Lab 2: Identifying the Presence of Influenza Using Polymerase Chain Reaction (PCR)

Student Researcher Background:

What is Polymerase Chain Reaction (PCR)?

Polymerase Chain Reaction or "PCR" is a powerful technique used by many scientists to **amplify** (or "copy") a particular gene or region of DNA in a test tube. Starting with only a few copies of DNA, PCR uses the same basic ingredients as DNA replication in the cell and results in ***billions*** of copies of your specific gene. ***All PCR experiments contain a few vital components:***

- A **DNA template**, which is DNA used as instructions to make more DNA. You will use 'patient' DNA provided to you as a template to amplify a small portion of the genome of the pathogenic agent.
- A **DNA polymerase**, which is an enzyme that assembles new DNA molecules. You will use a special kind of DNA polymerase called ***Taq*** polymerase to copy your gene.
- Forward and reverse **primers**, or small pieces of DNA used to start or "prime" DNA synthesis, which bind specifically to the 5' and 3' sequences on either side of the DNA of the influenza neuraminidase gene. These primers tell the DNA polymerase where to start copying your gene.
- **Deoxynucleotide triphosphates (dNTPs)**, which are the building blocks of DNA, and are used to build the new DNA molecules during your PCR.
- **Buffer** (usually in a 10X concentrate, which you dilute with pure water) that helps to stabilize the pH of the solution and reproduce the conditions in a cell so that the *Taq* DNA polymerase can function.

Today you will be using **2X OneTaq Master Mix** (***Figure 2.1***), which contains all the ingredients for your PCR ***except*** the DNA template, primers, and nuclease-free water. The 2X OneTaq Master Mix in Figure 2.1 is green because it has a special dye added to it. Some 2X OneTaq Master Mix is clear.

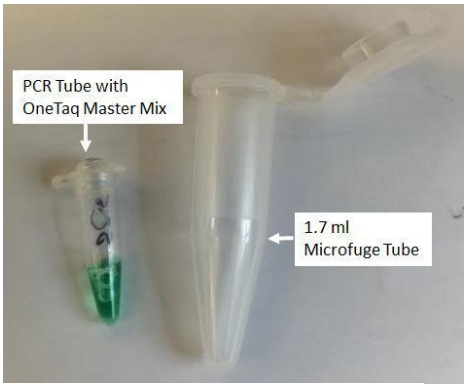

Figure 2.1: 0.2 ml PCR Tube with OneTaq Master Mix Compared to a 1.7 ml Tube.

How Do You Know Which Primers to Use?

Scientific experiments build on what is already known. In order to make the Taq DNA polymerase specifically copy ***your*** gene of interest and not just any piece of DNA, you need to use primers that are known to bind to the DNA right next to the gene you want to copy. You will be using a mixture containing a Forward and a Reverse primer, called a primer pool. Each primer in the primer pool was originally derived from the specific DNA sequence of the influenza neuraminidase gene.

Primer pools: Collections or mixtures of primers, usually used in PCR.

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Procedure:

[**Note:** Work in groups of 2 students.]

1. Obtain ice in your ice container. PCR primers should be kept cold.

2. PCR tubes are very small, as seen in Figure 2.1, and do not permit complicated labels. Use the table below (***Table 2.1***) to list each patient sample that will be tested by you and your lab partner, and the number that you will use to label your PCR tubes. ***Your PCR tube should also include your initials***, such as "JT" in the examples below.

Table 2.1: DNA Samples and PCR Test Tube Labels:

PCR Tube Label	Sample Description
*+	Positive Control
*0	Negative Control
* 1, 2, 3, or 4	Patient 1, or Patient 2, or Patient 3, or Patient 4

**** Be sure to include your initials in addition to the tube number.***

Example:

JT1	Joe Ting's Patient #1 sample

3. The PCR tubes are the small, ***0.2 ml clear tubes provided by your teacher*** (***See Figure 2.1***). Obtain six tubes for your group: one for each patient sample, one for a positive control, and one for the negative control.

4. Label each PCR tube as you described in ***Table 2.1*** (for example, "JT1" or "JT2").

5. Label one PCR tube with one of your lab partner's initials and a "0" (for your group's Negative Control, for example, "JT0").

6. Label one PCR tube with one of your lab partner's initials and a "+" (for your group's Positive Control, for example "JT+").

7. Add each of the following components to your clear PCR tubes, in the order listed below. Try to keep the components on ice as much as possible.
   a. 12.5 µl of 2X One Taq Master Mix
   b. 10.0 µl of your DNA sample (labeled "+", "0", "P1", "P2", "P3" and "P4")
   c. 2.5 µl of the primer pool (labeled "Primer")

8. Mix your PCR tubes containing your PCR mixture by closing the lid and flicking the tube with your finger.

9. Spin down the contents of your clear PCR tubes with your PCR mixture. If a centrifuge is not available, tap the tubes on your bench top to bring all of the liquid to the bottom of the tubes.

