"Okay—is there anybody ELSE whose homework ate their dog?"

Biotechnology:
Restriction Enzymes

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?**
  - ◆ **biotech = direct manipulation of genes**

Because all organisms use the same genetic code, scientists can make a plant **glow like a firefly.**

Application of Recombinant DNA

- **Combining sequences of DNA from 2 different sources into 1 DNA molecule**
  - ◆ **often from different species**
    - ▪ **human insulin gene in E. coli (humulin)**
    - ▪ **frost resistant gene from Arctic fish in strawberries**
    - ▪ **"Roundup-ready" bacterial gene in soybeans**
    - ▪ **BT bacterial gene in corn**
    - ▪ **jellyfish glow gene in Zebra "Glofish" – GFP!**


Biotechnology Today

- **Genetic Engineering**
  - ◆ **manipulation of DNA**
  - ◆ **if you are going to engineer DNA & genes & organisms, then you need a <u>set of tools</u> to work with**
  - ◆ **this unit is a survey of those tools…**
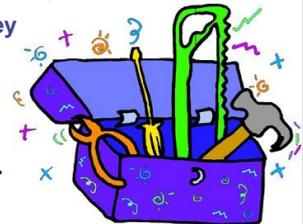
Our tool kit…

Bioengineering Tool Kit

- **Basic Tools**
  - ◆ **restriction enzymes**
  - ◆ **ligase**
  - ◆ **plasmids / cloning**
  - ◆ **DNA libraries / probes**
- **Advanced Tools**
  - ◆ **gel electrophoresis**
  - ◆ **PCR**
  - ◆ **DNA sequencing**
  - ◆ **Southern blotting***
  - ◆ **microarrays***
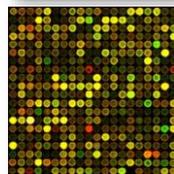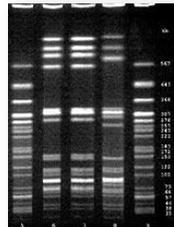
Cut, Paste, Copy, Find…

- **Word processing metaphor…**
  - ◆ **cut (Ctrl + X)**
    - ▪ **restriction enzymes**
  - ◆ **paste (Ctrl + V)**
    - ▪ **ligase**
  - ◆ **copy (Ctrl + C)**
    - ▪ **via plasmids**
      - ◆ **bacteria**
      - ◆ **transformation**
    - ▪ **via PCR**
  - ◆ **find (Ctrl + F)***
    - ▪ **Southern blotting**
    - ▪ **probes**

Cutting DNA

- **Restriction enzymes**
  - ◆ **restriction endonucleases**
  - ◆ discovered in 1960s
  - ◆ evolved in bacteria to cut up foreign DNA ("action <u>restricted</u> to foreign DNA")
    - ▪ protection against viruses & other bacteria
      - ◆ bacteria protect their own DNA by **methylation** & by <u>not</u> using the base sequences recognized by the enzymes in their own DNA (Lab #14)
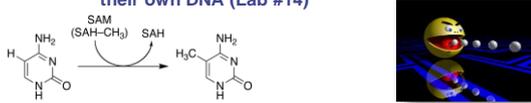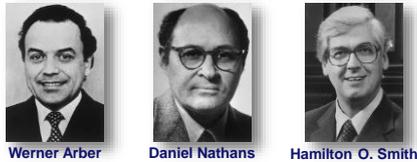
Restriction Enzymes

- **Action of enzyme**
  - ◆ cut DNA at specific sequences
    - ▪ <u>restriction site</u>
  - ◆ symmetrical "palindrome"
  - ◆ produces "ends"
    - ▪ <u>sticky ends</u>
    - ▪ <u>blunt ends</u>
- **Many different enzymes**
  - ◆ named after organism they are found in
    - ▪ EcoRI, HindIII, BamHI, SmaI

Madam I'm Adam

CTGAATTCCG
GACTTAAGGC

CTG AATTCCG
GACTTAA GGC
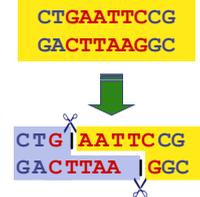
Discovery of Restriction Enzymes

