Lab #1: Amplifying the ALU intron for Hardy-Weinberg Analysis, Part 1

OBJECTIVES:
- Review the following terms and concepts presented in Biology 211: enzymes, DNA structure and replication, role of DNA polymerase, primers, cell structure, PCR, gel electrophoresis, homologous chromosomes, introns, Mendelian Genetics, alleles
- Application of modern biotechnology techniques (DNA extraction and PCR) toward population genetics analysis. Learn the technique of Polymerase Chain Reaction (PCR)

INTRODUCTION:
In this Lab exercise, we will attempt to isolate our own DNA and then use the Polymerase Chain Reaction (PCR) to analyze our own genetic make-up! Recall that PCR is a powerful technique that mimics cellular DNA replication to make millions of copies of short, specific regions of DNA. We will use this technique to amplify a short, specific region of our own genomic DNA. Amplifying this DNA will allow us to compare the frequencies of this allele in our class (population) and compare it to those predicted by the Hardy-Weinberg Equation.

A Quick Review of PCR:
In 1983, Kary Mullis at Cetus Corporation developed the molecular biology technique known as the polymerase chain reaction (PCR). PCR revolutionized genetic research, allowing scientists to easily amplify short specific regions of DNA for a variety of purposes including gene mapping, cloning, DNA sequencing and gene detection.

The objective of PCR is to produce a large amount of DNA in a test tube (in vitro) starting from only a trace amount. This template can be any form of double-stranded DNA such as genomic DNA. A researcher can take trace amounts of genomic DNA from a drop of blood, a single hair follicle, or a cheek cell and make enough to study. Prior to PCR, this would have been impossible!

PCR is conducted in three steps: 1) Denature the template DNA, 2) Allow the primers to anneal, and 3) Extend (copy) the template DNA. In the first step, the template DNA is heated up to break the hydrogen bonds holding the two strands together. This allows each strand to serve as a template for generating copies of the DNA. In the second step, the temperature is reduced to allow the primers to anneal, or bind, at their complimentary sequence on the template. In the third step, the temperature is raised again to allow the DNA polymerase to bind at the primer and add nucleotides at its 3` end. These steps are repeated 20-40 times, generating literally millions of copies of DNA from each template molecule!

The Target of our PCR:
Recall that humans have 23 pairs of chromosomes, or a total of 46 chromosomes. These chromosomes contain somewhere around 23,000 genes. Interestingly, these genes occupy only ~5% of our DNA. The other 95% of our DNA consists of non-coding DNA, or DNA that doesn’t directly code for a particular protein. Introns are an example of some of this non-coding DNA. As you might imagine, the sequence of our introns is much more variable than the sequence of the coding portions of our genes (exons) as mutations in introns don’t necessarily impact the structure of the protein. We carry, and pass along, such mutations silently as they don’t affect our phenotype.

Over time, many short inserted sequences have accumulated in our DNA. For example, most of us carry approximately 500,000 copies of a 300 bp sequence known as the Alu sequence in our DNA. The origin and function of these sequences are still unknown. Despite this, these repeated Alu sequences have proved
interesting for geneticists because when they are present in particular genes, they can be associated with diseases. The pattern of insertions can also be used to study the relatedness of individuals.

The target of our PCR will be a specific locus on chromosome 16 that sometimes contains an Alu sequence. At this site, some individuals have a single Alu sequence inserted. Note that in this case, the presence or absence of the Alu sequence has nothing to do with a disease state and simply serves as a convenient measure of molecular variation. Individuals may have this insert in both homologous chromosomes, just one homologous chromosome, or in neither of their homologous chromosomes. In today’s experiment, we’ll use primers (short regions of single-stranded DNA) that flank the Alu insertion site. These primers will provide the binding site for DNA polymerase to begin copying our template DNA. If the template DNA does not have the insertion, the amplified fragment will be 641 base pairs (bp) long. If the insertion is present, the amplified fragment will be 941 bp in length.

METHODS
NOTE: Please notify your instructor before beginning this lab if you have a family member who is also in this class.

