Biotechnology

Gel Electrophoresis

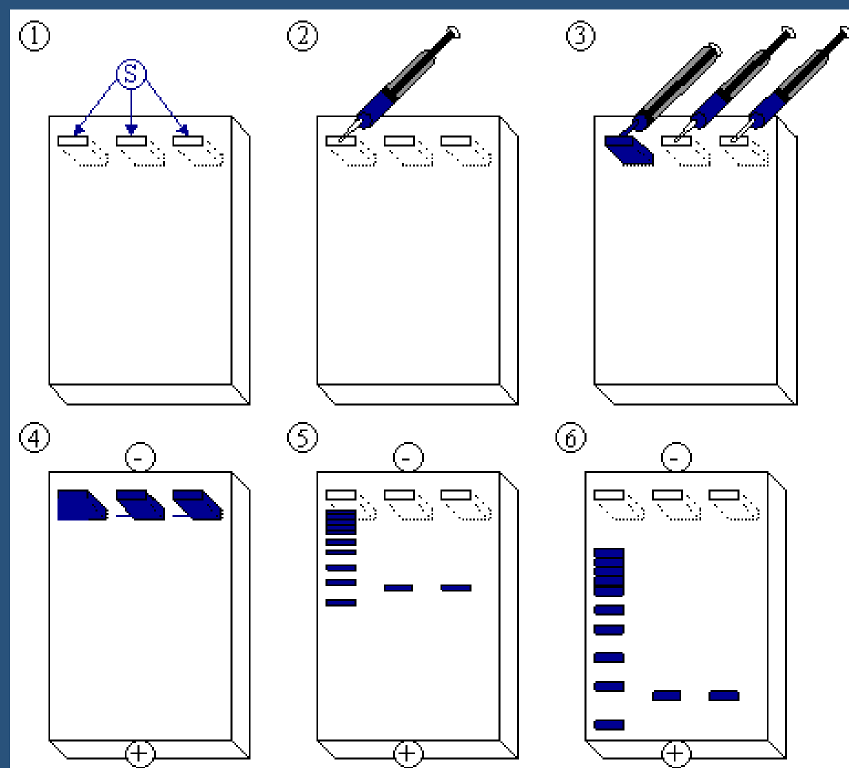
Gel Electrophoresis

- A technique that separates macromolecules
- on the basis of their rate of movement
- through a gel
- under the influence of an electric field.

  - Example of macromolecules
    - nucleic acids or proteins

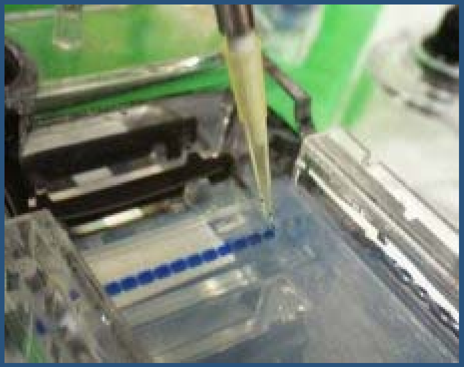
Gel Electrophoresis

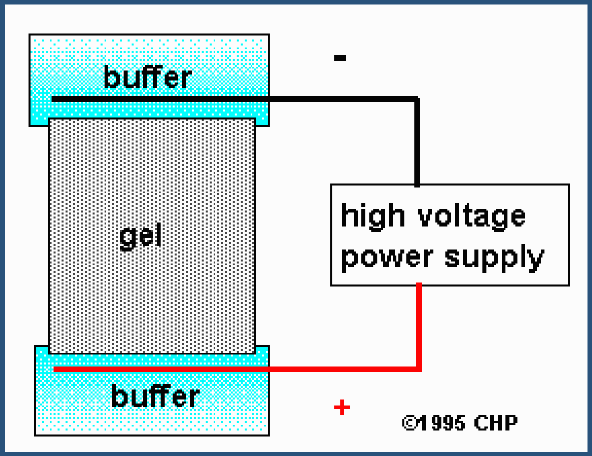
- It sorts a mixture of DNA molecules into bands, each band consisting of DNA molecules of the same length.