10. Working with your teacher and the rest of your class, put all 6 of your group's tubes into the **thermocycler**. The PCR reaction conditions are as follows:

1 cycle	95°C	2 min	[initial denaturation]
30 cycles	95°C	30 sec	[denaturation]
	50°C	15 sec	[low-temp annealing]
	68°C	60 sec	[elongation]
1 cycle	68°C	5 min	[final elongation]
1 cycle	4°C	overnight	

Thermocycler: Type of machine used to automate polymerase chain reaction that cycles through all of the temperatures required to complete the PCR.

Answer each of the following questions:

Question 1. What did you do in this laboratory experiment and why?

Question 2. What skills did you learn or practice?

Question 3. What are we testing for in the Negative Control PCR? What do you expect to happen in this PCR?

Question 4. What are we testing for in the Positive Control PCR? What do you expect to happen in this PCR?

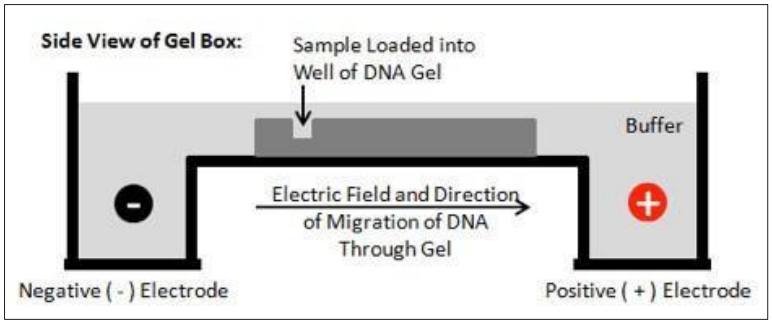
Lab 3: Analyzing PCR Results with Agarose Gel Electrophoresis

Student Researcher Background:

What is Agarose Gel Electrophoresis?

Agarose gel electrophoresis is a procedure used to determine the presence and size of pieces of DNA. By analyzing the results of PCR on an agarose gel, genetic researchers are able to determine: (1) whether the PCR reaction was successful, indicated by the presence of a DNA band on the gel; and (2) whether the PCR reaction resulted in a **PCR product** (piece of DNA) of the expected size or molecular weight.

Agarose is a powdered material made from seaweed. After mixing the agarose powder in buffer and heating it to melting in the microwave, the liquid is poured into a small mold (similar to the procedure used when making Jell-O®). A comb is placed in the liquid to make small holes or **wells** in which to put the DNA. After placing the gel in the gel box (see ***Figure 3.1***), DNA samples are mixed with loading dye and a single DNA sample is loaded into each well of the gel. Loading dye is often concentrated, such as 3X or 6X, and is diluted with water and/or your DNA sample. An electric current is then passed through the gel; since DNA is negatively charged, it flows through the gel to the positive electrode (anode). The rate at which the DNA moves through the gel is determined by its size or molecular weight—smaller molecules travel more quickly, and larger molecules travel more slowly.



PCR product: The DNA copied or "amplified" during PCR.

Figure 3.1: Running a DNA Gel.

How Do You Know the Molecular Weight of Your DNA Samples?

We include a **molecular weight standard** on the gel to determine the size of the DNA bands or PCR products. Molecular weight standards, often abbreviated "MW," contain pieces of DNA of known sizes. By comparing your DNA band to the band(s) of the molecular weight standard, you can estimate the size and concentration of your DNA band(s). You can also make a graph of the molecular weight standards relative to the distance each molecular weight standard band traveled through the gel. This allows for a more accurate estimation of the size of your DNA band(s).

PART I: Pouring a 0.8% DNA Gel

[**Note:** Work in groups of 2 students (the same students who performed the PCR together). If your teacher has already poured your DNA gels, skip *Part I* and go to *Part II*.]

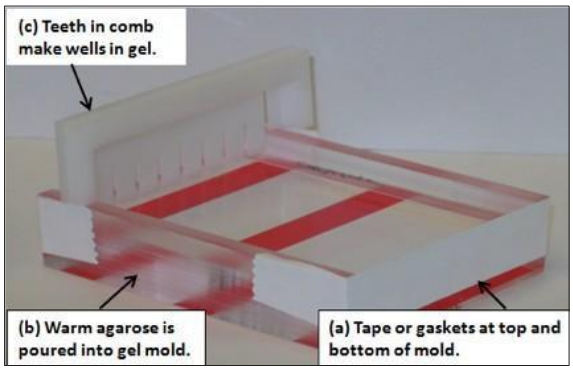


Figure 3.2: Procedures for Pouring an Agarose DNA Gel.

1. Obtain the following items:
   a. 1 tube of powdered agarose (400 mg)
   b. 50 ml DNA Gel Buffer
   c. Erlenmeyer flask or glass bottle
   d. Labeling tape

2. Use the labeling tape to label your flask or bottle with your group's name or number.

3. Combine the agarose and buffer in your flask or bottle and gently mix them together by swirling.

4. Microwave the mixture on high for 1 minute. Check the liquid to see if all of the agarose has melted. If the agarose hasn't completely melted yet, microwave again for 20 seconds, and check again. Repeat microwaving for 20 seconds at a time until all the agarose has melted.

