

"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?
 - ♦ direct manipulation of genes for practical purposes



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

Biotechnology Today

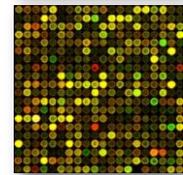
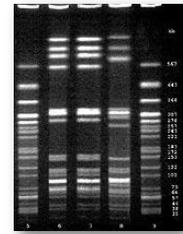
- Genetic Engineering
 - ♦ manipulation of DNA
 - ♦ if you are going to engineer DNA & genes & organisms, then you need a **set of tools** to work with
 - ♦ this unit is a survey of those tools...



Our tool kit...

Bioengineering Tool Kit

- Basic Tools
 - ♦ restriction enzymes
 - ♦ ligase
 - ♦ plasmids / cloning
 - ♦ DNA libraries / probes
- Advanced Tools
 - ♦ PCR
 - ♦ DNA sequencing
 - ♦ gel electrophoresis
 - ♦ Southern blotting
 - ♦ microarrays



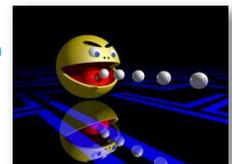
Cut, Paste, Copy, Find...

- 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 **restricted** to foreign DNA")
 - protection against viruses & other bacteria
 - ♦ bacteria protect their own DNA by **methylation** & by **not** using the base sequences recognized by the enzymes in their own DNA



Restriction Enzymes

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

Madam I'm Adam

CTGAATTCGG
GACTTAAGGC

CTG|AATTCGG
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

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

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

Gel Electrophoresis

DNA Sample A, DNA Sample B, DNA Sample C

100,000
50,000
25,000
10,000
5,000
2,500
1,000
500

1 2 3 4 5 6 7 8 9

DNA and restriction endonuclease

Gel

Glass plates

Power source

Completed gel

Longer fragments

Shorter fragments

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

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, Hind III

100,000
10,000
1,000
100

0 10 20 30 40 50

Distance (mm)

100,000
10,000
1,000
100

bp

DNA Hind III

DNA II

DNA I

RFLP

- Restriction Fragment Length Polymorphism
 - differences in DNA between individuals

Allele 1

Allele 2

Difference in base sequence

(a) DNA from two alleles

(b) Electrophoresis of restriction fragments

(c) Completed gel

- 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

Single base-pair change

Sequence duplication

(a) Three different DNA duplexes

(b) Cut DNA

(c) Gel electrophoresis of restriction fragments

Larger fragments → Smaller fragments

RFLP Use in Forensics

- Evidence from murder trial
 - Do you think suspect is guilty?

blood sample 1 from crime scene

blood sample 2 from crime scene

blood sample 3 from crime scene

"standard"

blood sample from suspect

blood sample from victim 1

blood sample from victim 2

"standard"