

Lab 5: Polymerase Chain Reaction

Introduction:

The Polymerase Chain Reaction (PCR) is a [molecular biology](#) technique for [enzymatically replicating DNA](#) without using a living [organism](#). The technique produces exponentially large quantities of a specific piece of DNA from trace amounts of starting material (template). The technique was made possible by the discovery of Taq polymerase, the DNA polymerase that is used by the bacterium *Thermus aquaticus*. This DNA polymerase is stable at the high temperatures needed to perform the amplification.

PCR amplification requires the presence of at least one DNA template strand. In this experiment we will use human genomic DNA. This lab consists of three parts: obtaining DNA (template), PCR amplification, and gel electrophoresis of amplified PCR samples. Your own cells will be the source of the template strands. PCR is a powerful tool because of its simplicity and specificity. Inexpensive reaction buffers, four DNA subunits (deoxynucleotide triphosphates of adenine, guanine, thymine, and cytosine), a DNA polymerase, two DNA primers, and minute amounts of the template strand are all that is needed to perform this reaction. The specificity of this technique is the ability to target and amplify one specific segment of DNA out of a complete genome.

In eukaryotes, genomic DNA is transcribed into RNA molecules containing both introns and exons. The functional segments of genes, the exons, code for proteins. Therefore, exon sequences are therefore similar among individuals. Introns often vary in size and number among individuals. This difference in intron sequences allows us to determine human genetic diversity. Intron sequences have been the target of random insertions by short repetitive interspersed elements (SINEs). One such repetitive element is called the Alu sequence. This is a DNA sequence about 300 base pairs long that is repeated almost 500,000 times within the human genome. In this lab, analysis of Alu repeats is used to estimate the frequency of an insert in a population and is a simple measure of molecular genetic diversity. This procedure is a simple PCR-based screen for a single Alu sequence within the PV92 locus on chromosome 16. The element is present in some individuals but not in others. Some may have the insert in the PV92 locus on one of their chromosome 16, others may have the insert in both alleles, and some do not have the insert in either chromosome.

Part I: DNA Template

Advance Preparation:

1. Aliquot InstaGene matrix (cheek cell preparation)
 - A. Thoroughly mix the InstaGene matrix.
 - B. Pipet 200 μ l of InstaGene into screwcap tube. Distribute one tube to two groups.
2. Prepare and aliquot InstaGene matrix with protease (hair follicle protocol)
 - A. Prepare a solution of InstaGene matrix containing 66 μ g/ml protease.
 - B. Pipet 200 μ l of InstaGene matrix with protease into screwcap tube. Distribute one tube to two groups.
3. Prepare and aliquot saline solution (cheek cell protocol)
 - A. Prepare a 0.9% saline solution.
 - B. Place 1 ml saline into separate cups for the two groups.

Cheek Cell DNA Template Preparation:

1. Label a 1.5 ml micro test tube with your group number / C (for cheek). Label screwcap tube similarly.
2. Scrape the inside of your cheek with plastic knife.
3. Pour saline solution into your mouth and rinse vigorously for 30 seconds. Expel the saline back into the cup.
4. Transfer 1 ml of your saline rinse into the micro test tube (NOT the screwcap tube). Scrape mouth again with a toothpick and swirl toothpick in micro test tube.
5. Spin your tube in centrifuge at full speed for 2 minutes. Remove your tube, you should see a match-head sized pellet of whitish cells at the bottom of the tube. If you don't see a pellet of this size, repeat the process.
6. Pour off the saline. Be careful not to lose your pellet. It's okay for a small amount of saline to remain.
7. Resuspend the pellet by flicking the tube so that no clumps of cells remain.
8. Set the micropipet to 20 μ l, transfer all of your resuspended cells to the screwcap tube containing InstaGene.
9. Screw the cap tightly on the tube. Shake to mix the tube contents.
10. Place the tube in the foam micro test tube holder and incubate at 56°C for 10 minutes in a water bath. At 5 minutes, shake the tubes gently, then place back into water bath.
11. Remove the tube, shake, and place into a 100°C water bath for 5 minutes.
12. Remove the tube and shake the contents to resuspend. Pellet the matrix by spinning at 6.0 rcf for 5 minutes in a centrifuge.