How:

1. Mixtures of nucleic acids or proteins are placed in wells near one end of a thin slab of a gel

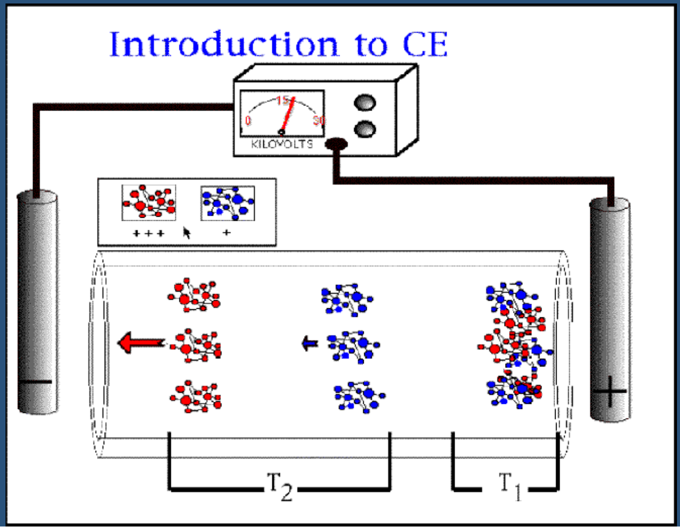




2. The gel is bathed in an aqueous solution (buffer).
3. This completes the electric circuit between the electrodes.
4. Electrodes are attached to both ends and voltage is applied

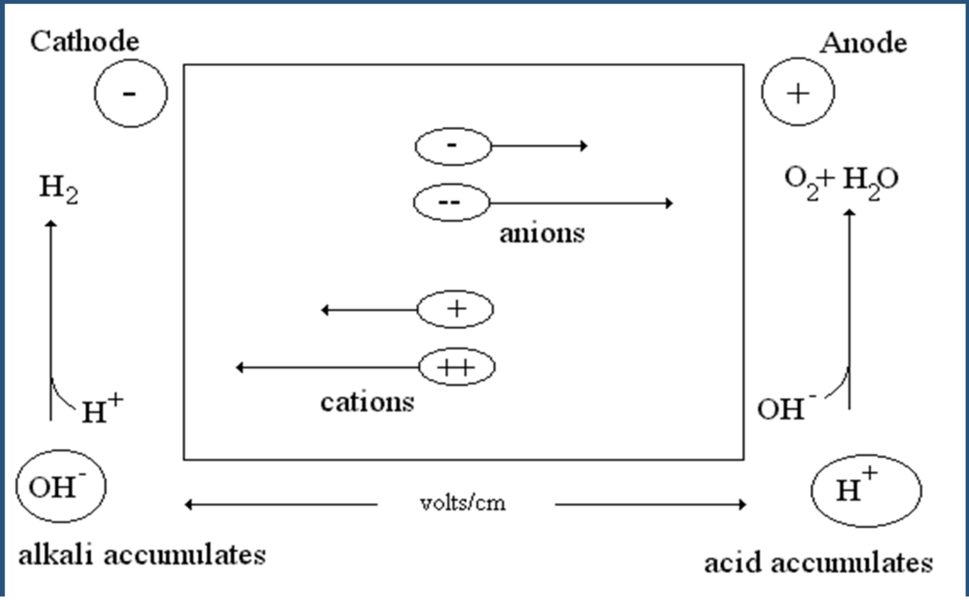
Gel Electrophoresis

- Each macromolecule then migrates toward the electrode of opposite charge at a rate determined mostly by
  - the molecule's charge and size
- How far a molecule travels while the current is on is inversely proportional to molecular size.



Gel Electrophoresis

- Nucleic Acids (DNA and RNA) are negatively charged : ”anions”
  - The “-” charge carried on the phosphate groups
  - The “-” charge is proportionate to their lengths.
- They will move toward the “+” electrode- ”anode.”



Gel Electrophoresis

- The polysaccharide Agarose is used to make the gel.
  - It is derived from algae.
  - Once poured, it contains microscopic pores which act as molecular sieves.
  - These will influence the rate at which the molecules will migrate.

Gel Electrophoresis

- Smaller molecules will be able to move through the pores more easily than larger ones:
  - The pores in gel impedes the longer fragments more than it does shorter ones.
  - If the fragments are the same size, the one with greater charge will migrate faster.

