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Polymerase Chain Reaction (PCR)

Life on the Edge

B

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Acknowledgements

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Editors
Dr. Jamila Cola, Georgia Institute of Technology
Dr. Loren Dean Williams, Georgia Institute of Technology School of Chemistry and Biochemistry
Ms. Alison Dowell, Georgia Institute of Technology, Science, Technology and Culture Undergraduate

Authors
Ms. Caitlin Prickett, Georgia Institute of Technology School of Chemistry and Biochemistry
Dr. Loren Dean Williams, Georgia Institute of Technology School of Chemistry and Biochemistry

Designers
Mr. Anthony Docal, Orbit Education Inc.
Ms. Aakanksha Angra, Georgia Institute of Technology, Biology Undergraduate Student
Mr. Timothy Whelan, Georgia Institute of Technology, Distance Learning and Professional Education

Photography Credits:

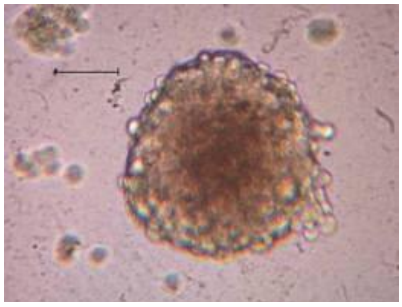
A. Picture of a DNA helix
B. Picture of a student checking the PCR tubes in the hot water bath.
C. Picture of a student pipetting.

National Standards Correlation

Life Science Content Standard C

The Cell

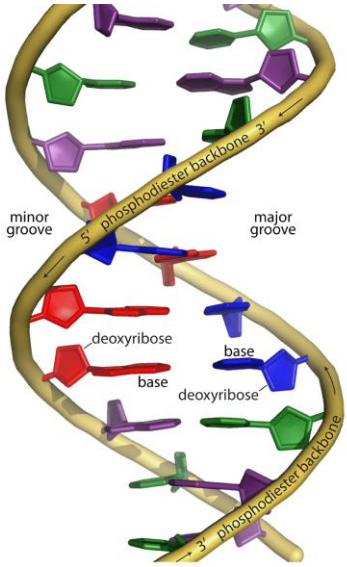
Cells store and use information to guide their functions. The geneticinformation to guide their functions. The genetic informationstored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires.



SK-N-SH cells grown in the NASARCCS.

The Molecular Basis of Heredity

In all organisms, the instructions for specifying the characteristicsof the organism are carried in the DNA, a large polymer formedfrom subunits of four kinds (A, G, C, and T). The chemical andstructural properties of DNA explain how the genetic informationthat underlies heredity is both encoded in genes (as a string of molecular“letters”) and replicated (by a templating mechanism). EachDNA molecule in a cell forms a single chromosome.Changes in DNA (mutations) occur spontaneously at low rates.Some of these changes make no difference to the organism, whereasothers can change cells an organisms. Only mutations in germcells can create the variatio that changes an organism’s offspring.



DNA’s double helix.

Purpose:

Students will become familiar with the technique used to amplify DNA through a polymerase chain reaction.

Objectives:

Students will master the skills necessary to perform a PCR reaction.

Overview:

The polymerase chain reaction (PCR) was developed in 1983 by Kary Mullis (who earned his Bachelor of Science degree in chemistry from Georgia Tech in 1966) for which he earned the Nobel Prize in Chemistry in 1993. PCR is a technique used in molecular biology to amplify a single or few copies of a piece of DNA, across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. The method relies on thermal cycling (thermocycling), consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA using a DNA polymerase. Primers (short DNA fragments) containing sequences complementary to the target region, along with a DNA polymerase, are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations.

Typically, PCR consists of a series of 20-40 repeated temperature changes, called thermal cycles, with each cycle commonly consisting of 2-3 discrete temperature steps. The temperatures used and the lengths of time that are applied in each cycle depend on a variety of parameters. These include the length of the template DNA to be amplified, the DNA polymerase used for DNA synthesis, dNTPs in the reaction, and the melting temperature of the primers.

PCR is now a common and indispensable technique used in medical and biological research labs for a variety of applications. These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in forensic sciences and paternity testing); and the detection and diagnosis of infectious diseases.

