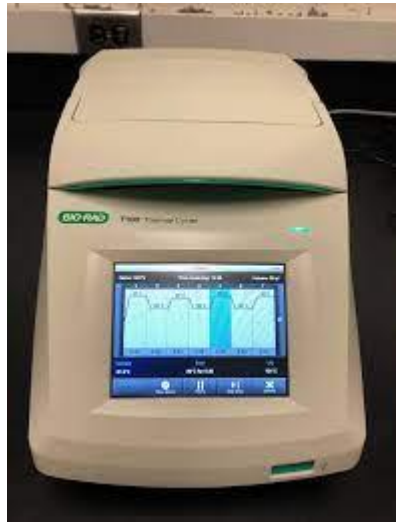
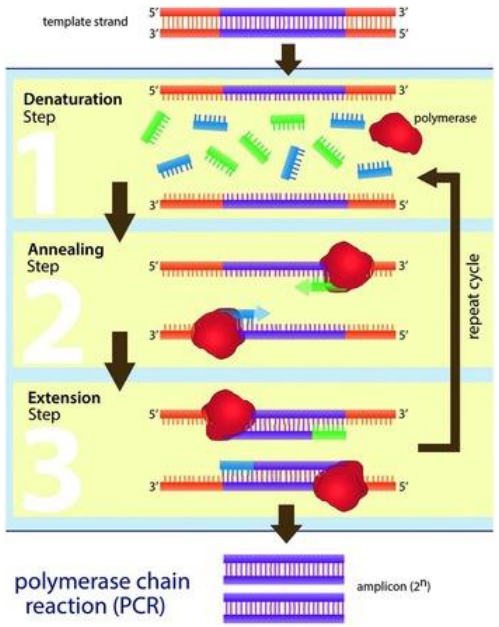




FDE 330
FOOD BIOTECHNOLOGY

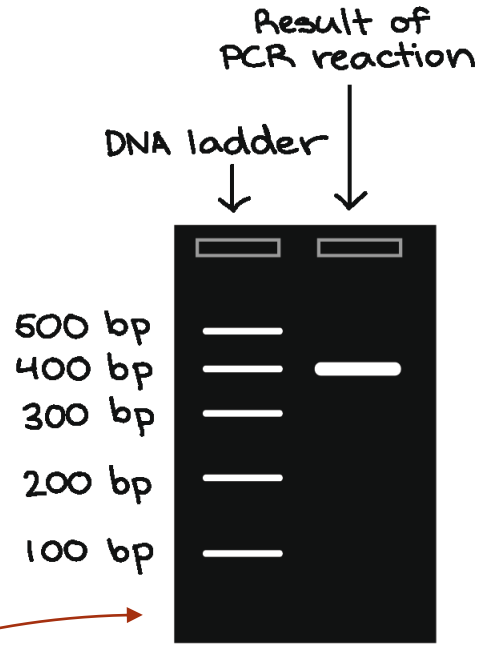
Gel Electrophoresis

Gel Electrophoresis

Gel Electrophoresis

Using gel electrophoresis to visualize the results of PCR

- The results of a PCR reaction are usually visualized (made visible) using gel electrophoresis.
- **Gel electrophoresis** is a technique in which fragments of DNA are pulled through a gel matrix by an electric current, and it separates DNA fragments according to size.
- A standard, or **DNA ladder**, is typically included so that the size of the fragments in the PCR sample can be determined.
- DNA fragments of the same length form a "band" on the gel, which can be seen by eye if the gel is stained with a DNA-binding dye. For example, a PCR reaction producing a 400 base pair (bp) fragment would look like this on a gel:



- A DNA band contains many, many copies of the target DNA region, not just one or a few copies. Because DNA is microscopic, lots of copies of it must be present before we can see it by eye. This is a big part of why PCR is an important tool: it produces enough copies of a DNA sequence that we can see or manipulate that region of DNA.

Gel Electrophoresis

- When working with DNA, it is often necessary to separate fragments of different lengths. Also, determining the length of DNA fragments is frequently useful. Both aims can be accomplished using gel electrophoresis.

