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?

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- 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?

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

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- The process of PCR was discovered in 1983 by Kary Mullis at the Cetus Corporation, however, the EYHQSPHQRIWKHLWFRPPHUFLDQKHUPDFHUWKH7&KHUPDEHU7ZR 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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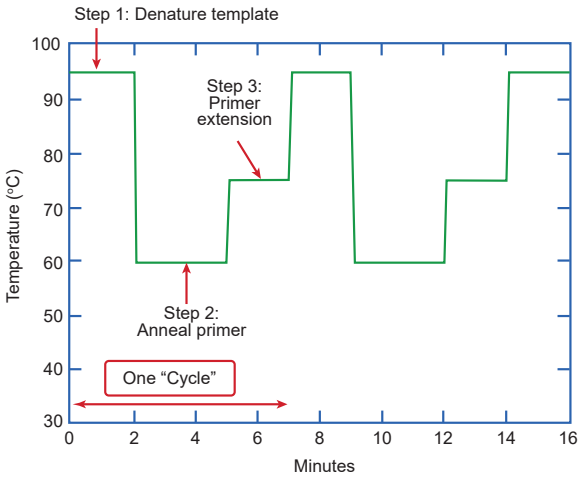
Exploring PCR

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?		

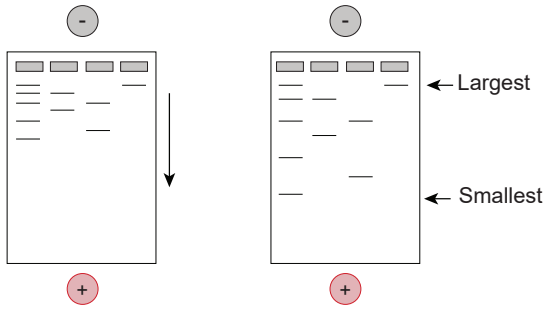
Exploring PCR

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?