In Part I of the experiment, we will be amplifying the DNA of *Thermus thermophilus*, or *Psychrobacter urativorans*. (Each group will perform a PCR reaction on one of these organisms). This is the DNA you extracted in the previous experiment. These reactions will be performed in a thermocycler. Here, we are amplifying a portion of the 16S ribosome (RNA gene) for *Thermus thermophilus*, and *Psychrobacter urativorans*, using universal bacterial primers. The expected sizes of our PCR products are as follows: *Thermus*

thermophilus—600 bp, and *Psychrobacter urativorans*—900 bp and 1200 bp. In part II of the experiment, we are targeting an 821 bp (basepair) sequence of lambda DNA. Lambda is a bacteriophage (virus that infects bacteria); its DNA is 48,502 bp long. Because the genome is relatively small, we can use manual PCR cycling in two steps: boiling water bath for denaturing, 55°C water bath for annealing. Replication occurs as the reaction warms up from annealing temperature to denaturing temperature. Take care not to go beyond the 20 seconds in boiling water, because the enzyme can be destroyed at boiling temperature.

In Part II of the experiment, we are targeting an 821 bp (basepair) sequence of lambda DNA. Lambda is a bacteriophage (virus that infects bacteria); its DNA is 48,502 bp long. Because the genome is relatively small, we can use manual PCR cycling in two steps: boiling water bath for denaturing, 55°C water bath for annealing. Replication occurs as the reaction warms up from annealing temperature to denaturing temperature. Take care not to go beyond the 20 seconds in boiling water, because the enzyme can be destroyed at boiling temperature.

We are using primers that target an 821 bp site from position 27219 to 28040. Upstream (forward) primer sequence is 5' ATC GCC GAA CGA TTA GCT CT 3'; downstream (reverse) primer sequence is 5' ATC CGG CAC AGT ATC AAG GT 3'. Primers attach to sites on the DNA strands that are at either end of the segment you want to copy. They are powerful tools for copying very specific DNA sequences since there is almost no chance that they will target the wrong site. Two primers attach to two different sites located on the DNA.

Prep Time for Teachers: Approximately 30 minutes
Class Time: Approximately 2 hours 45 minutes for activity and discussion

Objectives

- **Students will understand the importance of each step in an experiment.**
- **Be able to use PCR machine as well as perform manual PCR.**

Skills

1. *Predicting* the outcome of an experiment
2. *Controlling* variables
3. *Conducting* an experiment
4. *Collecting, recording,* and *graphing* data
5. *Drawing* conclusions and *communicating* them to others

The First Two Cycles of a Polymerase Chain Reaction

Part I—PCR via Thermocycler:

1. Amplification of *Thermus thermophilus* DNA
    1. Put on disposable gloves.
    2. Use a permanent marker to label your 0.65 mL PCR tube. Label tube with "T". Place your initials on the tube.
    3. Add the following to a tube containing a PCR bead [Note: *Use a new pipette tip for each addition.*]:
        a. 14 µL of sterile, distilled water, from "W" tube
        b. 25 µL of buffer, from "B" tube
        c. 3 µL of primer mix, from "P" tube
        d. 7 µL of *Thermus thermophilus* DNA, from "T-DNA" tube
    4. Snap the cap onto the tube, pushing down firmly to ensure a tight fit.
    5. Mix the tube contents by gently flicking the tube with a finger.
    6. Centrifuge the tube for 20 seconds to bring the components to the bottom of the tube.
    7. Place the PCR tube into the rack next to the thermocycler. (The camp leader will start the thermocycler.)
    8. Immediately proceed to Part II—Manual PCR.

2. Amplification of *Psychrobacter urativorans* DNA
    1. Put on disposable gloves.
    2. Use a permanent marker to label your PCR tube containing the PCR bead. Label tube with "P". Place your initials on the tube.
    3. Add the following to a tube containing a PCR bead [Note: Do not mix the tube contents until all of the components (below) have

been added to the tube containing the bead. *Use a new pipette tip for each addition.*]:

a. 3 µL of primer mix, from "P" tube
b. 8 µL of *Psychrobacter urativorans* DNA, from "P-DNA" tube
c. 14 µL of sterile, distilled water, from "W" tube

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4. Snap the cap onto the tube, pushing down firmly to ensure a tight fit.
5. Mix the tube contents by gently flicking the tube with a finger.
6. Centrifuge the tube for 20 seconds to bring the components to the bottom of the tube.
7. Place the PCR tube into the rack next to the thermocycler. (The camp leader will start the thermocycler.)
8. Immediately proceed to Part II—Manual PCR.

