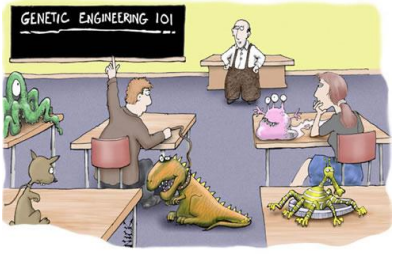


"Okay—is there anybody ELSE whose homework ate their dog?"

Biotechnology:
Restriction Enzymes

The BIG Questions…

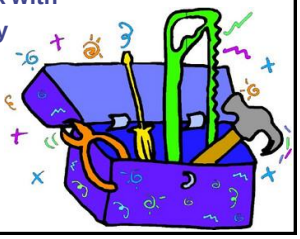
- How can we use our knowledge of DNA to:
  - diagnose disease or defect?
  - cure disease or defect?
  - change/improve organisms?
- What are the techniques & applications of biotechnology?
  - direct manipulation of genes for practical purposes

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

Biotechnology Today

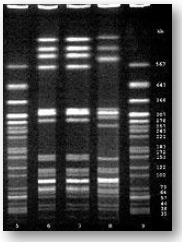
- Genetic Engineering
  - manipulation of DNA
  - if you are going to engineer DNA & genes & organisms, then you need a set of tools to work with
  - this unit is a survey of those tools…

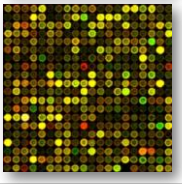
Our tool kit…



Bioengineering Tool Kit

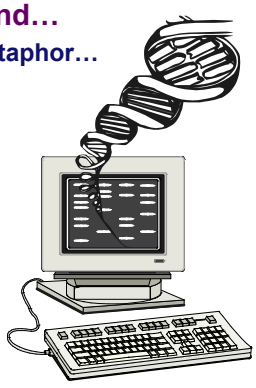
- Basic Tools
  - restriction enzymes
  - ligase
  - plasmids / cloning
  - DNA libraries / probes
- Advanced Tools
  - PCR
  - DNA sequencing
  - gel electrophoresis
  - Southern blotting
  - microarrays





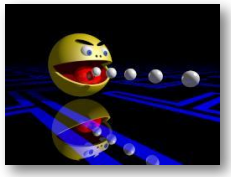
Cut, Paste, Copy, Find…

- 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

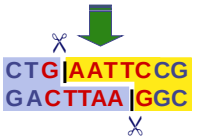


Restriction Enzymes

- 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

CTGAATTCCG
GACTTAAGGC



1960s|1978

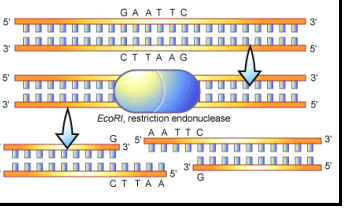
Discovery of Restriction Enzymes


Werner Arber


Daniel Nathans

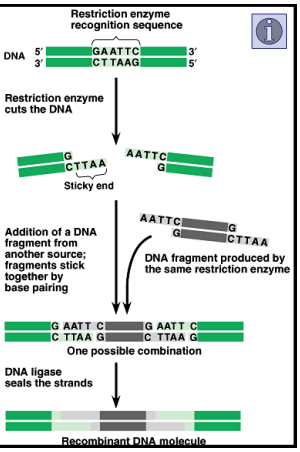

Hamilton O. Smith

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

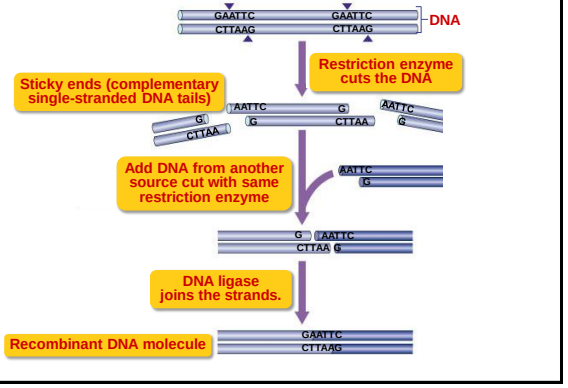


Paste DNA

- Sticky ends allow:
  - H bonds between complementary bases to anneal
- Ligase
  - enzyme "seals" strands
    - bonds sugar-phosphate bonds
    - covalent bond of DNA backbone

