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Genetic Testing for Non-Major Biology Students

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LAB 7: DNA TESTING

I. INTRODUCTION

What is genetic testing and why is it important to you? Genetic testing is the examination of your DNA, the chemical instruction manual that your body uses to function. DNA is a two-stranded molecule formed into a double helix shape like a twisted ladder. People often get genetic tests to look for changes in their DNA caused by mutations. Genetic tests can help understand risks from genetic conditions, the chance of a genetic condition in your family, cancer, or determining the cause of a disease. Examples of genetic links to human health include obesity, breast cancer, dyslexia, and an array of others.

What is a polymerase chain reaction (PCR)? The polymerase chain reaction (PCR) is a technique used to amplify (increase in number) a specific piece of DNA. Think of it like a technology that can find a needle in a haystack and then make millions of identical copies of that needle.

In this lab, you will be amplifying your own DNA in a tube. You will be looking for a particular piece of DNA that has a different size in different people. Once the lab is complete, you will be able to interpret the size of this particular piece of DNA in your genome. The genome is the term used to represent a person's complete set of DNA. DNA is the blueprint that allows our bodies to function. Molecular biology is the study of genes and the molecular details that regulate the flow of genetic information from DNA to RNA to proteins, from generation to generation.

Within the molecular framework of biology, DNA, RNA, and proteins are closely tied to each other. Because proteins and enzymes (enzymes speed up chemical processes in the body) ultimately play such a critical role in the life process, scientists study proteins in an attempt to understand how they work. With this understanding, it was believed we could cure, prevent, and overcome disease and physical handicaps as well as explain exactly how and why organisms exist, reproduce, and die.

Think of DNA→ to RNA→ to proteins like this:

- DNA: The complete cookbook that a restaurant uses that has thousands of recipes.
- RNA: The daily menu at a restaurant, it is a very small amount of meals compared to the size of the massive cookbook.
- Proteins: A single meal on the daily menu that you order

The objective of PCR is to produce a large amount of DNA in a test tube (in vitro), starting from only a tiny amount. A researcher can use tiny amounts of DNA from a drop of blood, a single hair follicle, or a cheek cell, and make enough DNA to study. In theory, only a single template strand is needed to copy and generate millions of new identical DNA molecules. It is the ability to amplify the precise sequence of DNA of interest that is the true power of PCR.

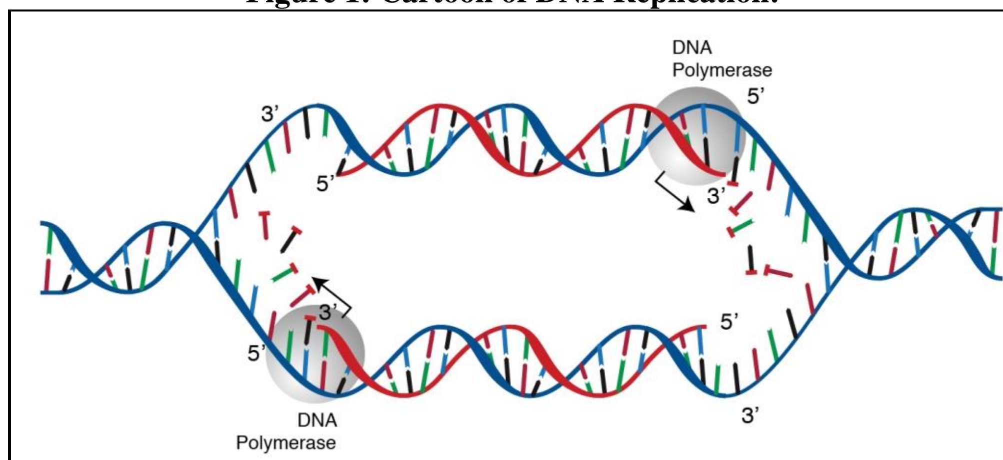
PCR has made an impact on many areas of biomedical research: gene mapping; cloning; DNA sequencing; and gene testing. PCR is now used as a medical diagnostic tool to detect specific mutations that may cause genetic disease; in criminal investigations and courts of law to identify suspects, and in the sequencing of the human genome. The development of PCR transformed molecular biology from a difficult science to one of the most accessible and widely used disciplines of biotechnology. PCR is widely used to detect COVID-19!