1960s 1978

Werner Arber   Daniel Nathans   Hamilton O. Smith

Restriction enzymes are named for the organism they come from:
EcoRI = 1st restriction enzyme found in E. coli
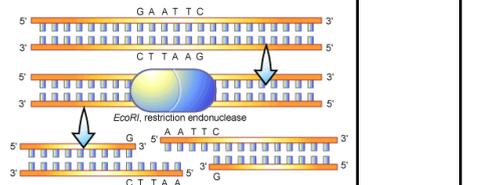
Paste DNA

- **Sticky ends allow:**
  - ◆ H bonds between complementary bases to anneal
- **Ligase**
  - ◆ enzyme "seals" strands
    - ▪ bonds sugar-phosphate bonds
    - ▪ covalent bond of DNA backbone
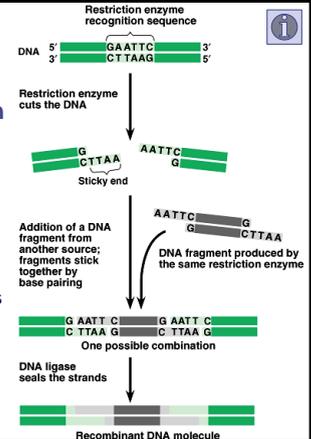
Biotech Use of Restriction Enzymes

- Sticky ends (complementary single-stranded DNA tails)
- Restriction enzyme cuts the DNA
- Add DNA from another source cut with same restriction enzyme
- DNA ligase joins the strands.
- Recombinant DNA molecule
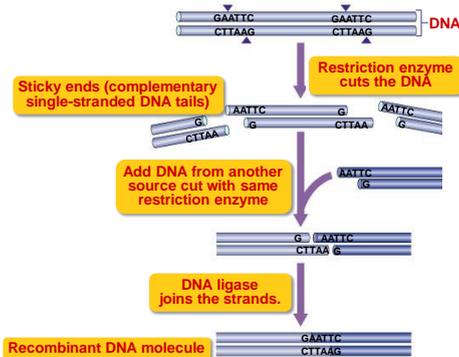
Gel Electrophoresis

- **Separation of DNA fragments by size**
  - ◆ DNA is negatively charged
    - ▪ moves toward + charge in electrical field
  - ◆ agarose gel
    - ▪ "swimming through Jello"
    - ▪ smaller fragments move faster

cut DNA 1st with restriction enzymes

Gel Electrophoresis

1. Mix agarose and buffer.
2. Boil mixture in microwave.
3. Cool the mixture to 65°C and pour into mold.
4. Gel solidified at room temperature.

Comb inserted into mold to make wells.
Comb removed; wells remain.
Finished gel

Gel Electrophoresis

DNA Sample A   DNA Sample B   DNA Sample C

DNA moves at a rate that is inversely proportional to the $\log_{10}$ of the number of base pairs

DNA and restriction endonuclease

Gel

Glass plates

Power source

Longer fragments
Shorter fragments

Completed gel

(a) Mixture of DNA fragments of different sizes in solution placed at the top of "lanes" in the gel

(b) Electric current applied, fragments migrate down the gel by size—smaller ones move faster (and therefore go farther) than larger ones

Measuring Fragment Size

- **compare bands to a known "standard"**
  - **usually lambda phage virus cut with HindIII**
    - **nice range of sizes with a distinct pattern**

DNA I   DNA II   DNA + Hind III

RFLP

- **Restriction Fragment Length Polymorphism**
  - **differences in DNA between individuals**

Allele 1

Difference in base sequence

Allele 2

(a) DNA from two alleles

(c) Completed gel

Allele 1
Allele 2

Longer fragments   Shorter fragments

(b) Electrophoresis of restriction fragments

- **change in DNA sequence affects restriction enzyme "cut" site**
- **will create different band pattern**

Polymorphisms in Populations

- **Differences between individuals at the DNA level**

Restriction endonuclease cutting sites

Larger fragments → Smaller fragments

Single base-pair change

Sequence duplication

(a) Three different DNA duplexes

(b) Cut DNA

(c) Gel electrophoresis of restriction fragments