5. Set the melted agarose aside where it can cool until it is only warm to the touch, usually about 55–60°C. Your teacher may have you place your melted agarose in a water bath to cool.

6. Add GelGreen to your melted, but slightly cooled gel solution. For 50mL of gel solution, use 5uL of GelGreen. Swirl the solution gently to completely mix in the Gel Green. GelGreen will bind to the DNA in the gel and the bands will glow when the gel is placed on an LED light.

7. Ask your teacher about the proper procedure for pouring a gel with your gel apparatus. This will usually involve the following steps (also shown in ***Figure 3.2***):
   a. Blocking the top and bottom ends of a gel casting tray with tape, blocks, or a rubber gasket.
   b. Pouring the warm agarose gel mixture into the mold.
   c. Placing the well comb into the notches on the gel casting tray. Combs typically have 6, 8, or 14 "teeth" to create "wells" in the gel in which to load your DNA.
   d. Waiting for the gel to solidify completely.

8. You can store your gel in the gel box or in the refrigerator for up to 2 weeks, as long as the gel is completely submerged in DNA Gel Buffer and protected from light.

PART II: Running a DNA Gel

9. Obtain an ice bucket and ice, and keep all of your tubes on ice as you work.

10. To each of your group's PCR reaction tubes add the following:
6uL of 5X loading dye

11. Use the table below [***Table 3.1***] to determine where you will load each sample from your group on your DNA gel. Add your name to the table below "**Student Group Name**." You will also be loading molecular weight standards on each gel. Molecular weight standards (sometimes abbreviated "MW") contain pieces of DNA of known sizes (see the ***Molecular Weight Standard Handout*** provided by your teacher). This will help you determine whether your PCR reactions produce DNA bands of the expected size (about 2,000 base pairs).

[**Note:** The Well Number is the slot on the gel in which you will load your samples (see ***Figure 3.3***). Most gels have 8 wells, but some have only 6, and others have up to 20. Check to see how many wells are in your gel.]

Well Number	Sample Name	Student Group Name
1	MW Marker	
2	PositiveControl	
3	Negative control	
4	Patient Sample 1	
5	Patient Sample 2	
6	Patient Sample 3	
7	Patient Sample 4	

Table 3.1: Samples Loaded onto DNA Gel.

12. Check that your gel is in your gel box and has enough DNA Gel Running Buffer in the gel box to just cover your gel.

Remember: "Run Towards Red." Be sure that the top of your DNA gel (where the wells are) is near the negative, or black, electrode on the gel box, and the bottom of your gel is near the positive, or red, electrode. That way the negatively-charged DNA will always "run towards red."

Figure 3.3: Loading Samples into a Well. Image Source: Navaho, Wikimedia Commons.

13. Have one member of the group load 12 µl of your MW marker into well #1 of your gel, using ***Table 3.1*** as your guide. ***Figure 3.3*** illustrates how to place your pipette tip ***just above the well*** before releasing the sample into the well. It is important not to push your pipette tip too deeply into the well, or you risk poking it through the bottom of the well and creating a leak.

14. Have one member of the group load 25 µl of your positive control PCR sample into well #2 of your gel, using Table 3.1 as your guide. Have another member of the group load 25uL of your negative control into well #3 of your gel, using Table 3.1 as your guide.

15. Have one member of the group load 25 uL of Patient Sample #1 into well #4 of your gel, using Table 3.1 as your guide.

16. Have one member of the group load 25 uL of Patient Sample #2 into well #5 of your gel, using Table 3.1 as your guide.

17. Have one member of the group load 25 uL of Patient Sample #3 into well #6 of your gel, using Table 3.1 as your guide

18. Have one member of the group load 25 uL of Patient Sample #4 into well #7 of your gel, using Table 3.1 as your guide

19. Put the lid on your gel box. Note that the lid can only be put on one way.

20. Plug in the gel power supply. The box should be set to 100 volts. Be sure the power supply is set to 100 volts and press “Run/Stop.”

21. Keep an eye on your gel. The gel should be set to run for about 25-30 minutes, though smaller gels may run faster. As the gel runs, the DNA loading dye will be visible in the gel. You should stop the gel by turning off the power supply when the bottom blue band of loading dye is about ½-inch from the bottom of the gel (about 75 - 80% of the way down the gel).

22. Your instructor will tell you how to visualize your gel. Review Molecular Weight handout from your instructor which shows the size of each DNA band in your molecular weight standard.

Answer each of the following questions:

Question 1. What did you do in this laboratory experiment and why?

Question 2. What skills did you learn or practice?

Question 3. What was the observed result with your PCR Negative Control and what does that mean?

Question 4. What was the observed result with your PCR Positive Control and what does that mean?

Be sure the complete the table on the following page as well.

Fill in Table 3.2 below with the data from your ELISA and PCR experiments:

Table 3.2: Results of Patient Samples

Patient ID	Positive by ELISA?	Positive by PCR?	Conclusion?