Polymerase Chain Reaction (PCR) Lab  
Designed by Kim Harrington

**Background Information**

One technique that we use to distinguish DNA from different individuals is the Polymerase Chain Reaction (PCR). We take DNA samples found at a crime scene and compare them to suspect DNA.

PCR is extremely sensitive and “makes it possible to identify a person who dropped a bloodspot the size of a pinhead or licked the back of a postage stamp. Crime scene samples that are decades old or ravaged by fire and decay are now yielding profiles that answer questions about identity or guilt.”

PCR is a method for generating multiple copies of specific regions of DNA. The PCR technique is based on the principles of DNA base pairing and DNA replication and it consists of four steps:

1. DNA is extracted from the sample. This is the template DNA. It is mixed with:
   a. A solution of four dNTPs (Adenosine, Thymidine, Guanosine and Cytosine)
   b. A heat-resistant DNA polymerase enzyme called Taq polymerase.
   c. PCR primers. These are short (about 20 bases long) single-stranded DNA molecules that have been synthesized in the laboratory and have defined DNA sequences. The primer sequences are chosen to flank the region that is to be amplified.

2. **DENATURATION**: The reaction mixture is heated to 95°C for 5 minutes, which causes the two strands of the DNA double helix to denature or separate, exposing the bases.

3. **ANNEALING**: The temperature is lowered to between 50°C and 70°C for a minute or so. This allows the primers to hybridize to their complementary base pair sequences present in the template DNA. The temperature must be chosen to allow the primer to sit on the correct site but not on other sites it doesn’t exactly match with.

4. **ELONGATION**: Taq polymerase extends the primers by adding dNTPs to the ends of the primers, filling in the gaps between the primers and creating double-stranded DNA in the region between the primers.

Steps 2 through 4 are repeated 20 to 40 times. At each cycle, the number of double-stranded DNA molecules doubles.
Because the PCR method selectively amplifies a specific short region of a DNA molecule, the template DNA for the first cycle can be impure, present in tiny amounts, or partially degraded. As long as one DNA molecule in the sample has an intact amplification region, it will work. These features make PCR particularly useful for forensic DNA analysis which can be performed on single hairs, a small number of cells in saliva or decayed samples from old crime scenes. Go to the following website and do the PCR activity http://nobelprize.org/educational_games/chemistry/pcr/ use this to answer questions #1-3 below and then play the game! It is called The Eye of the Donkey.
The specific strategy for parts of the DNA to be amplified in forensic analysis differs. A common strategy is the use of Short Tandem Repeats (STRs). STRs are regions within a person’s DNA that contain specific DNA sequences randomly repeated a number of times. STRs have repeat units that are two to seven base pairs long repeated seven to 40 times per STR region. A subset of the STR loci in a person’s genome are used for DNA profiling. At present, the FBI specifies 13 STR loci as a core set to be used in forensic analysis. For example, one STR locus has 10 alleles (10 different repeat lengths) and has a four base pair repeat unit (TCTA). When all 13 core STR loci are used to generate a DNA profile, the probability that two randomly chosen profiles will match is approximately one in $6 \times 10^{14}$ (one in 600 trillion). See [http://www.cstl.nist.gov/biotech/strbase/](http://www.cstl.nist.gov/biotech/strbase/) for more information about forensic profiling.

The amplified fragments are then run on an electrophoresis gel to separate them. Electrophoresis separates DNA by size. Because DNA is ionized and negatively charged at physiologic pH when placed in an electric field it will migrate toward the positive electrode. Small pieces of DNA experience less resistance and move faster (farther) than large pieces.

Go to this web site [http://gslc.genetics.utah.edu/units/biotech/gel/](http://gslc.genetics.utah.edu/units/biotech/gel/) and do the activity. Answer the questions (5 through 8) on the prelab while doing the activity and turn it in at the beginning of the lab.

**RFLP Prelab Questions**

These questions must be answered from the lab introduction materials and turned in at the beginning of lab.

1. What does PCR do?
2. What are the main steps in PCR?
3. Why must you heat the sample to 95°C?
4. What is agarose?
5. What do you see as the gel is running?
6. What staining solution does the illustration use?
7. What are your estimates for the size of bands compared to the standard?

**Lab Exercise**

We are working in a forensic lab that tests samples for law enforcement in Washington State. We primarily work with DNA samples from blood, hair, saliva or cells found at crime scenes to try and identify the perpetrator of the crime.

