Learning Outcomes

- Recombinant DNA
- Restriction Enzymes
- Vectors
- Clones
- Subunit Vaccines
- Polymerase Chain Reaction
Biotechnology and Recombinant DNA

- Biotechnology:
  - The use of microorganisms, cells, or cell components to make a product
  - Foods, antibiotics, vitamins, enzymes

- Recombinant DNA Technology:
  - Insertion of genes to produce desired proteins
  - Often inserted into bacteria like E.coli
Restriction Enzymes

• Cut specific sequences of DNA
• Destroy bacteriophage DNA in bacterial cells
• Hundreds of restriction enzymes now known
  - Staggered cut
  - Sticky ends - un-paired bases

Sticky Ends
Plasmid Vectors

- Carry desired gene from one cell to cell for insertion
- Plasmids are used to insert genes into bacteria
- Genetically engineered viruses are used to insert genes into animal cells

Figure 9.3

Polymerase Chain Reaction (PCR)

- To make multiple copies of a piece of DNA enzymatically
- Heat stable form of DNA Polymerase from *Thermus aquaticus*
- RNA primers synthesized to match known base sequences in DNA
- Deoxynucleotide Triphosphates
- Thermocycler
Chain Reaction

- Incubate target DNA at 94°C 1 min
- DNA strands separate
- Incubate at 60°C 1 min
- Primers attach to single stranded DNA
- Incubate at 72°C 1 min
- DNA polymerase copies target DNA
- Repeat

Applications of PCR

- Clone DNA for recombination
- Amplify DNA to detectable levels (CSI)
- Diagnose genetic disease
- Detect pathogens
- RNA primer synthesized specific sequence
  - unique to pathogen
  - Known mutation
  - Will only attach if primer anneals
DNA can be inserted into a cell by:

- **Transformation**
  - Cells take DNA from environment
  - \( \text{CaCl}_2 \) + Heat Shock

- **Electroporation**
  - Electrical currents form pores in plasma membrane

- **Protoplast fusion**
  - Enzymatically digest cell wall
  - Plant cells and algae
  - Cells fuse, genes recombine

- **Vectors**
  - Modified viruses

DNA insertion

- **Gene gun**
- **Tungsten Bullets**
  - Plant cells

- **Microinjection**
  - Animal cells
Genetic Engineering

1. Resistant DNA and foreign DNA are mixed up with the same restriction enzymes. The reaction is run on gel to separate the digested DNA into fragments. If necessary, enzymes (e.g., alkaline phosphatase) are added to inactivate the restriction enzymes.
2. Purified DNA is treated with ligase and cloned into a plasmid. A new strain of bacteria is used to produce the cloned DNA. Foreign DNA has been inserted into the plasmid.
3. The recombinant plasmid is introduced into bacteria, which become ampicillin-resistant.
4. AEsplacarbohydrates are spread on a nutrient agar plate containing ampicillin and a gelatinase substrate and incubated to allow for the gelatinase to convert the gelatin substrate into a clear gel.
5. The colonies that grow up the plasmid will grow in the presence of ampicillin. Colonies that produce gelatinase is called a strain.

Making a Gene Product

1. Make replica of master plate on nitrocellulose filter.
2. Treat filter with detergent (SDS) to lyse bacteria.
3. Treat filter with sodium hydroxide (NaOH) to separate DNA into single strands.
4. Add radiolabeled probe.
5. Probe with hybridized DNA to select clones containing gene of interest.
6. Wash film to remove unbound probe and expose film to X-ray film.
7. Compare developed film with replica on master plate to identify colonies containing gene of interest.
Gene Libraries

- Gene libraries
  - fragments of an entire genome stored in plasmids or phages
- cDNA is made from mRNA by reverse transcriptase

Therapeutic Applications

- Subunit vaccines
- Nonpathogenic viruses carrying genes for pathogen’s antigens as vaccines
- Gene therapy to replace defective or missing genes
- Human Genome Project
  - Nucleotides have been sequenced
  - Human Proteome Project may provide diagnostics and treatments
### Agricultural Applications

