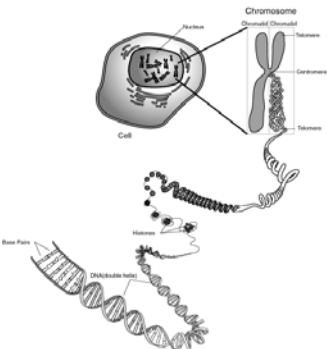


DNA Restriction Analysis

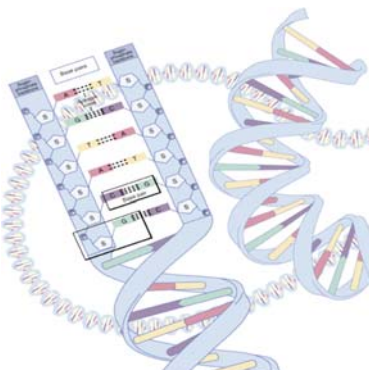
DNA is Tightly Packaged into Chromosomes Which Reside in the Nucleus



The diagram illustrates the packaging of DNA. At the bottom, a DNA double helix is shown with 'Base Pairs' labeled. This is wrapped around 'Histones' to form a 'Nucleosome'. These nucleosomes are further packed into a 'Chromatin Fiber'. The fiber is then highly condensed into a 'Chromosome', which is shown as an X-shaped 'Chromatid Chromatid' structure within a 'Cell' nucleus.

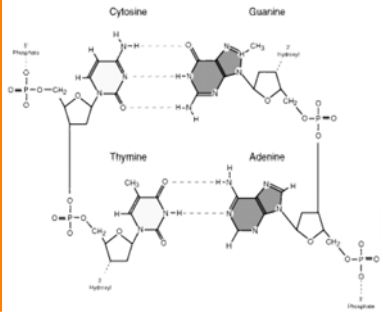
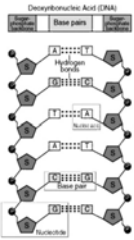
Model of DNA

DNA is Comprised of Four Base Pairs

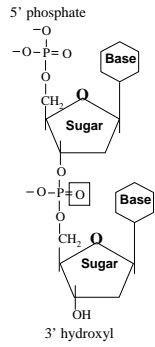


The image shows a 3D model of a DNA double helix. A legend identifies the four nitrogenous bases: Adenine (A), Thymine (T), Guanine (G), and Cytosine (C). The bases are shown as colored blocks (A: blue, T: red, G: yellow, C: green) that pair up to form the rungs of the DNA ladder.

Deoxyribonucleic Acid (DNA)

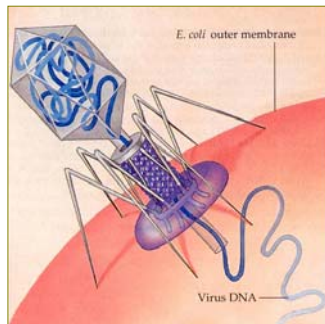


DNA Schematic



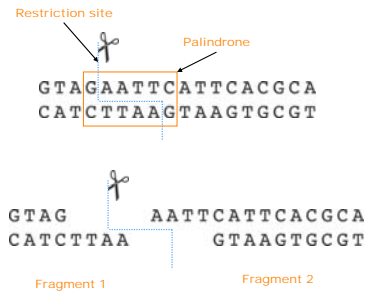
DNA Restriction Enzymes

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



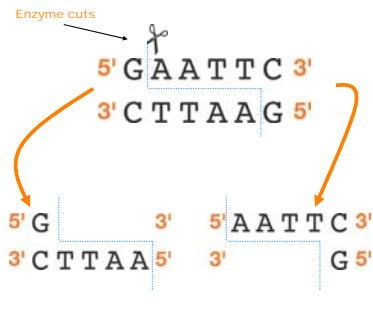
Enzyme Site Recognition

- 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

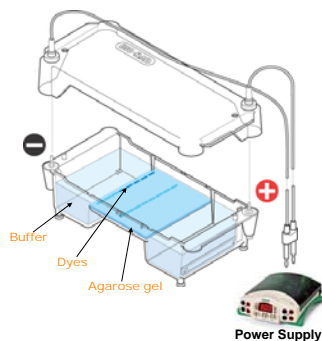
- Restriction Buffer provides optimal conditions
- NaCl provides the correct ionic strength
- Tris-HCl provides the proper pH
- Mg²⁺ is an enzyme co-factor

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

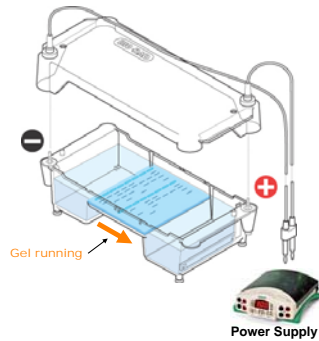
Agarose Electrophoresis Loading

- 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

