PCR Introduction, Procedure and Report.

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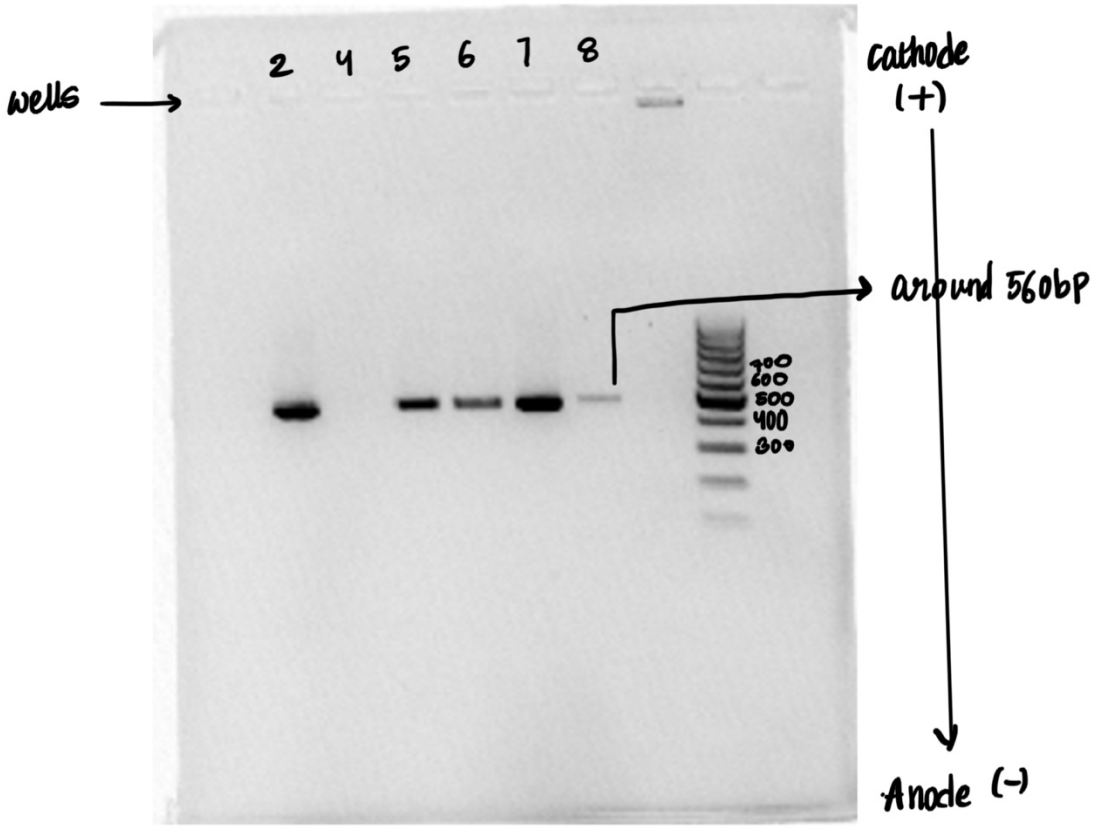
04/06/2023

Presentation of the picture of the gel, with proper labeling of each lane, wells, the sizes of the marker bands, the anode and cathode :



Enzymes work properly in certain temperatures and DNA will also bind together in those temperatures. These are unique for each organism. If the temperature is increased to a level where proteins do not function properly, the DNA will unbind. This is called denaturation.

Annealing is the second step of the PCR process, where the temperatures are cooled down, in this experiment 55-58 degrees, and the primers bind to the ends of the DNA strands. Hydrogen bonding occurs between the nucleotides of the DNA strand.

This experiment uses Taq DNA polymerase, it is isolated from the *Thermus aquaticus,* a bacterium that grows in hot springs. This DNA polymerase does not have proof-reading function which leads to high mutation rate (1/10^4). On the other hand, the normal DNA replication process found in living cells has proof reading function which leads less mutation.

The basis of separation of DNA molecules in the agarose gel:

- The molecular size of DNA – the distance the DNA fragments travelled is inversely proportional to the $\log_{10}$ of their molecular weight. So, the smaller pieces will move faster, and the heavier pieces move slower.
- DNA fragments are negatively charged, so they will be drawn towards the positive electrode (anode) in the gel.

In this experiment we used Ethidium Bromide which intercalates into the DNA molecules. The UV radiation at 254 nm is absorbed by the DNA and transmitted to the bound dye(Ethidium Bromide). The energy is re-emitted at 590 nm in the red-orange region of the spectrum.

Results:

- The predicted size of the PCR product is 440 bp.
- The estimated size was 560 bp.
- We are using a 100 base pair ladder and not the 500 because the Human mitochondrial genome is really small, it will give us more precise and accurate measurement than 500.
- After 30 cycles, $2^{30} = 1.07 * 10^9$ of DNA molecules will be present.