**Table 9.2**

<table>
<thead>
<tr>
<th>Agricultural Products</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bt cotton and Bt corn</td>
<td>Plants have transgenic genes from Bt. rhizogenes, too kills insects that eat plants.</td>
</tr>
<tr>
<td>Maize, sorghum, etc.</td>
<td>Cause for pest degradation is removed so pests have longer shelf life.</td>
</tr>
<tr>
<td>Pseudomonas syringae, Xanthomonas bacteria</td>
<td>Seeds contain protein that inhibits undesirable ice formation on plants.</td>
</tr>
<tr>
<td>Pseudomonas fluorescentis</td>
<td>Has transgenic gene from insect pathogen. Bacillus rhizogenes; turns kills controlling insects that attack bacteria.</td>
</tr>
<tr>
<td>Rhizobium meliloti bacteria</td>
<td>Modified for enhanced nitrogen fixation.</td>
</tr>
<tr>
<td>RoundUp® (glyphosate) resistant crops</td>
<td>Plants have bacterial gene; allows use of herbicide on weeds without damaging crops.</td>
</tr>
</tbody>
</table>

---

### Genetic Engineering Using Agrobacterium

**Figure 9.18**

1. The plasmid is inserted into a bacterium.
2. The bacterium is infected with the plasmid carrying foreign gene.
3. The bacterium is introduced into the plant cell by the cytoplasm or a plant cell.
4. Foreign DNA is not by the same enzyme.
5. A plant is generated from a cell clone. All of the cells carry the foreign gene and may express it as a new trait.
Safety Issues and Ethics

- Avoid accidental release
- Genetically modified crops must be safe for consumption and for the environment
  - “Superweeds”
- Who will have access to an individual’s genetic information?

<table>
<thead>
<tr>
<th>Product</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha interferon</td>
<td>Therapy for leukemia, melanoma, and hepatitis, produced by E. coli and</td>
</tr>
<tr>
<td></td>
<td>Bacillus subtilis (spore).</td>
</tr>
<tr>
<td>Antigens</td>
<td>Assist with immune system.</td>
</tr>
<tr>
<td></td>
<td>Produced by genetically modified sheep.</td>
</tr>
<tr>
<td>Baculovirus</td>
<td>Treatment for multiple cancers, produced by recombinant cell culture.</td>
</tr>
<tr>
<td>Bone morphogenic protein</td>
<td>Induces new bone formation, useful in healing fractures and</td>
</tr>
<tr>
<td></td>
<td>reconstructive surgery, produced by recombinant cell culture.</td>
</tr>
<tr>
<td>Colony stimulating factor (CSF)</td>
<td>Counteracts effects of chemotherapy, improves resilience in</td>
</tr>
<tr>
<td></td>
<td>leukemia, produced by E. coli and B. subtilis.</td>
</tr>
<tr>
<td>Erythropoietin (EPO)</td>
<td>Treats anemia, produced by recombinant cell culture.</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Treatment of hemophilia, improves clotting, produced by recombinant cell</td>
</tr>
<tr>
<td></td>
<td>culture.</td>
</tr>
<tr>
<td>Human growth hormone</td>
<td>Helps wounds, burns, scars, produced by E. coli.</td>
</tr>
<tr>
<td></td>
<td>Produced by E. canis that carries hepatitis gene on a plasmid.</td>
</tr>
<tr>
<td>Human insulin</td>
<td>Therapy for diabetes, better tolerated than insulin extracted from animals, produced by E. coli.</td>
</tr>
</tbody>
</table>
Gardisil a Subunit Vaccines

- Gardasil offers women protection v HPV 6,11,16 and 18
  - Genital warts
  - Cervical Cancer
- Virus gene for HPV protein L1 moved to Yeast
- Yeast cells synthesize HPV protein
- Injected as a vaccine