

**Activity 2.1.2 Copying Our Genes**

Introduction

Housed in the nucleus of your cells, DNA holds the key to your genetic destiny. The DNA code tells an amazing story about the person you will grow up to be. Each gene controls production of a protein, which ultimately affects a trait in your body. The tools of modern day molecular biology give scientists the ability to peek into a person’s genes and explore this amazing sequence at the most basic level.

Developed in 1984 by scientist Kary Mullis, the *polymerase chain reaction* (PCR) is a lab technique that produces numerous copies of a specific segment of our DNA in a relatively short period of time. It is a three-step process that is repeated over and over and that produces identical copies of the target sequence. The revolutionary technique harnesses the power of an unusual enzyme derived from bacteria that thrive in hot springs. Because these bacteria live in an extremely hot environment, their polymerase enzyme, the enzyme that helps replicate DNA, can withstand the near boiling temperatures necessary to complete a PCR reaction. In PBS, you estimated the number of DNA segments produced in 30 cycles of PCR. Remember, this exponential growth results in millions of copies of the gene of interest.

PCR is an amazing medical intervention, allowing scientists to explore our genome. PCR is so specific and powerful that only a tiny amount of genetic material is necessary as a starting material. This technique allows scientists to amplify tiny amounts of DNA to detect pathogens, to detect trace amounts of DNA at crime scenes, and to diagnose genetic conditions. Mullis went on to win the 1993 Nobel Prize in chemistry for his groundbreaking work.

In this activity, you will learn the basics of PCR. In the next activity, you will use PCR to amplify a segment of your own DNA and to complete your own genetic test.

Equipment

* Computer with Internet access
* Markers or colored pencils
* Laboratory journal
* Optional items:
  + Edvotek Kit #330 – The Molecular Biology of DNA Amplification by PCR (optional)
* DNA Template for Amplification
* Primer Mix
* Tubes with PCR Reaction beads
* Standard DNA Markers
* Enzyme Grade Ultrapure Water
  + Thermal cycler
  + Agarose gel
  + Horizontal gel electrophoresis equipment and power supply
  + Balance
  + Microcentrifuge
  + Microcentrifuge tubes (0.5ml)
  + PCR tubes (0.2ml)
  + Flasks or beakers (250ml)
  + Microwave, hot plate, or burner
  + Ice bucket and ice
  + 65°C waterbath or water-filled beaker
  + Micropipettor
  + Safety glasses

Procedure

Part I: PCR Basics

1. View the animations of PCR listed below.

* Polymerase Chain Reaction – Dolan DNA Learning Center <http://www.dnalc.org/resources/animations/pcr.html>
* The Polymerase Chain Reaction – Sumanas, Inc. <http://www.sumanasinc.com/webcontent/animations/content/pcr.html>

1. Create a flow chart of the three main steps of PCR in your laboratory journal. Draw a simple diagram of each step, using different color markers or pencils to highlight key components in the reaction. Make sure to identify the temperature at which each step occurs, as well as the function of each of the following reagents and/or equipment in the overall process:

* Taq polymerase
* DNA primers
* DNA nucleotides or dNTPs
* Thermal cycler

1. Answer the Conclusion questions 1- 5.

Optional Part II: PCR Reaction Using a Thermal Cycler

1. Note that your group will work closely together with three other groups to complete this lab. Each group will be responsible for a different number of PCR reaction cycles (*0* (*control)*, *10*, *20*, and *30*). Decide which group will be responsible for each.
2. Obtain a 0.2ml PCR tube from the designated area. Label the tube *0 (control)*, *10*, *20*, or *30*, depending on which you are responsible for, and include your initials or a group designation. If you are responsible for the control sample, obtain and label two 0.2ml PCR tubes.
3. Practice drawing up and expelling small volumes, such as 2µl, with a micropipettor. Make sure you can see the tiny drop when you dispense the solution.
4. If you are responsible for the control reaction, proceed to Step 8. If you are responsible for the 10, 20, or 30 cycle reaction, proceed to Step 10.
5. If you are responsible for the control reaction, set up two reactions by adding the following to the two 0.2ml tubes labeled *0*.

