Chapter 20.

Biotechnology:
DNA Technology & Genomics
The BIG Questions…

- How can we use our knowledge of DNA to:
  - diagnose disease or defect?
  - cure disease or defect?
  - change/improve organisms?
- What are the techniques & applications of biotechnology?
  - direct manipulation of genes for practical purposes
**Biotechnology**

- The genetic manipulation of organisms
  - humans have been doing this for thousands of years
    - plant & animal breeding
Evolution & breeding of food plants

Evolution in morphology of *Zea mays* from ancestral teosinte (left) to modern corn (right). The middle figure shows possible hybrids of teosinte & early corn varieties.
Evolution & breeding of food plants

- “Descendants” of the wild mustard
  - Brassica spp.
Animal husbandry / breeding
Biotechnology today

- Genetic Engineering
  - manipulation of DNA
  - if you are going to engineer DNA & genes & organisms, then you need a set of tools to work with
  - this unit is a survey of those tools...
Bioengineering Tool kit

- **Basic Tools**
  - restriction enzymes
  - ligase
  - plasmids / cloning
  - DNA libraries / probes

- **Advanced Tools**
  - PCR
  - DNA sequencing
  - gel electrophoresis
  - Southern blotting
  - microarrays

AP Biology
Cut, Paste, Copy, Find…

- **Word processing metaphor…**
  - cut
    - restriction enzymes
  - paste
    - ligase
  - copy
    - plasmids
      - bacteria
      - transformation
    - PCR
  - find
    - Southern blotting / probes
Cut DNA

- **Restriction enzymes**
  - discovered in 1960s
  - evolved in bacteria to cut up foreign DNA ("restriction")
    - protection against viruses & other bacteria
      - bacteria protect their own DNA by methylation & by not using the recognition sequences
    - hundreds of different enzymes
      - EcoRI, HindIII, BamHI, Smal
    - cut at restriction site
      - specific sequence of DNA
      - symmetrical "palindrome"
      - produces **sticky ends**

AP Biology
Werner Arber discovered restriction enzymes. He postulated that these enzymes bind to DNA at specific sites containing recurring structural elements made up of specific basepair sequences.

Hamilton Smith verified Arber’s hypothesis with a purified bacterial restriction enzyme and showed that this enzyme cuts DNA in the middle of a specific symmetrical sequence. Other restriction enzymes have similar properties, but different enzymes recognize different sequences. Ham Smith now works at Celera Genomics, the company who sequenced the human genome.

Dan Nathans pioneered the application of restriction enzymes to genetics. He demonstrated their use for the construction of genetic maps and developed and applied new methodology involving restriction enzymes to solve various problems in genetics.
Adaptive value of restriction enzymes?

Blunt vs. sticky ends?

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Recognition Sequence</th>
<th>Microorganism</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ava</em></td>
<td>AG<strong>C</strong>T</td>
<td><em>Arthrobacter luteus</em></td>
</tr>
<tr>
<td><em>BamHI</em></td>
<td>G<strong>G</strong>ATCC</td>
<td><em>Bacillus amyloliquefaciens</em> H</td>
</tr>
<tr>
<td><em>BglII</em></td>
<td>GCC<strong>N</strong>N<strong>N</strong>NGCC</td>
<td><em>Bacillus globis</em></td>
</tr>
<tr>
<td><em>BglII</em></td>
<td>AG<strong>A</strong>CT</td>
<td><em>Bacillus globis</em></td>
</tr>
<tr>
<td><em>EcoRI</em></td>
<td>G<strong>A</strong>AA<strong>T</strong>TC</td>
<td><em>Escherichia coli</em> RY13</td>
</tr>
<tr>
<td><em>EcoRI</em></td>
<td>I<strong>C</strong>C<strong>T</strong>GG</td>
<td><em>Escherichia coli</em> K245</td>
</tr>
<tr>
<td><em>EcoRV</em></td>
<td>GA<strong>T</strong>AC</td>
<td><em>Escherichia coli</em> 362P/74</td>
</tr>
<tr>
<td><em>HindIII</em></td>
<td>GG<strong>A</strong>CC</td>
<td><em>Haemophilus aegyptius</em></td>
</tr>
<tr>
<td><em>HindIII</em></td>
<td>GG<strong>I</strong>CC</td>
<td><em>Haemophilus aegyptius</em></td>
</tr>
<tr>
<td><em>HpaII</em></td>
<td>C<strong>C</strong>G<strong>G</strong></td>
<td><em>Haemophilus influenzae</em> R Higgins</td>
</tr>
<tr>
<td><em>HpaII</em></td>
<td>C<strong>C</strong>G<strong>G</strong></td>
<td><em>Haemophilus parainfluenzae</em></td>
</tr>
<tr>
<td><em>MboI</em></td>
<td>C<strong>C</strong>GG</td>
<td><em>Moraxella species</em></td>
</tr>
<tr>
<td><em>PstI</em></td>
<td>CTGC<strong>A</strong>G</td>
<td><em>Providencia stuartii</em> 164</td>
</tr>
<tr>
<td><em>PvuII</em></td>
<td>CA<strong>C</strong>TG</td>
<td><em>Proteus vulgaris</em></td>
</tr>
<tr>
<td><em>SalI</em></td>
<td>G<strong>I</strong>CG<strong>A</strong>C</td>
<td><em>Streptomyces albus</em> G</td>
</tr>
<tr>
<td><em>TaqI</em></td>
<td>T<strong>C</strong>GA<strong>A</strong></td>
<td><em>Thermus aquaticus</em></td>
</tr>
<tr>
<td><em>XhoI</em></td>
<td>C<strong>T</strong>G<strong>A</strong>G</td>
<td><em>Xanthomonas hiocleia</em></td>
</tr>
</tbody>
</table>