- This technique involves the addition of a sample containing DNA to a gel (agarose or polyacrylamide) that is suspended in a buffer.

- *Polyacryamide gel electrophoresis* is usually used to separate proteins by charge and or size, but the size of DNA and RNA fragments or separation of nucleic acid molecules are estimated by applying an electric field to move the negatively charged molecules through an agarose matrix using *agarose gel electrophoresis*.

- Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel.

- Gel electrophoresis uses a gel as an anticonvective and sieving medium during electrophoresis and the movement of a charged particle in an electrical field.

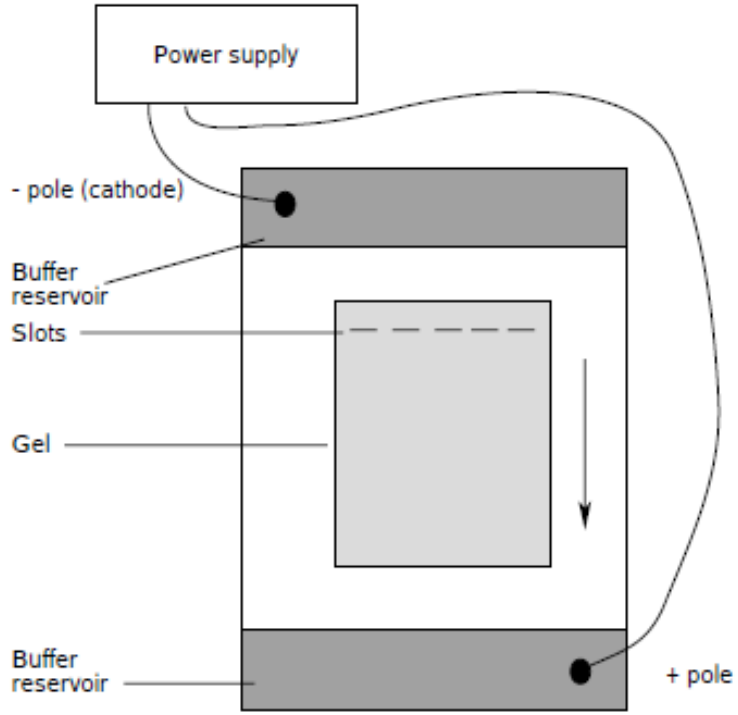
- DNA gel electrophoresis is usually performed for analytical purposes after isolation of crude DNA from the lysed cells, amplification of DNA via PCR, but may be used as a preparative technique prior to use of other methods such as mass spectrometry, restriction fragment length polymorphism (RFLP), PCR, cloning, DNA sequencing, or Southern blotting for further characterization.

Agarose Gel Electrophoresis

- **In agarose gel electrophoresis**, DNA is loaded into small slots (wells) cut into the gel.

- An electrical current is then applied, and the charge that DNA carries (due largely to the phosphate groups of the backbone) will move the DNA fragments toward the positive pole.

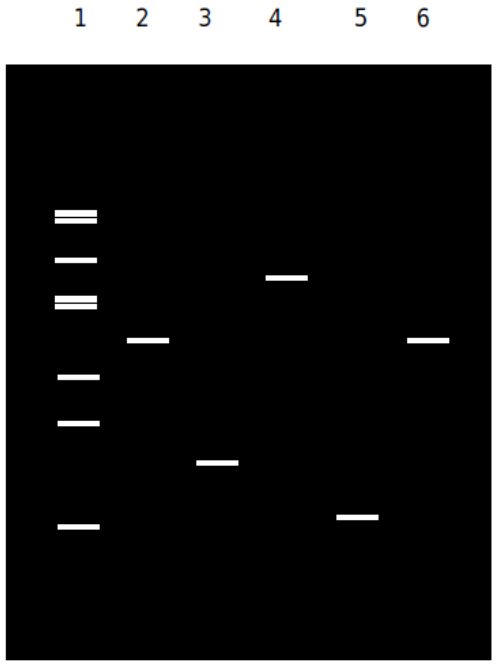
- However, the fragments do not move through the pores of the gel at equal rates; small fragments flow rapidly, whereas large fragments are impeded by the gel structure, and move slowly. Thus, fragments of different sizes are separated.



