tr>
</tbody>
</table>
Many Uses for PCR

- DNA to be amplified by PCR does not have to be purified and can be present in small amounts
  - Used in clinical diagnosis, forensics, conservation
  - Samples can be small or old (insects in amber)

Fig. 13-12, p. 302
Separation of Restriction Fragments by Gel Electrophoresis
DNA Sequencing is one form of genome analysis

- **DNA sequencing**
  - A technique for determining the nucleotide sequence of a fragment of DNA
  - Basic method used in Human genome project and other genome projects

- The Sanger method of DNA sequencing is the most commonly used method and can be automated
1. The fragment of DNA to be sequenced is mixed with primers, DNA polymerase, and the four deoxynucleotides. Chemically modified nucleotides each labeled with a differently colored fluorescent dye are also added to the mixture.

2. The polymerase copies the DNA into new strands again and again. Synthesis at each new strand stops when a modified nucleotide gets added to it.

3. There are now many fragments of DNA in the mixture. Each is a truncated copy of the DNA template; each is tagged with a modified nucleotide.

4. Electrophoresis separates the fragments into bands according to their length. All fragments in each band are the same length, and all have the same modified nucleotide at their 3' end. Thus, each band is a certain color.

5. A computer detects and records the color of each band on the gel. The order of colors of the bands represents the sequence of the template DNA.
In 1977, Fred Sanger sequenced the 5,400 nucleotides in the genome of a virus.

Automated methods allowed the human genome (3.2 billion nucleotides) to be sequenced.

DNA sequencing is one of the basic methods in recombinant DNA technology.
13.7 DNA Microarrays are used to Analyze Gene Expression

- DNA microarrays are used to analyze thousands of genes in a single experiment.
- A typical microarray is a small glass panel containing thousands of arrayed gene sequences.
- Detection is by hybridization.
DNA Microarrays are used to for many purposes.

**Table 13.2** Uses of Microarrays

<table>
<thead>
<tr>
<th>Application</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Expression</td>
<td>Assay expression levels of thousands of genes in a single experiment. Used to study development, disease versus normal states.</td>
</tr>
<tr>
<td>SNP Detection</td>
<td>Surveys alleles and/or populations to detect single nucleotide polymorphisms (SNPs). Used in genotyping, identifying predisposition to disease, forensic analysis, selection of patient-specific cancer therapy.</td>
</tr>
<tr>
<td>Alternative Splicing</td>
<td>Exon junction microarrays detect mRNAs for alternative splice forms of a gene in different tissues, stages of development.</td>
</tr>
<tr>
<td>Fusion Genes</td>
<td>Used to detect fusion genes for precise diagnosis of some leukemias and other cancers such as prostate cancer. Often used to help establish prognosis.</td>
</tr>
<tr>
<td>Organism Identification</td>
<td>Used to detect mislabeled food, often from endangered species, in the food supply. Also used to assay contaminating microorganisms in food.</td>
</tr>
</tbody>
</table>
DNA Microarrays are used to compare gene expression patterns among different cells.

Actual DNA microarray result