DNA Replication:

Every cell in your body contains a copy of your DNA. When your body makes new cells (cell division) your DNA has to be replicated so the daughter cells (new cells) have the same DNA as the parent cell. When DNA replicates, it makes almost perfect copies of itself.

To begin DNA replication, the double-stranded DNA has to be unwound so that the two strands are separated. When the strands are separated by an enzyme, a protein then tells another protein, DNA polymerase, to begin adding nucleotides (A, C, G, and T) onto the single strand to create a new double-stranded molecule of DNA. DNA replication ends with two complete molecules of DNA after starting with one DNA molecule.

Figure 1: Cartoon of DNA Replication:



Polymerase Chain Reaction (PCR):

It is estimated that there are 20,000 individual genes in the human genome. The true power of PCR is the ability to target and make millions of copies of (or amplify) a specific piece of DNA (or gene) out of a complete genome. In this activity, you will amplify a region within your chromosome 16. The recipe for PCR amplification of DNA contains a simple mixture of ingredients. To replicate a piece of DNA, the reaction mixture requires the following components:

1. DNA template: Containing the intact sequence of DNA to be amplified. (Your own DNA).

2. Individual deoxynucleotides: A, T, G, and C, the raw materials of DNA.

3. DNA polymerase: An enzyme that assembles the nucleotides into a new DNA chain.

4. Magnesium ions: A cofactor (catalyst) required by DNA polymerase to create the DNA chain. A catalyst increases the rate of a chemical reaction.

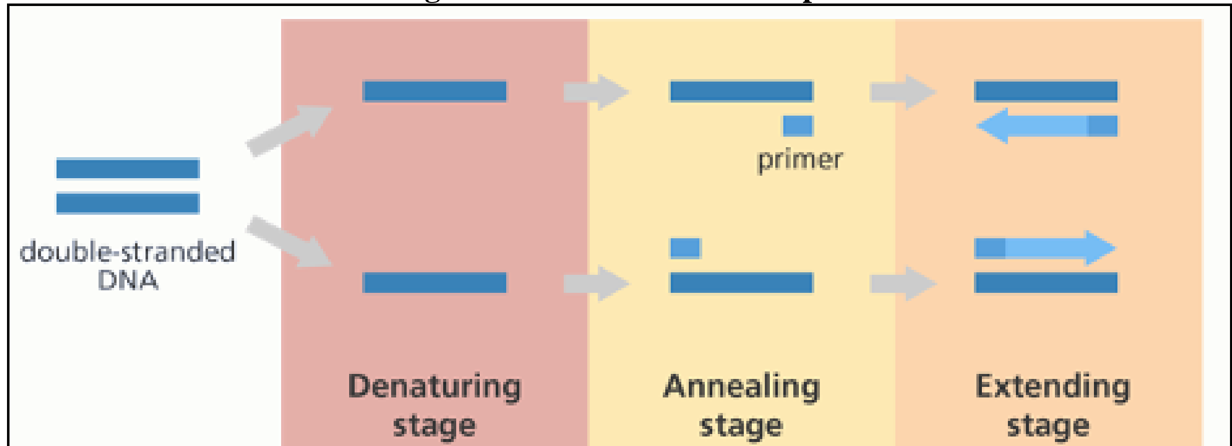
5. Oligonucleotide primers: pieces of DNA complementary to the template that tell DNA polymerase exactly where to start making copies.

6. Salt buffer: provides the optimum ionic environment and pH for the PCR reaction to take place.

The PCR reaction we are doing contains a complete master mix that is already combined and ready for you to add your own DNA to begin the PCR process. The complete master mix contains Taq DNA polymerase, deoxynucleotides, oligonucleotides, oligonucleotide primers, magnesium ions, and the buffer. Every time a cycle is completed with your DNA added, DNA copies are doubled, going from 2 to 4 to 8 to 16, and so on, until after 20 cycles, you will have more than a MILLION copies of your DNA. The primers you are given in the PCR kits in the lab target the exact sequence of DNA that match the DNA that you are testing on chromosome 16.