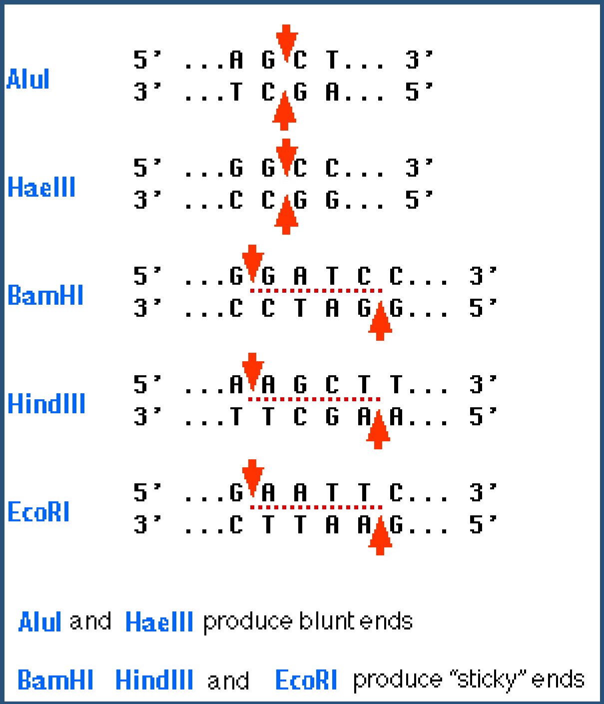
Gel Electrophoresis

- The higher the applied voltage, the faster the molecules travel.
- The buffer serves to make the water a better conductor of electricity and to control the pH.
  - The pH is important to the charge and stability of the traveling molecules.

Uses:

1. DNA Fingerprinting:

   -Chop up DNA with proteins called restriction enzymes



These cut DNA at specific sequences of base pairs (like those to the left) called restriction sequences.

DNA sequences are very alike in regions with genes that code for proteins.

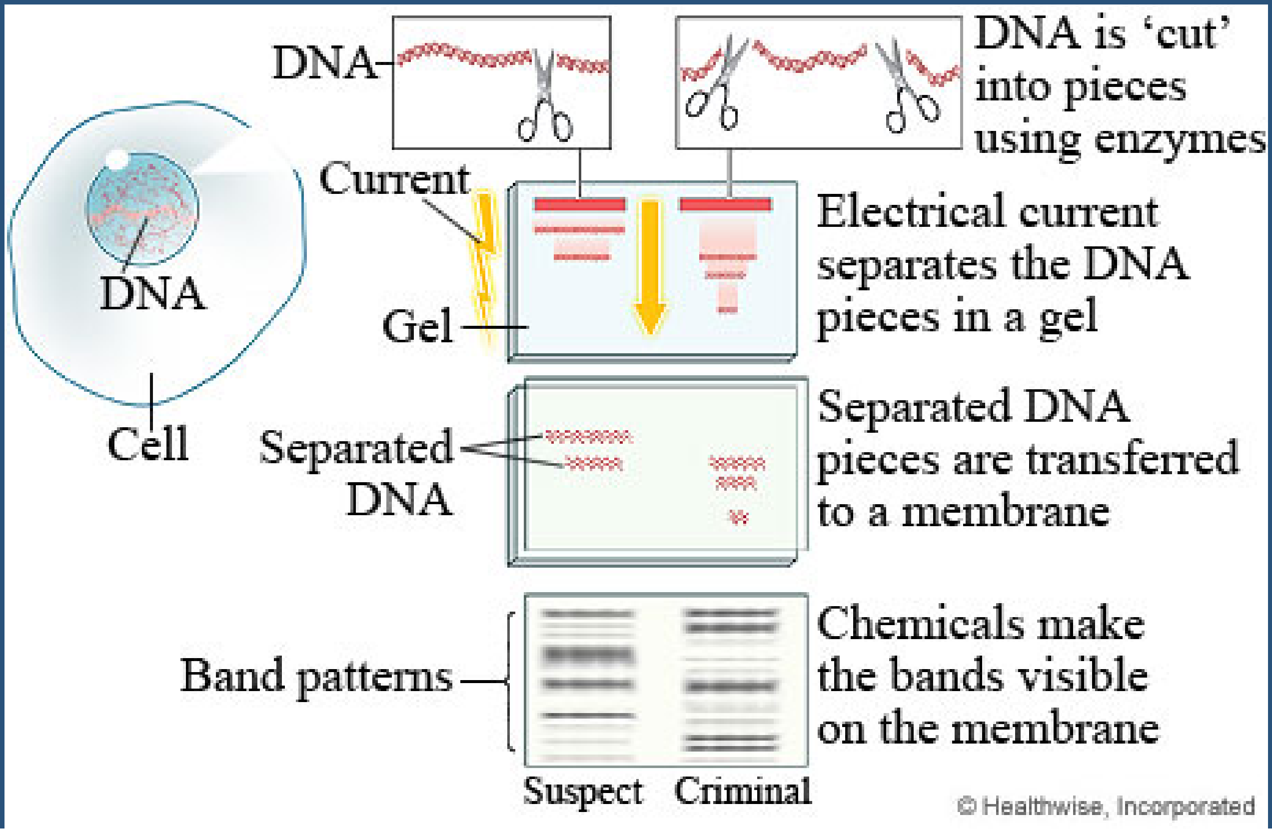
In non-coding regions ("junk DNA"), sequences are more variable due to mutations accumulating.