* 2 µl DNA Template for Amplification
* 2 µl Primer Mix
* 5 µl 10x Gel Loading Solution
* 10µl Enzyme Grade Ultrapure Water

1. Place the control reaction sample on ice as instructed by your teacher. Proceed to Step 22.
2. If you are responsible for the 10, 20, or 30 cycle reaction, obtain a 0.5 mL PCR reaction tube. Tap the tube gently and check to make sure you have a pellet or a bead in the bottom of the tube.
3. Set up the PCR reaction by adding the following to the 0.5 mL PCR reaction tube.

* 10 µl DNA Template for Amplification
* 10 µl Primer Mix
* 7 µl Enzyme Grade Ultrapure Water

1. Tap the reaction tube gently, to mix the contents.
2. Use a micropipettor to carefully transfer the contents of the PCR reaction tube to the 0.2ml PCR tube labeled 10, 20, or 30. Insert the PCR tube into the thermal cycler. Proceed to Step 14.
3. Program the thermal cycler for a total of 30 cycles. Your teacher may have completed this step for you. Each cycle will consist of:

* 94°C for 45 seconds
* 45°C for 45 seconds
* 72°C for 45 seconds

1. Start the thermal cycler after all of the tubes are loaded.
2. Pause or stop it after the 10th cycle and remove the PCR tube labeled *10* from the thermal cycler. Continue the program.
3. Add 5µl of 10x Gel Loading solution and 8µl of Enzyme Grade Water to the sample in the *10* tube. Store the tube on ice.
4. Pause or stop the thermal cycler after the 20th cycle and remove the PCR tube labeled *20* from the thermal cycler. Continue the program.
5. Add 5µl of 10x Gel Loading solution and 8µl of Enzyme Grade Water to the sample in the *20* tube. Store the tube on ice.
6. Remove the PCR tube labeled *30* from the thermal cycler at the end of the program.
7. Add 5µl of 10x Gel Loading solution and 8µl of Enzyme Grade Water to the sample in the *30* tube. Store the tube on ice.

Optional Part III: Gel Electrophoresis

1. Note that you will work with the three other groups from Step 4 for the remainder of this activity.
2. Follow your teacher’s instructions to make two 0.8%, 6-well agarose gels.
3. Place the solidified gels into the electrophoresis chamber. If necessary, remove rubber dams or tape from the sides of the gel trays.
4. Pull up on the combs to expose the wells. Be careful not to tear or damage the gels.
5. Fill the electrophoresis chamber with electrophoresis buffer. Make sure the gels are completely submerged.
6. Locate your PCR sample tubes and the tube of Standard DNA Fragments.
7. Heat the DNA standard fragments and your PCR samples for two minutes at 65°C. Allow the samples to cool before loading them onto the gel.
8. Load 20µl of each sample in the following order into each of the two gels:

|  |  |
| --- | --- |
| Lane | Tube |
| 1 | Marker – Standard DNA Fragments |
| 2 | Control Reaction Sample – 0 Cycles |
| 3 | Reaction Sample – 10 cycles |
| 4 | Reaction Sample – 20 cycles |
| 5 | Reaction Sample – 30 cycles |

1. Copy the above gel loading table into your laboratory journal.
2. Run and stain your gel according to your teacher’s instructions.
3. Predict what you think you will see in each lane of the gel and describe these predictions in your laboratory journal.
4. Estimate the number of DNA copies that will be found in Tubes 2-5. Show your calculations in your lab journal. Remember that you started with a segment of double-stranded DNA.
5. View your gel results using a white light box or transilluminator.
6. Write a conclusion for the experiment in your laboratory journal. Be sure to analyze what is visible on your gel and relate this to the process of PCR.
7. Answer Conclusion questions 6 - 8.

Conclusion

1. Explain how a scientist can target a specific gene or region of the DNA in a PCR reaction.
2. Explain how a thermal cycler helps with the process of PCR.
3. Brainstorm how you could run a PCR reaction if you did not have access to a thermal cycler.
4. Explain how the process of PCR can be used in the identification of a disease pathogen. Provide evidence and examples from the outbreak investigation in Unit 1.
5. How do you think PCR can be used to diagnose genetic diseases and disorders?
6. Based on what you know about the process of PCR, what reagents must have been included in the PCR bead you used in the experiment? Explain.
7. Why was it necessary to run a control in this experiment? What does the control tell you?
8. Devise a method for determining the molecular weight of the fragment you amplified and describe that method below. Make sure to think about the information that is available by examining your gel.