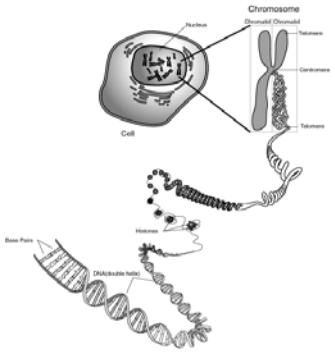
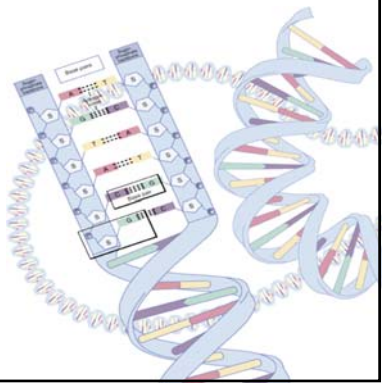
DNA Restriction Anaysis

DNA is Tightly Packaged into Chromosome s Which Reside in the Nucleus

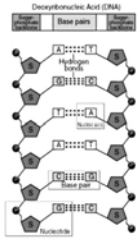


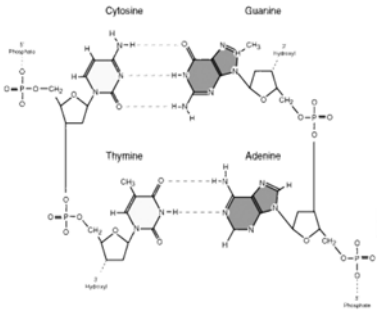
Model of DNA

DNA is Comprised of Four Base Pairs

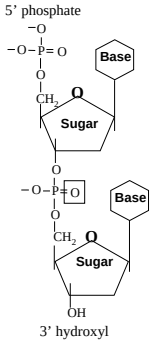


Deoxyribonucleic Acid (DNA)



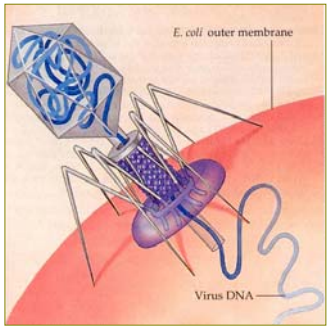


DNA Schematic



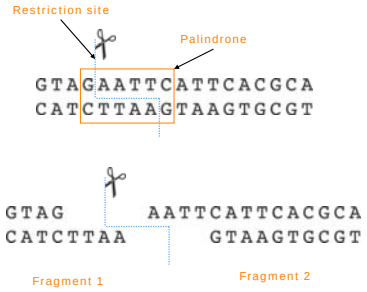
DNA Restriction Enzymes

- **Evolved by bacteria to protect against viral DNA infection**
- **Endonucleases = cleave within DNA strands**
- **Over 3,000 known enzymes**



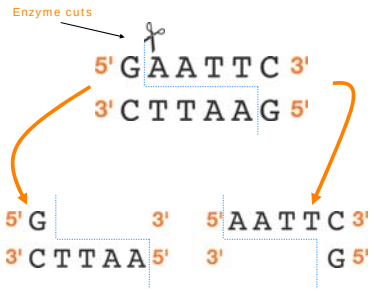
Enzyme Site Recognition

- Each enzyme digests (cuts) DNA at a specific sequence = restriction site
- Enzymes recognize 4- or 6- base pair, palindromic sequences (eg GAATTC)

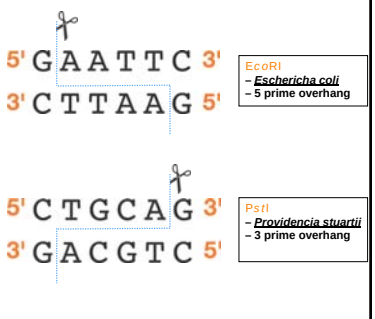


5 vs 3 Prime Overhang

- Generates 5 prime overhang



Common Restriction Enzymes



The DNA Digestion Reaction

- **Restriction Buffer provides optimal conditions**
- **NaCl provides the correct ionic strength**
- **Tris-HCl provides the proper pH**
- **Mg^{2+} is an enzyme co-factor**

DNA Digestion Temperature

Why incubate at 37°C?

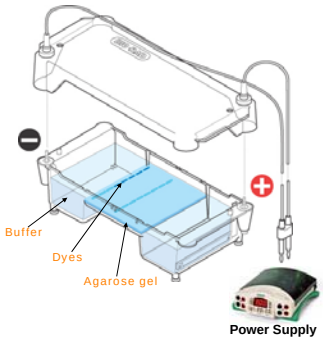
- **Body temperature is optimal for these and most other enzymes**

What happens if the temperature is too hot or cool?

- ***Too hot*** **= enzyme may be denatured (killed)**
- ***Too cool*** **= enzyme activity lowered, requiring longer digestion time**

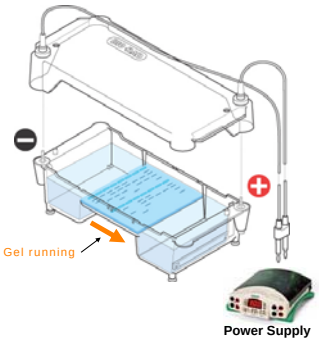
Agarose Electrophoresis Loading

- Electrical current **carries negatively-charged DNA through gel towards positive (red) electrode**



Agarose Electrophoresis Running

- **Agarose gel sieves DNA fragments according to size**
  - **Small fragments move farther than large fragments**



Analysis of Stained Gel

Determine restriction fragment sizes

- **Create standard curve using DNA marker**
- **Measure distance traveled by restriction fragments**
- **Determine size of DNA fragments**
- **Identify the related samples**

Molecular Weight Determination

Size (bp)	Distance (mm)
23,000	11.0
9,400	13.0
6,500	15.0
4,400	18.0
2,300	23.0
2,000	24.0