Part II—Manual PCR :

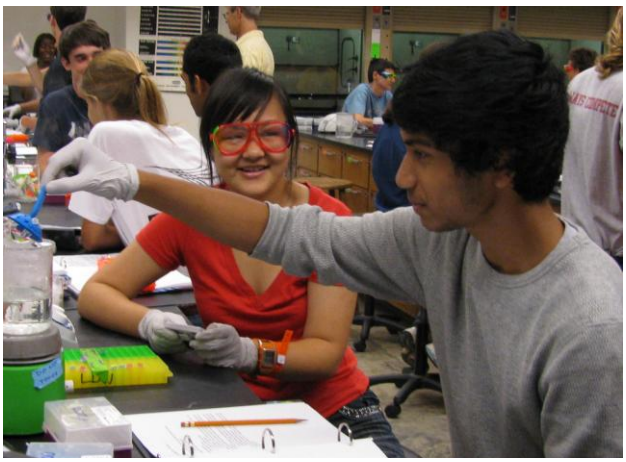
Each student performs the following procedure.

1. Put on disposable gloves.
2. Use a permanent marker to label your PCR tube containing the PCR bead. Label tube with "L". Place your initials on the tube.
3. Add the following to a tube containing a PCR bead [Note: Do not mix the tube contents until all of the components (below) have been added to the tube containing the bead. *Use a new pipette tip for each addition.*]:
   a. 4 µl of primer mix, from "P" tube
   b. 10 µl of lambda DNA, from "DNA" tube
   c. 11 µl of sterile, distilled water, from "W" tube
4. Snap the cap onto the tube, pushing down firmly to ensure a tight fit.
5. Mix the tube contents by gently flicking the tube with a finger.
6. Vortex for 5 seconds.
7. Centrifuge the tube for 20 seconds to bring the components to the bottom of the tube. (The reaction is fully dissolved and mixed when it appears clear.)
8. Use a push pin and puncture the lid of tube "L".
9. Immediately place PCR tube in ice. ☐ Proceed to "Protocol".

**Individual roles for each student per group are as follows:

Student 1: "Time-keeper", uses timer to alternate between 20 seconds and 1 minute and announces when it is time to transfer the floatie containing tubes. (Sits on the right-side of set-up)

Student 2: "Transferee of tubes", ensures tubes are constantly in contact with water baths and



transferred promptly within cycles. (Sits at the center of set-up)
Student 3: "Cycle tracker", records number of cycles completed. *Must be focused* (Sits at the left-side of set-up)

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Part II—Manual PCR : (*continued)*
Protocol

1. Place your tube (tube "L") into the foam floatie and begin the cycling:
    a. 20 seconds in boiling water bath
    b. 1 minute in 55°C water bath
2. *Repeat for a total of 30 cycles.*

Important Information

i. One complete PCR cycle:
    a. 20 seconds in boiling water bath
    b. 1 minute in 55°C water bath
    c. Cycle complete! *(This is when you would denote completion of "Cycle 1" on your cycle chart.) *Only mark a step when fully completed!**
ii. "Student 3" (a.k.a. the cycle tracker) must also monitor the 55°C water bath and ensure that the temperature remains constant; this is accomplished by adding ice to the water bath.
    a. **If ice is needed to reduce temperature, only add the ice when the PCR tubes are in the boiling water bath. Do not add ice to the water bath containing PCR tubes; this will cause an uneven temperature surrounding the tubes and affect the overall reaction.**
iii. ** It is vital for each student to be aware of their responsibilities and pay close attention during manual PCR. Constant repetition in this experiment can cause focus to waiver!**

The Polymerase					

Chain Reaction—Cycle Chart					
Cycle	Completed				
1					
2					
3					
4			Remember:		
5			1) Boiling (95°C) H_2O -- 20 seconds		
6			2) 55°C H_2O -- 1 minute		
7			**Cycle Completed**		
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