PCR amplification occurs in 3 steps: a denaturation step, an annealing step, and an extension step.

Figure 2: Cartoon of PCR Steps:



Denaturation: The reaction mixture containing your DNA and the complete master mix is heated to 94°C for 1 minute. Which unravels the double-stranded DNA into two single-stranded molecules. This is similar to the first step of DNA replication. The high temperature usually deactivates all proteins and enzymes that would be used in DNA replication, but the Taq DNA polymerase is naturally active at high temperatures, so it is not deactivated.

Annealing: During this step, the oligonucleotide primers in the complete master mix find their complementary sequences on the single strands of DNA (think of it as a lock and key). Similar to an RNA primer in DNA replication, the oligonucleotide primer allows the Taq DNA polymerase to figure out where to bind to begin the extension step.

Extension: After the Taq DNA polymerases bind to the oligonucleotide primers the Taq DNA polymerase adds nucleotides (A, T, G, and C) to the strand, creating a copy of DNA. For this step, the temperature is 72°C, the best temperature for Taq DNA polymerase activity.

These 3 steps complete one cycle of PCR. A complete PCR amplification undergoes many of these three cycles to make millions of copies of DNA. We do PCR in a special machine that holds the tubes and can very rapidly heat and cool to complete each step. Even with 40 rounds of the three steps, this will only take a few hours in the PCR machine in MCK.

Table 1: How DNA Replication is Similar Compared to PCR

DNA Replication	Polymerase Chain Reaction (PCR)
DNA replication occurs inside the body (in vivo)	PCR occurs in the laboratory (in vitro)
Unwinding of DNA by an enzyme	Denaturation- unwinding your DNA using heat at 94°C
DNA polymerase binds to the open DNA strand so it can add new nucleotides (A, C, G, and T)	Annealing- cooling the DNA to 60°C so Taq DNA polymerase can bind to the open strand so it can bind new nucleotides (A, C, G, and T)
DNA polymerase binds nucleotides to extend the strand to create a replicate of the template strand	Extension- heating the DNA to 72°C where the Taq DNA polymerase binds nucleotides, extending the strand to create a replicate of the template strand
A copy is created of the template strand resulting in two copies of DNA	Many copies are created of the template strand.

Gel Electrophoresis of Amplified PCR Samples:

It is estimated that the 23 pairs, or 46 chromosomes, of the human genome (23 chromosomes come from the mother and the other 23 come from the father) contain approximately 20,000 genes. Each chromosome contains a series of specific genes. The larger chromosomes contain more DNA, and therefore more genes, compared to the smaller chromosomes. Each of the homologous chromosome pairs contains similar genes.

Each gene holds the code for a particular protein. Interestingly, the genes only comprise 5% of the total chromosomal DNA. The other 95% is noncoding DNA. This noncoding DNA is found not only between but within genes, splitting them into segments. The exact function of the noncoding DNA is not known, they are called introns. Exons are the portion of DNA that is coded into proteins in your body.

