Introduction to PCR

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Introduction to PCR

Goals

In this lab, students will be expected to extract DNA from a saliva sample, amplify specific sequences of DNA, and test amplification with gel electrophoresis.

Learning Objectives

Students will learn and perform:

- Primer design for PCR amplification
- DNA extraction from Saliva samples
- Centrifugation
- PCR amplification and cycling
- Gel electrophoresis Analysis

Safety

ALWAYS WEAR NECESSARY PPE

This safety sheet has been designed to provide you with the tips and guidelines to follow in order to maintain the utmost safety

when doing this experiment. This lab will combine a DNA amplification method with gel electrophoresis. Both of these techniques require you to be very careful about the reagents you are using and how you are using them.

In this lab, you will be utilizing chemicals and reagents that can definitely be troublesome if you do not properly use them in the correct ways. This is why it is essential that you know the type of reagents you will be working with and the potential harm they can cause you. With a sound understanding of your reagents and their specific uses in the protocol, you will not only gain a better understanding of how hazardous the reagent itself is but you will have a better understanding of the protocol as a whole.

Refrigerators will be needed to store the consumables inside of. In order to minimize the risk of cross-contamination, try not to store these items in the same places as food. Ideally, lab settings have entire refrigerators dedicated only to DNA, RNA, and other reagents. Since this course is being done inside your homes, we must try our best to minimize these contaminants as possible, make sure food and DNA that must be stored together are in good proximity apart and that the consumables are in another form of sealed containment(i.e plastic bag or box).

Tips and Hazards

- Wait a few seconds before taking PCR tubes out of Bento lab thermocycler; it is still hot
- When using the centrifuge make sure to include a counterweight
- Use a cloth, towel, or heat resistant gloves to take out agarose gel from the microwave; or wait for it cool where you can touch it comfortably

Background

The **Polymerase Chain Reaction** (PCR) is a technique that scientists have utilized to amplify DNA. When there are small amounts of DNA present in a sample, it is necessary to make copies of this DNA in order to be able to study the DNA in detail. PCR has become a commonly used technique in the medical lab, clinical lab, and even forensics labs.

PCR takes advantage of thermal cycling, where reactants are exposed to different heating and cooling temperatures to either denature the DNA or activate enzymes essential to the reaction. The primary reagents involved in a PCR lab are oligonucleotides called primers and DNA polymerase. The first thing that happens is that the DNA duplex is denatured, and the two strands are unannealed from each other. The primers are then added so that when the temperature is lowered they can ligate onto their complementary regions. Now, these new primers will serve as templates for DNA polymerase to enzymatically begin adding nucleotides to it and begin the process of creating a new strand of DNA. As each nucleotide is added, it serves as a template for the next nucleotide, creating a chain reaction where the original DNA sequence is amplified exponentially.

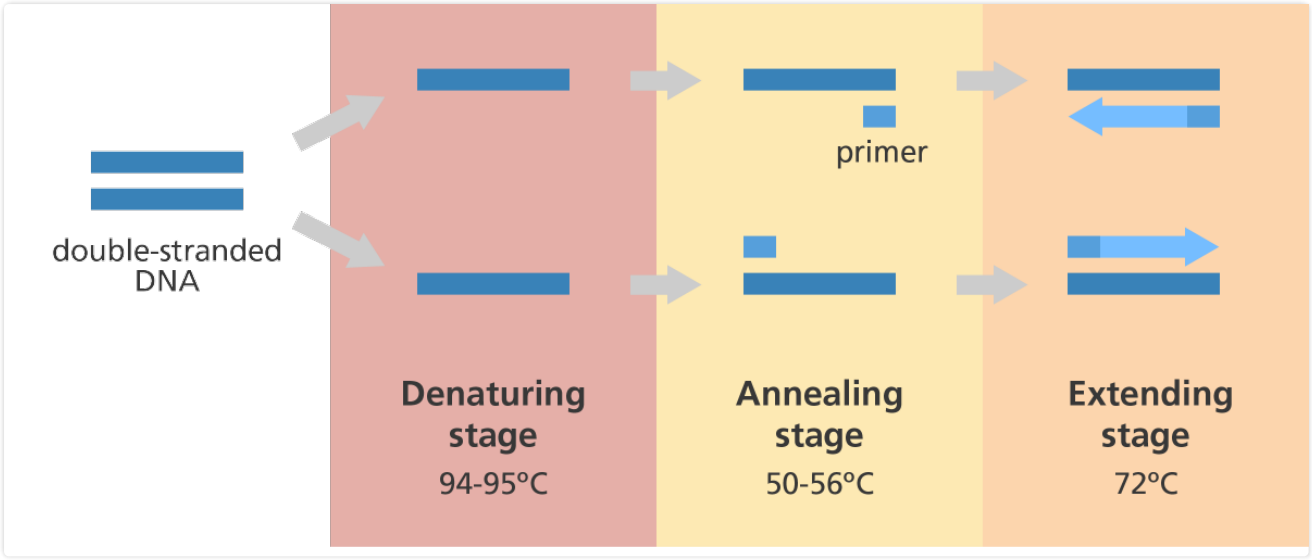
Key Components

- **DNA Template:** Target DNA region one intends to amplify
- **DNA Polymerase:** Enzyme that polymerizes more DNA strands
- **DNA Primers:** Two DNA primers that are complementary to the 3' end of both strands of DNA; what serves as the template for DNA polymerase to bind and elongate the strand
- **dNTPs:** The building blocks of what DNA polymerase makes the new strand of DNA from