Hair Follicle DNA Template Preparation:

1. Label a 1.5 ml micro test tube with your group number / H (for hair). Label screwcap tube similarly.
2. Collect 2 hairs from either your eyebrows or eyelashes making sure that they have a noticeable sheath.
3. Place your tube in the foam micro test-tube holder and incubate at 56°C for 10 in a water bath. At 5 minutes, shake the tubes gently, then place back into water bath.
4. Remove the tubes, shake, and place into a 100°C water bath for 5 minutes.
5. Remove the tubes and shake the contents to resuspend. Pellet the matrix by spinning at 6.0 rcf for 5 minutes in a centrifuge.

Part II: PCR Amplification

Advance Preparation (Complete steps 1 & 2 while student samples are in boiling water bath):

1. Prepare complete master mix by adding primers
 - A. Pipet 550 µl of master mix into a labeled micro test tube.
 - B. Label micro test tubes “Master” for each of the groups and place the tubes on ice.
 - C. Add 11 µl of the primer mix to the master mix, pipet this solution up and down a 2-3 times to mix. The solution should be yellow.
2. Set up control PCR reactions
 - A. Label the control PCR tubes: +/+, -/-, and +/-.
 - Pipet 20 µl of the ++ template into each ++ PCR tube.
 - Pipet 20 µl of the -- template into each -- PCR tube.
 - Pipet 20 µl of the +/- template into each +/- PCR tube.
 - B. Pipet 20 µl of the complete master mix into each of the control tubes.
 - C. Place the tubes on ice until ready to load into the Gene Cyclor.

Cheek / Hair DNA Template:

1. Label a PCR tube and a capless micro test tube with group # / C or H, place the PCR tube in the capless tube, and place both in foam holder.
2. Transfer 20 µl of the DNA template (supernatant) from the screwcap tube into the bottom of the PCR tube. Be very careful not to transfer any of the matrix beads into the PCR tube.
3. Locate the tube of yellow matrix mix on ice and transfer 20 µl of the master mix into the PCR tube. Mix by pipetting up and down 2-3 times. Cap the tube tightly.
4. Place tube into the thermal cyclor with the control reactions. The reactions will undergo 40 cycles of PCR amplification.

Part III: Electrophoresis of Amplified PCR Samples

Advance Preparation:

1. Prepare agarose gels prior to lab
 - A. Prepare electrophoresis buffer. 1x TAE buffer is needed to make the agarose gel and is also required for each electrophoresis chamber. Add 3 ml of TAE to 147 ml of de-ionized water.
 - B. Make the agarose solution. Make a 1% solution by adding 1 g of agarose to 100 ml of of 1X TAE buffer.
2. Add 10 μ l of PV92 XC loading dye to each amplified positive control sample.

Amplified PCR Samples:

1. Obtain your PCR tube from the thermal cycler and place in the capless micro test tube. Centrifuge for ~3 seconds at 2.0 rcf.
2. Add 10 μ l of PV92 XC loading dye into your PCR tube and mix gently.

(CAUTION: WEAR GLOVES WHILE WORKING WITH GEL!)

3. Using a clean tip for each sample, load samples into wells of the gel:

Lane	Sample	Load Volume
1	MMR	10 μ l
2	(+/+) control	10 μ l
3	(-/-) control	10 μ l
4	(+/-) control	10 μ l
5	Group 1	20 μ l
6	Group 2	20 μ l
7	Group 3	20 μ l
8	Group 4	20 μ l

4. Secure the lid on the gel box and turn on the power supply and electrophorese at 100 V for 45 minutes.