Gel electrophoresis
The gel is submerged in buffer, and DNA fragments migrate toward the anode (indicated by the arrow).

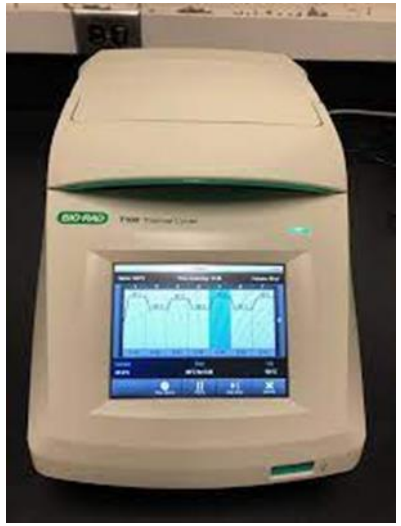
Agarose Gel Electrophoresis

- A number of samples can be added to each gel, creating discrete **lanes**.
- Fragments of known length can be added to certain lanes, allowing measurement of DNA fragment length in the other lanes.
- Once electrophoresis is completed, DNA in the gel can be visualized by adding **ethidium bromide** to either the gel or the DNA sample. Ethidium bromide binds to DNA and fluoresces visibly when exposed to ultraviolet light.

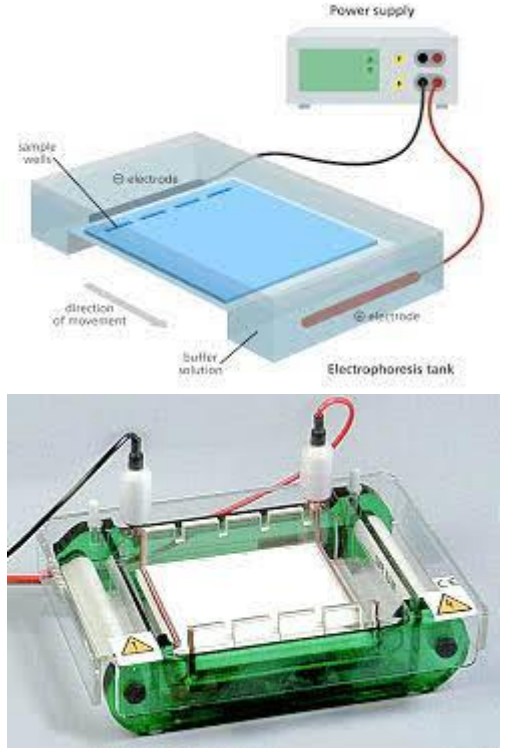


- Molecular biologists measure DNA size in units of base pairs or kilobase (kb) pairs (thousands of base pairs).
- Gel electrophoresis can accurately separate and measure small fragments of DNA, but for segments larger than 40 kb, special techniques (e.g., pulsed-field gel electrophoresis) are required.

Separation of DNA fragments by gel electrophoresis
Lane 1 contains fragments of known molecular weight. This ladder can be used to measure the size of DNA fragments in lanes 2 through 6.

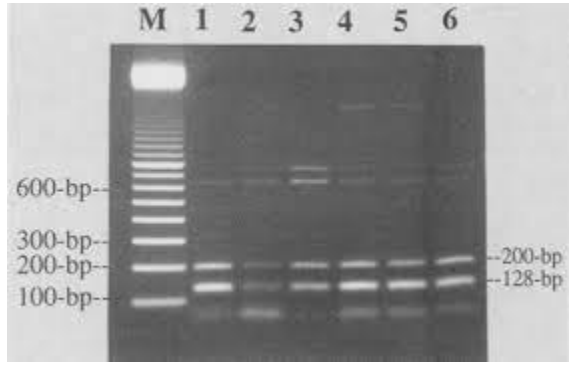


PCR



Agarose Gel Electrophoresis

UV Gel Imaging System



Analyzing and Interpreting the gel results of PCR products

Animation Links

- https://www.youtube.com/watch?v=QwT-Tj89VLo
- https://www.youtube.com/watch?v=saJIWFUGEbw