3-D Molecular Designs
...where molecules become real ™

Biotechnology Kit©

Exploring PCR

PCR: Nature's Photocopier

Genes are identifying factors that can help scientists distinguish between two organisms. But, since genes are so small, scientists need a large quantity of DNA to study them. PCR stands for **Polymerase Chain Reaction** (PCR). Today, you will look at what that means through this activity - but for now, think of it as a biotechnological tool that can produce many copies of a selected DNA sequence, like a photocopier!

Before we make a single copy though: In the space below, write down all of the components you will need to make a copy of the target DNA.

Think back to DNA Replication.

Goal: To model the flow of the PCR process through the first three cycles and understand the applications of PCR in the biotechnology industry.

Introduction

Your DNA holds many, many secrets inside of it; but the DNA itself is very small. Even though, if you stretched out all of the DNA in one of your cells, it would be approximately six feet long - the average size of a gene is only 1,000 base pairs, or just 340 nm!

You started off your exploration of DNA looking at how it serves as the genetic basis for inheritance and how its code will ultimately determine the structure of proteins which determines how an organism will function. DNA can also provide information about your ancestry, your criminal activity, and even what type of bacteria is causing your skin infection, allowing the doctor to prescribe the most effective medicine. But how do you get this information from such a small macromolecule?

To solve these and many more issues, scientists will often look at a very small section of the genome. But, remember how small a gene is? How can you make enough DNA to tell the difference between two individuals? The answer is - you need a very small photocopier that can make a lot of copies.

Scientists use a piece of equipment called a thermocycler in this reaction.

- Break down the word "**thermocycler**". How do you think this piece of equipment works?

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- Knowing what you know about DNA and its structure/function, why would this piece of equipment be selected to be used in the PCR reaction?

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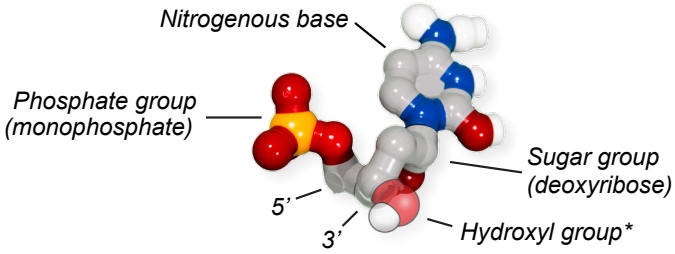
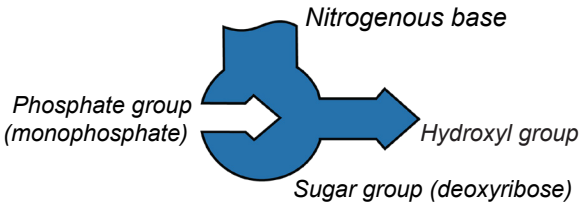
Exploring PCR

Getting Started

Today you are going to model the PCR process using foam nucleotides. Before we start, acquaint yourself with the foam pieces.

Compare the foam nucleotide to the plastic model on left and chemical drawings on the previous page.





**The hydroxyl group (OH) shown in the above illustration has been added to the photo of the nucleotide, since this particular model of a nucleotide doesn't show the hydroxyl group.*

Examine the diagrams to the right.

- In the image representing the foam nucleotide on the bottom right, label the locations of the 3' and 5' carbons.
- Think back - how did two nucleotides bind? Yes, the 3' and 5' carbons were important locations, but ***how exactly*** did they bind?

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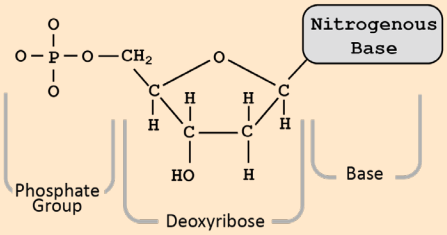
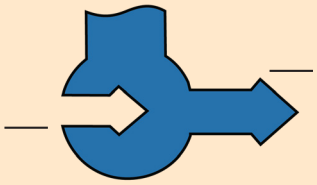
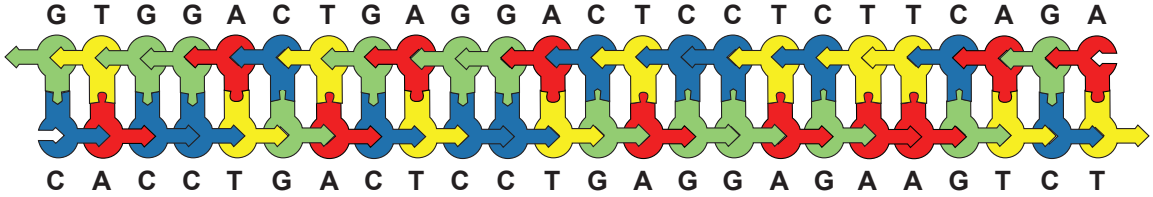




Now assemble the template DNA strand using the following 5' to 3' code.



Finally, organize the following items:

- Make two separate piles for Primer One and Primer Two.
  - *Primer One: TGAC*
  - *Primer Two: TTCT*
- Make three separate piles for the cycle flags (Cycle One, Cycle Two, and Cycle Three).
- Open your bags of nucleotides, but keep them off to the side.