PCR can be broken down into three stages. Each step serves its purpose in the overall reaction and requires its own specific temperature range that the thermal cycle will create for the sample. These stages can be further described below:

Stages

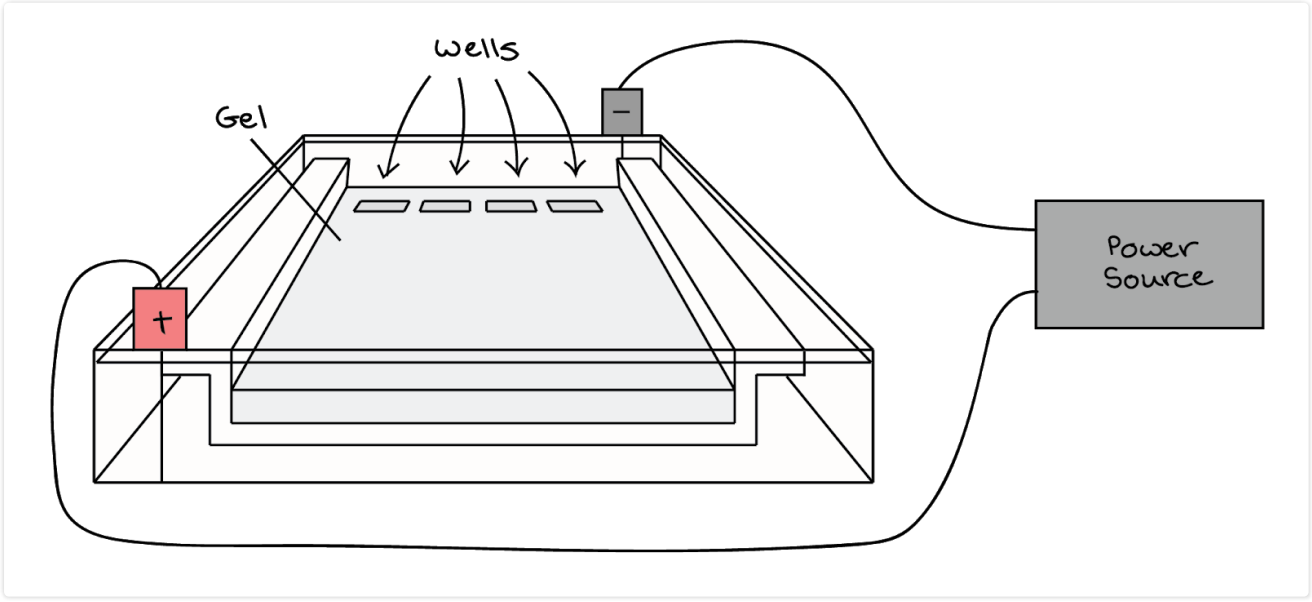
- **Denaturation:** The first step of the reaction. This requires that the temperature be raised to 94-98°C. This is the stage where the DNA duplex is denatured by breaking the hydrogen bonds between complementary base pairs. The results are two single strands of DNA.
- **Annealing:** The second step of the reaction. The temperature is lowered in order to allow the DNA primers to anneal to their complementary regions. The thermal cycler brings the temperature down to about 50-65°C. This step is very crucial to the overall process because it essentially lays out the template for which a new strand will be created. You want to make sure that the annealing temperature is low enough so that the primers are able to hybridize to the strand but it also needs to be high enough so that the binding of this primer is specific.
- **Elongation:** The final step of the reaction. This is the step where the DNA polymerase will go over the primer and make use of the dNTP's in the solution to create a new DNA strand. The temperature will primarily depend on the polymerase you are using as they have different activation temperatures. For Taq polymerase, a DNA polymerase commonly used for PCR, the temperature for this stage is 72°C.



Your Genome website

These three steps together coincide with one cycle of the reaction. In order to get the millions of copies one desires, multiple cycles need to be done. The formula needed to calculate the number of copies of DNA made after a given cycle is 2n, where n represents the number of cycles.

Gel electrophoresis is a technique used to separate DNA fragments (or other macromolecules, such as RNA and proteins) based on their size and charge. Electrophoresis involves running an electrical current through a gel containing the molecules of interest. The gel is composed of many pores. The pores allow the molecules to move through them. Based on their size and charge, the molecules will travel through the gel in different directions or at different speeds, allowing them to be separated from one another. All DNA molecules have the same amount of charge per mass. Because of this, gel electrophoresis of DNA fragments separates them based on size only. The smaller the molecule the faster and further it travels, while the bigger the molecule the slower and closer it travels. Using electrophoresis, we can see how many different DNA fragments are present in a sample and how large they are relative to one another. (Khan)



khan

For gel electrophoresis, there is a power source that allows for a running current to occur within the gel. The current is produced by 2 electrodes placed at different ends of the box containing the gel electrophoresis. DNA has an overall negative charge, so the DNA will move from the negative electrode in the direction of the positive electrode. In the gel, we produce multiple wells. Each well holds one sample of DNA and one well of DNA must always hold a DNA ladder. A DNA ladder is a reference for DNA samples, it displays multiple bands of DNA where the size is already known. This creates a reference for the DNA samples.

Resources

Bento lab Resources page

Gel electrophoresis (Khan; article)

Disclaimer:

The information provided on this document is intended for the educational purposes of the BME 22L laboratory course. It is worth noting that the information listed on this document is subject to change and is not finalized. Therefore, the information on this document should not be used outside of this course.