Part A: Extracting Cheek Cell DNA
Our first challenge will be to extract our genomic DNA from our cheek cells. To do this, follow these steps:

1. Each member of your team should obtain one micro test tube and one tube containing 200 μl of the InstaGene matrix and label them with your initials.
2. Obtain a cup containing saline solution. Pour into your mouth and rinse vigorously (like a mouth wash) for 30 seconds. Expel back into cup.
3. Transfer 1 ml of your saline rinse into your micro test tube with your initials (not the tube with the InstaGene matrix!).
4. Cap micro test tube securely and place in centrifuge and spin your tube for 2 minutes.
5. After spinning, you should see a small whitish pellet. If no pellet is visible, repeat steps 3 & 4.
6. Pour off the saline supernatant leaving behind the pellet and a small amount (about 50 ml) of saline at the bottom of the tube. Re-suspend the pellet in the liquid by flicking the tube so that no clumps remain.
7. Using a 2-20 μl micropipette, transfer 20 μl of your re-suspended cells into the tube containing the InstaGene matrix. Close cap and shake well.
8. Place your tube in the 56º C heat block for 10 minutes. Approximately halfway through this incubation, remove the tube and shake briefly before returning to the heat block.
9. Remove the tube, shake again, and transfer it to the 100º C heat block for 6 minutes.
10. Remove the tube, shake again, and spin the tube at ~6,000 x g for 5 minutes.

Successful PCR depends on separation of all cellular debris and Instagene matrix. IT IS VERY IMPORTANT NOT TO SKIP THIS STEP. MAKE SURE TO SPIN FOR THE FULL 5 MINUTES! Once your sample has spun, try not to dislodge the pellet from the bottom (this is the debris you want to keep separate from the supernatant).
Lab: PCR of the Alu Insert for Hardy-Weinberg Analysis

Part B: Amplifying the Target Sequence by PCR

With our DNA successfully isolated from our cheek cells, we’re ready to begin the PCR. We’ll set-up this reaction today and allow it to run overnight. We’ll analyze our samples using gel electrophoresis during our next lab session.

A). Set up group controls:

Each lab group will need to obtain and label three CONTROL tubes (small PCR tubes you set into larger tube-holders so they sit upright in the foam or plastic rack). Note that PCR tubes have thin walls to facilitate heat transfer. This also facilitates the rupture of the tubes, so handle them with care!

1. Writing very small on only the top of the tube include a small group symbol on all tubes (so you can identify the tubes later) and one tube each with the following: ++, +/−, −/−

2. Using a fresh pipette tip for each sample transfer 10 μl of a homozygous control (+/+), homozygous control (−/−), and a heterozygous control (+/−) to their appropriate tubes.

NOTE: you may need to share control tubes with another group, OR controls may have already been prepared by the lab technician or your instructor.

B. Prepare your sample -

1. Label 1 PCR tube (on the top of the tube) with your group symbol and your initials. You will need to be able to identify your sample later.

2. Using a micropipette, remove 20 μl of your DNA supernatant solution (from tube you spun for 5 minutes) and transfer it to the PCR tube. AVOID THE PELLET! Do not transfer any of the InstaGene matrix or cell debris at the bottom of the tube.

3. Locate the tube of PCR Master Mix (MM). Note that this should be kept on ice! Remove 20 μl of the yellow PCR Master Mix and add it to the PCR tube containing your sample.

4. Pipette up and down to gently mix, avoid air bubbles if possible! Make certain to change pipette tips between samples!

5. Remove 20 μl of the yellow PCR Master Mix and add it to the 3 PCR control tubes for the group (note that the Master Mix may already be in the controls - your instructor will let you know).

6. Again, pipette up and down to gently mix, avoid air bubbles if possible! Make certain to change pipette tips between samples!

7. Cap the PCR tubes tightly (watching those thin walls!) and place them in the green thermocycler rack. Make sure to note where, in the rack, your team placed your samples. Make sure your group’s tubes are easily identifiable, and that you can recognize your own tube. The thermocycler will automatically heat and cool the samples, performing 40 cycles of PCR.

Clean Up:

• Used pipette tips, Master Mix tubes and InstaGene sample tubes should be put in the garbage.
• Ice can be poured down the sink.
• All other equipment (including Styrofoam sample holders, Styrofoam cups and PCR sample tube holders) should be returned to cart.
• Workstation should be wiped down if any solutions have been spilled.
AN OVERVIEW OF METHODS

Carefully collect cheek cells with a pipet tip
Add cheek cells to InstaGene matrix in micro test tube
Vortex or vigorously agitate to extract genomic DNA

**Incubate at 56°C for 10 minutes then repeat vigorous agitation**

**Incubate at 100°C for 6 minutes then repeat vigorous agitation**

Centrifuge samples for 5 minutes to pellet matrix

DNA template preparation

Transfer supernatant with genomic DNA to 200 µl PCR tube

Add primers to master mix containing:
- Nucleotides
- Reaction buffer
- DNA polymerase

Add complete master mix to student DNA samples and positive controls

Place tubes in thermal cycler and amplify target DNA sequence

DNA amplification