* The recognition sequence is abbreviated so that only one strand, reading 5' to 3', is given. The cleavage site is represented by an arrow (†) and the modified base, where it is known, is indicated by an asterisk (*V* = 5'-methylcytosine and *C* = 5-methylcytosine). R, Y, and N represent purine nucleotide, pyrimidine nucleotide, and any nucleotide, respectively.

Paste DNA

- **Sticky ends allow:**
  - H bonds between complementary bases to anneal

- **Ligase**
  - enzyme “seals” strands
    - joins sugar-phosphate bonds

*AP Biology*
Copy DNA

- **Plasmids**
  - small, self-replicating circular DNA molecules
    - naturally occur in bacteria
    - insert DNA sequence into plasmid
  - transformation
    - insert recombinant plasmid into bacteria
    - culture recombinant bacteria = clone of cells
    - permits production of multiple copies of a specific gene or DNA sequence
Recombinant plasmid

- Antibiotic resistance genes as a selectable marker
- Restriction sites for splicing in gene of interest
Gene cloning

1. Isolation of plasmid DNA and DNA containing gene of interest
2. Gene inserted into plasmid
3. Plasmid put into bacterial cell
4. DNA of chromosome
5. Gene of interest (black)
6. Recombinant DNA (plasmid)
7. Bacterial chromosome

1. Cells cloned with gene of interest
2. Identification of desired clone
3. Various applications
4. Gene used to alter bacteria for cleaning up toxic waste
5. Protein dissolves blood clots in heart attack therapy
6. Human growth hormone treats stunted growth
7. Recombinant bacterium

Gene for pest resistance inserted into plants
Basic research on gene
Copies of gene
Copies of protein
Basic research on protein
Cloning a Human Gene

- Use both ampicillin resistance & color
  - if DNA is correctly inserted within lacZ gene then colonies will be white
    - intact lacZ gene produces functional enzyme: lactose → blue
    - broken lacZ gene does not produce functional enzyme lactose ≠ blue

LacZ system was developed to make cloning more efficient. Scientists were frustrated by having to search for bacteria which were successfully transformed with plasmids containing the human gene. LacZ system was developed to quickly distinguish bacteria that contained “original” plasmids vs. bacteria that contained plasmids with the human gene inserted.
What if you don’t have your gene conveniently on a chunk of DNA ready to insert into a plasmid?

Have to find your favorite gene (YFG) out of the entire genome of the organism...
DNA libraries

- Cut up genomic DNA from many cells with restriction enzyme
- Clone all fragments into plasmids at same time
  - shotgun cloning
- Create a stored collection of genomic DNA fragments
**Find DNA in library**

- **Locate YFG**
  - if you know sequence of protein...
    - can guess part of DNA sequence
    - “back translate” protein to DNA
  - if you have sequence of similar gene from another organism...
    - use part of this sequence
- **Nucleic acid hybridization with probe**

**Complementation**

if you have a mutant that lacks YFG, you can transform bacteria with plasmids from the library until one “cures” (complements) the mutation
Screening the library

- **Nucleic acid hybridization**
  - identify transformed bacteria using a probe
  - use tagged complementary DNA probe
    - radioactive P32 or fluorescence
  - heat-treated DNA for testing = denaturation to unwind strands
  - DNA hybridization between probe & denatured DNA

AP Biology
DNA probes

- **Probe**
  - short, single stranded DNA molecule
  - mix with denatured DNA

- **DNA Hybridization**
  - probe bonds to complementary DNA sequence

- **Label**
  - probe is labeled for easy detection

![Diagram of DNA probes](image)
cDNA libraries

- Collection of only the coding sequences of expressed genes
  - extract mRNA from cells
  - reverse transcriptase
    - RNA → DNA
    - from retroviruses
  - clone into plasmid
- Applications
  - need edited DNA for expression in bacteria
  - human insulin

Could you imagine how much that first insulin clone was worth to Genentech? One little piece of DNA in a plasmid worth billions! It put them on the map & built a multi-billion dollar biotech company.