Exploring PCR

Cycle One

You are now ready to begin PCR. PCR reactions typically run for 30 cycles and take up to two hours to run, depending on the thermocycler being used. Today, you are going to model the first three cycles.

Vocabulary to think about:

Before you begin, define the following terms:

Denaturationain

_____

_____

_____

Annealing

_____

_____

_____

Extension

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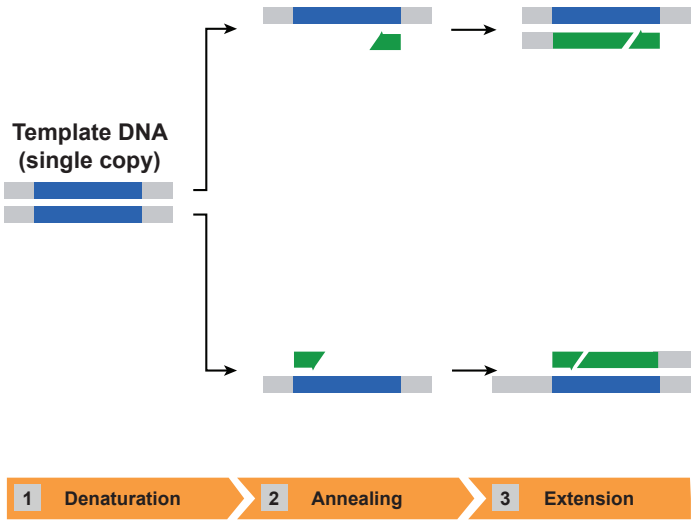
_____

_____

Cycle One



Steps

1. **Denature** the double stranded DNA (dsDNA). By separating the strands, you are representing the thermocycler heating up to 95°C and breaking the hydrogen bonds that hold together the base pairs of the target DNA.
2. **Anneal** the primers. Using both gray primers, Primer One and Primer Two, find a complementary binding site on the target DNA. These primers provide a 3' starting point so that the polymerase can elongate and add free nucleotides (dNTPs). The thermocycler will reduce the temperature to 55 o C for annealing.
3. **Extend** the sequence by adding free nucleotides (dNTPs) to the 3' end of Primer One and Primer Two. Add bases along the template DNA until you reach the end of the strand. The thermocycler increases the temperature during this phase to **72°C**.
4. Place a Cycle One flag in each of the gray primers to help you keep order as the cycles progress.

Look at your two new strands. Use this space to make observations and write down any questions that come up. How do you think this process will work?

Exploring PCR

Before you move onto Cycle Two, answer the following questions and complete Cycle One of the PCR Storyboard.

Think about what you just modeled and answer the following questions. You may need to look up some of the information.

- Scientists conduct a PCR reaction inside an Eppendorf Tube.
  - What is an Eppendorf Tube? How big is it?
  - In your model, what would represent the Eppendorf Tube?

_____

_____

- When your cells conduct DNA Replication, DNA Polymerase III is the enzyme that adds nucleotides to the growing chain.
  - What type of polymerase is used in PCR? Why can DNA Polymerase III not be used?
  - In your model, what would represent the polymerase?

_____

_____

- What do you think is the importance of each of the temperatures in this process?

_____

_____

- The process of PCR was discovered in 1983 by Kary Mullis at the Cetus Corporation, however, the development of the first commercial thermal cycler, the TC1 DNA Thermal Cycler (TC1), was not until 1987. How do you think the reaction was carried out for these early years of discovery? What motives do you think propelled scientists (and engineers) to not only develop, but perfect the thermocycler?

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- How can you change the model to include a thermocycler? Remember, the thermocycler is the apparatus that adjusts the temperature of the reaction and is where the Eppendorf tube is placed.

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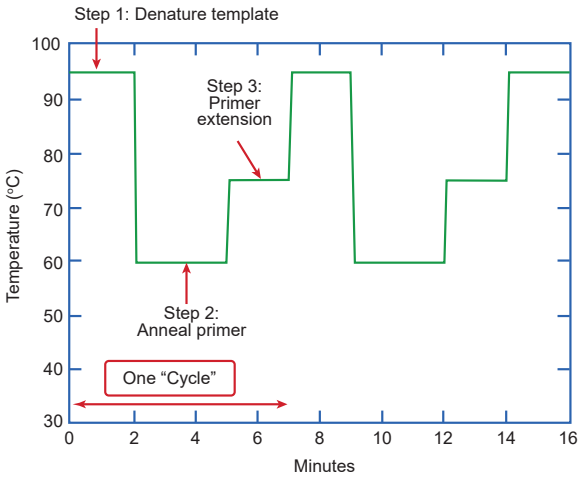
Exploring PCR

Cycle Two

Each cycle of PCR will work in much the same fashion - with some key differences starting to occur. However, the temperature cycles will remain the same as the sample cycles through the three steps of the PCR reaction. The total time of a cycle is dependent on how fast the apparatus can change temperature (both heat up and cool down).



