

Restoring the American Chestnut

Low-tech PCR

In 1983 Kary Mullis conceived and developed an ingenious method for amplifying fragments of DNA called polymerase chain reaction (PCR). In *Scientific American*, Mullis summarized the procedure: "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents, and a source of heat." In 1993 Mullis was awarded the Nobel Prize in chemistry for his work on PCR. PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications. These include DNA cloning for genome sequencing, functional analysis of genes, the diagnosis of hereditary diseases, the identification of genetic fingerprints (used in solving crimes and paternity testing), and the detection and diagnosis of diseases. It is a scientific technique used to amplify, or create millions of identical copies of, a particular DNA sequence, all within a tiny reaction tube. You can think of this procedure as similar to the DNA replication that occurs in your body cells every time they divide. However, PCR is different in two major ways. First, PCR only copies a specific region of DNA (rather than your entire genome). And second, it makes millions of copies of DNA (rather than just one).

The purpose of the exercise is to *simulate* a PCR machine.

The essential ingredients for a successful PCR are:

1. *DNA polymerase enzyme:*

This enzyme can endure temperatures up to 94°C without being denatured, unlike most other enzymes. This enzyme can add complementary nucleotides to a DNA strand during DNA synthesis.

2. *Buffer and magnesium chloride:*

These solutions serve to optimize the conditions in which the enzyme functions, as enzymes are very sensitive to pH and salt concentrations.

3. *Four basic deoxynucleotides, also known as dNTPs:*

The four basic nucleotides are the building blocks of DNA (A, T, G, and C). This is what the DNA polymerase enzymes add in the final step.

4. *Primers:*

These are short pieces of single-stranded DNA which act as starter material from which the DNA polymerase will begin adding nucleotides. They match up to the original DNA placed in the reaction and there are 2 different primers. One is meant to match the top, or 5' side (this is the forward primer), and the other is meant to match the bottom, or 3' side (this is the reverse primer).

5. *DNA:*

This is the original copy of the DNA, also known as the template. This is what you are making copies of.

Restoring the American Chestnut

Different temperatures are needed to produce certain changes in the DNA. This process is called thermocycling. Three different temperatures are needed to complete different parts of the reaction:

Step 1: Denaturing: DNA starts off consisting of _____ strands. Raising the temperature (94°C) breaks the ___ bonds connecting the DNA bases (3 bonds connect C and ___; 2 bonds connect A and ___). The result is 2 single strands of DNA. (*Fill in the blanks.*)

Step 2: Lower temperature (48° C) allows primers to anneal, or base pair, to DNA. The primers will block the DNA strands from reattaching to each other.

Step 3: 72° C provides needed temperature for elongation using DNA polymerase. Individual nucleotides are base paired onto the existing strand starting at the primer and continuing to the end of the segment.

Procedure:

You and your partner will simulate what happens inside of a PCR reaction while one group demonstrates the thermocycling method for the entire class.

1. The group that will demonstrate the thermocycling method will be given a microcentrifuge tube which already contains all of the ingredients needed for PCR -- except a DNA sample to copy. They will need to collect this sample from someone else in the class.

To collect a DNA sample: Put a bit of the deionized water in one of your test tubes. Take the cotton swab and gently scrape the inside of your cheek with it. Then, slosh the tip of the swab in the tube with the water. Stopper the tube and put it in the boiling water for a couple of minutes to rip open the cells and let your DNA out into the solution. Tap the tube gently with your finger to evenly distribute the DNA in the solution and use a stirring rod to measure out a tiny amount and transfer to the microcentrifuge tube. Turn off hot plate when finished with this step.

Groups that are simulating what occurs in the PCR reaction will need to prepare their DNA samples as well. Cut out the DNA and tape it together as indicated. It should result in a double-stranded fragment that is sixteen base pair long with the following nucleotide sequence:

TAGTCACGCTAAGTCT
ATCAGTGCATTGAGA

2. The group demonstrating thermocycling has been provided with three water baths. One member of this group will need to continually check the temperature to see that it is accurate. Ice is available if the temperature becomes too high. The first step in thermocycling is to denature the DNA, changing it from double-stranded to single-stranded. These students need to put their reaction tube into the 94° C bath for one minute.

During this time, groups that are performing the simulation need to denature a strand of DNA by separating it into two complementary single strands (cut in half lengthwise). Slide one strand in front of you and slide the complementary strand in front of your partner.

3. Now it is time for the annealing step. At the end of one minute, the demonstration group will transfer its tube to the 48° C bath for one minute. During this step, the forward and reverse primers will pair with a section on each side of the original DNA strand.

Restoring the American Chestnut

Simulation groups should locate the forward (yellow) primer and find where it pairs under the top original strand. Next, they should locate the reverse (green) primer and find where it pairs above the bottom original strand.

4. The final step is elongation. The demonstration group needs to transfer its tube to the 72° C bath for ninety seconds. During this time, free nucleotides base pair to the remaining portions of the original DNA.

Simulation groups will now add nucleotides from the bag labeled “Free Nucleotides.” Choose one nucleotide at a time without looking and place it where it pairs up to the original DNA strand. When all nucleotides are in place, tape to secure all pieces. There should now be two complete sets of double-stranded DNA.

How do the two pieces of DNA compare to each other? (Check, they should be identical).

This is the end of the 1st cycle!!!

5. All groups will now complete this procedure two more times. The demonstration group will need to check the temperature of the water baths and have someone keep up with the time. Simulation groups will need to cut apart the two strands of DNA that were just created. Be sure not to mix up the pieces. All strands of DNA created at the end should be identical to the original strand.

Follow-up Questions:

1. How many strands do you have at the end of the 2nd cycle? _____
2. How many strands do you have at the end of the 3rd cycle? _____
3. How many copies of DNA would you have present after 10 cycles? _____
4. On the back side of this paper, construct a graph that shows the number of copies of DNA per cycle for 15 cycles, if the number can be found by 2^x . x = the number of cycles, the solution equals the number of copies of DNA.
5. An actual PCR process goes through about 30 cycles, how many copies of DNA would this produce? _____
6. Draw an untwisted strand of DNA: (include letters for N-bases)

7. Draw what this strand looks like after it is denatured: (include letters for N-bases)

Restoring the American Chestnut

8. Draw what the strand of DNA from above with “annealed” primers attached to it looks like:

9. Draw the final result of 1 cycle of PCR.

10. What are the 5 main ingredients of a PCR reaction?

11. List the 3 steps of PCR and briefly describe what happens in each.

12. List some beneficial uses of PCR technology.