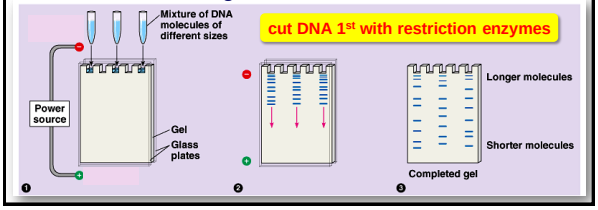


Biotech Use of Restriction Enzymes

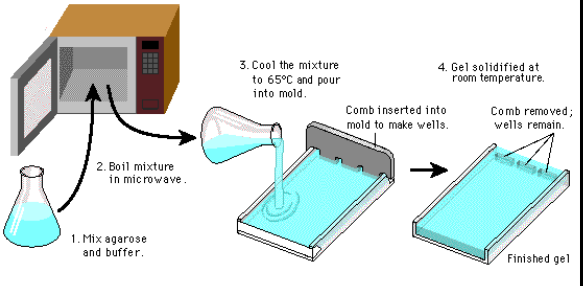


Gel Electrophoresis

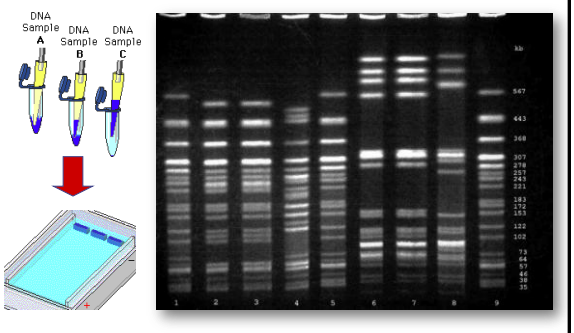
- Separation of DNA fragments by size
  - DNA is negatively charged
    - moves toward + charge in electrical field
  - agarose gel
    - "swimming through Jello"
    - smaller fragments move faster

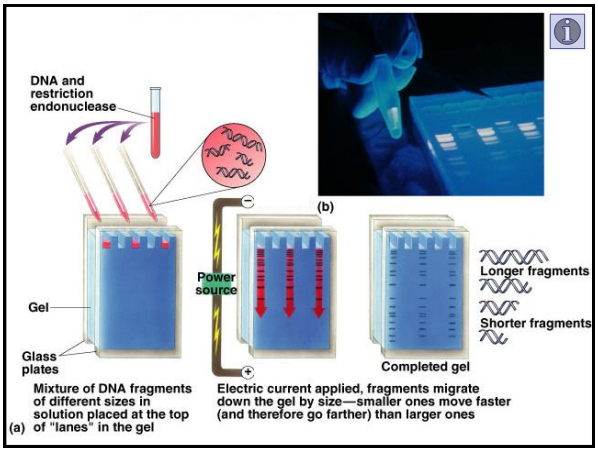


Gel Electrophoresis



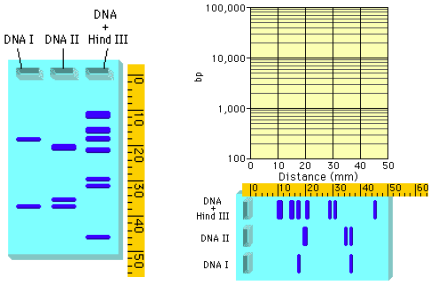
Gel Electrophoresis





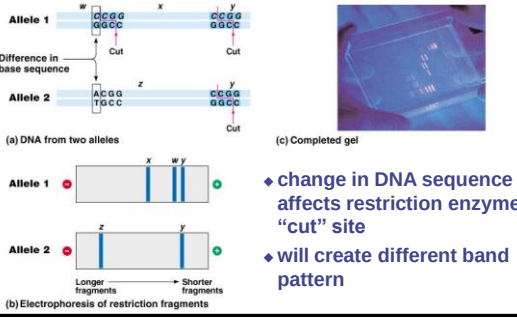
Measuring Fragment Size

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



RFLP

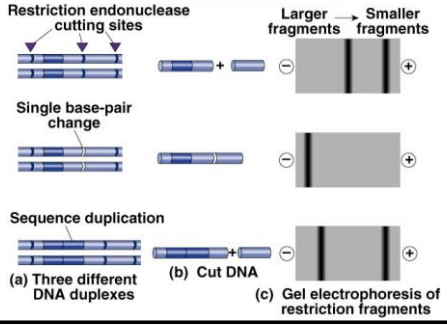
- Restriction Fragment Length Polymorphism
  - differences in DNA between individuals



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

Polymorphisms in Populations

- Differences between individuals at the DNA level



RFLP Use in Forensics

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