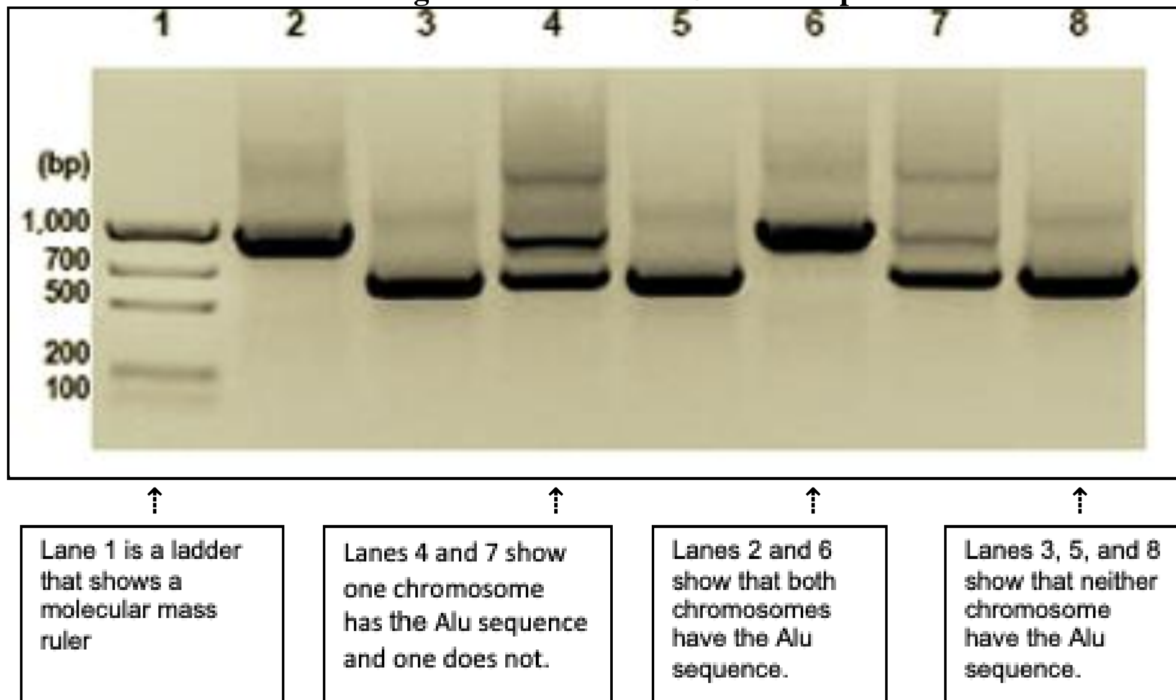
The specific sequence (intron) we are testing for in you is called an "Alu sequence." In this activity, you will look at an Alu sequence in a region of your chromosome 16. The Alu sequence is present in some people, but not all. Some people have the Alu sequence in one copy of chromosome 16 (one allele), others may have it in both copies of chromosome 16 (two alleles), while some may not have it on either copy of the chromosome. The presence or absence of this insert can be detected using PCR followed by agarose gel electrophoresis.

In this lab, you will not have "real genetic results" because we are targeting a section of your DNA that is in a non-coding region (introns). This means the DNA we are looking at does not code for anything that your body produces. Whether you have the Alu sequence or not, it does not matter since it does not make a product, so it is nothing to worry about.

Gel electrophoresis in an agarose gel separates DNA pieces according to their size. The agarose gel is a slab of gel with compartments in it that you will insert the PCR mixture into to determine whether or not you have the Alu sequence in your DNA. When the gel is placed into a buffer solution, an electrical current is passed through the gel. Your DNA is negatively charged and when electric currents are applied, DNA is drawn towards the positive side (opposites attract). The agarose gel allows smaller DNA pieces to move more easily than the larger ones, therefore, smaller particulars can move further down the gel than larger ones.

If both of your chromosomes contain the Alu sequence, each PCR product will be 941 base pairs. If neither of your chromosomes has the Alu sequence, each PCR product will be 641 base pairs. If you have an Alu sequence on one chromosome and not the other, you will see one band that is 941 base pairs and one that is 641 base pairs, the gel will show two bands if this is the case. The increase in size is due to a 300 base pair sequence contributed by the Alu insert if it is present.

Figure 3: Results of a Gel Electrophoresis



II. PROCEDURE

Week 1 – recover DNA from each student for PCR. PCR for both sections will run overnight and then your tubes will be in the refrigerator until next week.
Week 2 – run PCR samples on gel electrophoresis to see results (see Figure 3).

III. DATA

My tube number is:

My DNA is in lane:

Now interpret your results! Check one of the following:

I have the Alu sequence on both of my chromosomes _______

I have the Alu sequence on one of my chromosomes _______

I have the Alu sequence on neither of my chromosomes _______

IV. PURPOSE

This is a two-week lab that focuses on genetic testing using techniques that are fundamental in biology by using the polymerase chain reaction (PCR) and gel electrophoresis. The goal is to connect concepts learned in lecture and lab to analyze your very own DNA just like a geneticist would interpret results.

SKILLS- to obtain throughout this lab:

- General lab protocol (pipetting, loading gels, and centrifugation)
- Experimental design
- How to interpret scientific information

KNOWLEDGE- you will learn and build upon:

- What PCR is and how it is related to DNA replication in cells
- The components that PCR requires
- Learn about positive and negative controls
- What gel electrophoresis is and how to understand results