Restrictions enzymes cut in these non-coding regions- giving you a unique DNA fingerprint.

Uses:

1. DNA Fingerprinting:

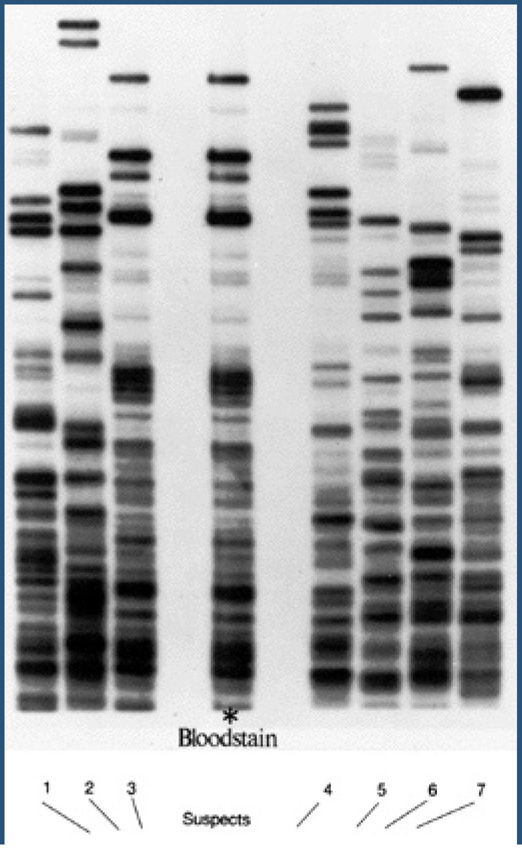
   -Chop up DNA with proteins called restriction enzymes



Uses:

1. DNA Fingerprinting:

   -Can be used to match the DNA found at a crime-scene to a suspect.



With this, it is as easy as matching up the lines with the evidence to the suspect.

The chances of two people having the same DNA fingerprint (if not identical twins) is about 1 in a billion.

Uses:

1. DNA Fingerprinting:

   -Can be used as a paternity test for the father.

This requires slightly more work.
The child receives half its DNA from the mother and half from the father- so half the lines will match the mother and vice versa with the father.

We usually know who the mother is, so you want to rule those lines out first in the child. Whatever lines remain in the child should match lines belonging to the father.

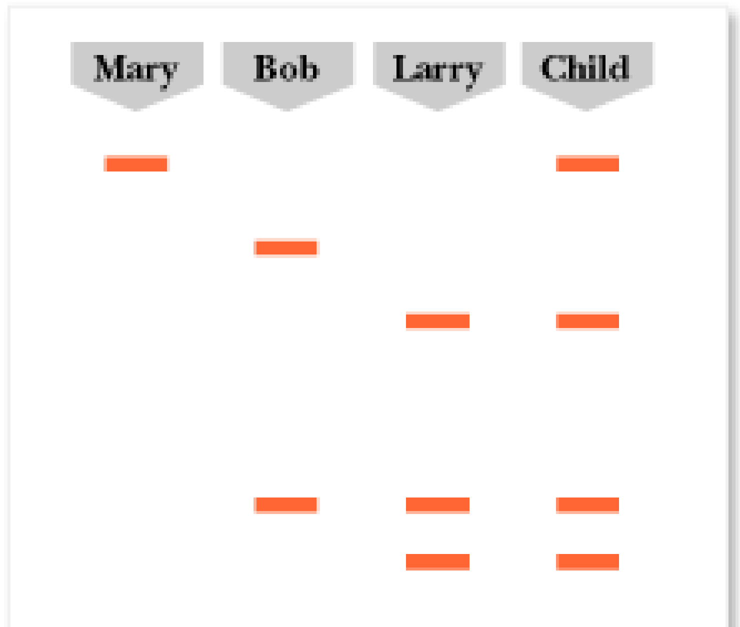


Figure 3

In this case Larry is the father of the child.

Uses:

1. DNA Fingerprinting

-Can be used to classify organisms based on DNA.

-Or to match DNA from body parts found at disaster sites/war zones to missing individuals.

Uses:

1. DNA Fingerprinting

-Finally it also be used to detect if a gene is present in a organism. This can be helpful in diagnosing a genetic disease like cancer.