Steps

1. **Denature** both dsDNAs produced in cycle One by pulling apart the foam strands.
2. **Anneal** primers. This time, pick up two gray Primer One pieces, and two gray Primer Two pieces. Find a binding site for all four primers on the separated DNA strands. Place the Cycle Two pins in the four grey primers that you just annealed.
3. **Elongate** the DNA strands. Working one strand at a time, add dNTPs to the 3' end of the growing nucleotide chain.
4. Complete the Cycle Two storyboard on the PCR Storyboard worksheet.

How many strands do you now have? Use this space to make observations and write down any questions that come up.

Cycle Three

Steps

1. **Denature** all four dsDNAs produced in Cycle Two by pulling apart the foam strands.
2. **Anneal** primers. This time, pick up four gray Primer One pieces, and four gray Primer Two pieces. Find a binding site for all *eight* primers on the separated DNA strands. Place the Cycle Three flags in the eight gray primers that you just annealed.
3. **Elongate** the DNA strands. Working one strand at a time, add dNTPs to the 3' end of the growing nucleotide chain.
4. Complete the Cycle Three storyboard on the PCR Storyboard worksheet.

How many strands do you now have? Use this space to make observations and write down any questions that come up.

Exploring PCR

Looking at the three cycles that you just modeled, answer the following questions. You may need to look up some of the information.

- Look back on all of the boxes that contain your questions and predictions. Where did you go right? Where did you go wrong? What misconceptions did you discover?

- Which of your strands do you think contains ***just*** the gene of interest with no "extra" DNA sequence added/overhanging to either side of the target sequence?

- What happens to the number of these target sequences each time through a PCR reaction?

- Based on your results from the previous question, make a prediction for how many of the target sequence will be in the Fourth Cycle? In the 20th Cycle?

- Scientists will run a PCR reaction 25-30 times. What do you think impacts how many cycles are required?

- How do you think scientists design/pick what primer they will use for a PCR reaction?

- How does the primer utilized in a PCR reaction differ from the primer used in DNA Replication?

Exploring PCR

PCR StoryBoard

Use the three boxes to illustrate the three cycles of PCR as if they were a comic strip. Refer back to the model that you just made for help with what to draw. On the lines beside each box, describe what is happening in each drawing. Use and underline the following words: **template, primers, dNTPs, Taq DNA polymerase, thermocycler, denaturation, annealing, extension, amplification.**

Cycle Number: _____

Number of PCR products produced: _____

Description of what is happening:

Cycle Number: _____

Number of PCR products produced: _____

Description of what is happening:

Cycle Number: _____

Number of PCR products produced: _____

Description of what is happening:

Exploring PCR

PCR vs. DNA Replication

On the surface, PCR and **DNA Replication**, the process by which your genome is replicated during cell division, seem like they are doing essentially the same function - creating copies of DNA. However, PCR often uses slightly different mechanisms to achieve the same result. To further understand the comparisons between PCR and DNA Replication, complete the table below.

	DNA Replication	PCR
When the two DNA strands are copied, the macromolecule must be split in half to expose the nitrogen bases. What process is used to accomplish this?	Helicase is used to break the hydrogen bonds between the two strands of DNA.	The reaction tube is heated to 95°C in a thermocycler. This breaks the hydrogen bonds holding the nitrogen bases together, giving you single-stranded DNA (ssDNA).
Which type of polymerase is used? AND What temperature does it prefer to function?		
Where does polymerase begin copying the DNA?		
What molecules are used as the building blocks of the new DNA strand?		
How does the polymerase know when it is time to stop replicating?		

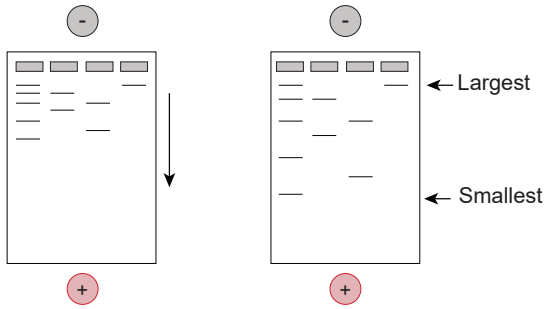
Exploring PCR

Extension: What comes <u>after</u> PCR?

After a sample undergoes a PCR reaction, scientists will likely run the samples on an agarose bed for gel electrophoresis.

- Organize your PCR products. *For this exercise, it does not matter what cycle the band's primer is a part of*. Use the lines below to explain how/why you organized your bands. Draw a sketch of how you organized it in the blank area below.

Although an agarose gel used in DNA electrophoresis looks like a solid, it has small pores inside of it. These pores help separate bands of different sizes. The smallest segments of DNA are able to squeeze through the pores and therefore travel the furthest, while the larger segments of DNA have a harder time navigating the pores within the agarose, and therefore do not travel as far.



- Now organize your bands the way they would appear if they were seperate bands in an agarose gel. Use the blank space below to sketch how you organized the segments and the lines to explain how/why you did.

- Think about the predictions you made about the number of target sequences that would occur by the end of the PCR process. What will happen when there are multiple copies of a given band size?

- Conversely, how do you think the larger bands, which will occur much less frequently, will appear on the gel?