A robbery occurred at a bank in University Place. The police arrived as the robbers were leaving the bank and shot one of the robbers. A few drops of blood were left at the scene which were taken into evidence. A car was stopped speeding through an intersection on Bridgeport Way. It is not known if the individuals in the car that was stopped were at the bank, but they were all three were felons and we can obtain their DNA from criminal data banks. A chapstick is believed to have fallen out of the bank robbers pocket at the scene and we have a DNA sample from that as well.

Our lab has received six DNA samples.

Sample 1: The robber’s blood from the crime scene.
Sample 2: A DNA sample from a patient treated for a similar gunshot wound the next day at Tacoma General Hospital.
Sample 3: Felon A in the car
Sample 4: Felon B in the car
Sample 5: Felon C in the car
Sample 6: Chapstick DNA
You will be assigned to one of six forensic groups. Do not share reagents or equipment with another forensic group unless specifically directed by your instructor. Always think about whether what you are doing might contaminate your sample. We don’t want this bank robber to get off because our lab messed up the evidence!

Procedure
Your forensic group will be assigned one of the six samples.

1. **Take a tube of sample DNA with your group number and a tube of PCR mix.**

You will be using two different micropipettors to transfer reagents. A few hints about using the micropipettors:

1. Place a tip gently on the end by holding your micropipettor and lowering it over a tip in the rack. Press down lightly and gently. The tip should attach to the micropipettor. Do not touch the end of the tip as you don’t want to contaminate it with your own DNA.
2. Make sure the micropipettors are dialed to the correct volume that needs to be dispensed. They should be set up for you. You should not need to change volumes during this lab. If you are unsure about your micropipettor volume, please check with your instructor.
3. To get reagents into the tip first push the button/plunger at the top of the micropipettor gently to the first stop. The moment that you feel any resistance, hold the plunger in that position. Put the tip in the reagent, close to but not touching the bottom of the vial. Holding it there, release the plunger, and allow the liquid to draw into the pipette tip.
4. Now put the pipette in the tube needing the liquid and gently push the button/plunger on the micropipettor top to the first stop. You can press a little further to get the last drop out. Keep the button pushed down and take the micropipette out of the solution. (If you let the button go while still in the liquid, it will suck the liquid back up)
5. Change the pipette tip if you are using different reagents as you don’t want to contaminate any tubes with reagents. If you are delivering the same thing to multiple tubes, you can use the same tip. The micropipettors that you are using have a separate button for ejecting the used tips. Your group should have a beaker in the supply tray for used tips.

**PCR setup:**

1. Your team should have a tube of DNA that has been numbered according to its source. **Using the navy blue pipettor (it should be set to 70 uL), add 70 uL of the PCR mix to your DNA tube.** When you add the PCR mix, use the pipettor to mix the DNA and mix together by drawing the mixture in and out of the pipette tip several times.
2. **Ensure that your tube is labeled on the lid with your group number.** This may be done for you already.
3. **Place the tube in the PCR machine for 5 minutes.**
4. **When the 5 minutes are up, use the gray and green pipettor to pipette 20 ul your DNA sample to each of the other 5 thin-walled tubes in your foam tray.**
5. **Distribute your tubes to each table in the lab, so that all groups (including your own) have a sample of your group’s DNA.**
6. When your rack has all 6 of the DNA samples from each of the other teams, you can **begin to load your electrophoresis gel.**

**Change tips between samples!**

- Use the gray and green pipette set to 20 uL. Start with the Mass Ruler and dispense 20uL into the first (Left) lane in the gel. Lower the pipet tip under the surface of the buffer into the well, but be very careful and gentle - don’t puncture the floor of the gel.
- Gently depress the pipet plunger and slowly expel the sample into a well. Keep the plunger depressed until the pipet is out of the gel box.
- Make sure there are no air bubbles in your pipet tip because the air will blow the sample right out of the gel.
- Refer to the illustration on the next page for how to load the lanes on your gel.
**Loading the Gel:**
Load 20 ul (or the whole tube DNA) in the correct lane as shown in the diagram below. The “M” stands for Molecular Mass Ruler, which should be in a 1.5 mL tube at your table. Just add 20 uL of the Mass Ruler. You should have seven lanes filled altogether.

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<thead>
<tr>
<th>M</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</table>

7. Once the samples are all loaded in the gel, place the lid on the chamber so that the red and black corners match, and make sure your chamber is plugged into the power supply unit. Then **turn the power supply ON**. Allow electrophoresis to proceed until the dye and DNA are out of the wells and held by the agarose molecules. Continue electrophoresis for 30-40 minutes until the DNA is at least half way into the gel. You can see this by looking at the side of the chamber and looking for the bluish/purple loading dye band.

8. At this point you can **turn off the power, remove the lid, and remove your gel from the apparatus by lifting the plastic casting deck**. **Caution:** The gel and buffer may be very hot!!! If so, allow them to cool. **Gently slide your gel from the casting deck into the staining tray.** Place your casting deck back into the electrophoresis chamber.

9. **Take your gel to the staining station, and carefully pour enough 100X FastBlast into the tray to just cover the gel.** Let it stain for 2-3 minutes with gentle agitation. Do not go longer than 3 minutes!!

10. Carefully decant your stain from your gel back into the the FastBlast container (please use the funnel provided) and gently transfer your gel to the rinsing container. This is the large tub that your groups supplies were in at the beginning of the lab. One way to transfer your gel without tearing it is to invert the empty rinsing container over your staining tray with the gel in it. **Press the two trays together and flip them over so that the gel falls into the rinsing container.**

11. **Fill the rinsing container with about 1/3 of the way with hot tap water.** Gently rock the container for about 5 minutes, after which, gently decant the water from the container into the sink (be sure not to let the gel pour out, too!). Add fresh hot tap water and rock for another 5 minutes. **Continue this process a couple of times.** Bands should begin to appear in about 15 minutes. Do not be discouraged if they do not appear in 15 minutes. Sometimes the destaining process could last several hours. If you do not see bands before the end of the lab period, place it in the polythene ziplock bag provided. You will be able to view your gel during a future class meeting.
While the gel is destaining, there is one other task that every forensic lab technician must do. In order to ensure that you have not contaminated your test samples with your own DNA, we must make a sample of your DNA for control purposes.

DNA Extraction:

1. Obtain a sample of Gatorade in a sterile disposable cup. You may need to pour your own. Pour just enough to cover the liner in the bottom of the cup.
2. Pour the Gatorade into your mouth.
3. Swish the Gatorade vigorously and then spit the Gatorade back into the cup.
4. Transfer the Gatorade with cheek cells into one of the clean vials of soap water.
5. Cap the vial tightly, and then gently roll it on the lab table for 2 to 3 minutes. Be very gentle, and do not shake the vial. Shaking the solution could shear the DNA strands and reduce the yield.
6. Open the vial, and hold it at a 45% angle. Very slowly add ice-cold ethanol to the vial, so it pours down the side of the vial and forms a layer that floats on top of the soapwater-and-spitty-Gatorade mixture.
7. The DNA will begin to precipitate at the interface of these two layers and should begin to float into the ethanol layer.

You will get a lot of DNA from your cheek cells in this procedure.
1. Copy the bands visualized on the gel onto this paper in the space below.

2. Use a ruler to make sure that you have the correct spacing for the bands. The DNA ladder that was used for reference has 5 bands at 5000 base pairs (bp), 2000 bp, 850 bp, 400 bp and 100 bp. Graph this data in Excel by Typing “Distance Traveled” in the first row in column A and “Base Pairs” in the first row in Column B. Enter your data into these columns. With your cursor still in one of the data cells, click on the "Insert" tab at the top of the screen and in the “charts” section, pick the Scatter plot. Choose the scatter plot that has smooth lines with Markers. Your graph should have base pairs on the y axis and distance traveled on the x axis. Now there should be a chart menu at the top of the screen with chart layout options. Pick Layout 9. This will provide a line of best fit, and an equation for that line. You can change the axis titles. Attach the excel graph here:
3. If you chose correct layout for your chart, Excel did a best fit analysis for you. The best fit should be linear and it will give you the linear equation. Write the equation below.

4. Use the equation above to calculate the size of the sample bands from your data. Report the band size in base pairs.

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<tr>
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<th>Sample 1</th>
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<th>Sample 4</th>
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<tbody>
<tr>
<td>Band 1 size</td>
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5. Does the data that you generated from the equation make sense when you look at how far the bands went in the gel and compare them to the DNA ladder?

6. Does this data help prove who did the crime? Why or why not?