3. PCR: Copying Your DNA

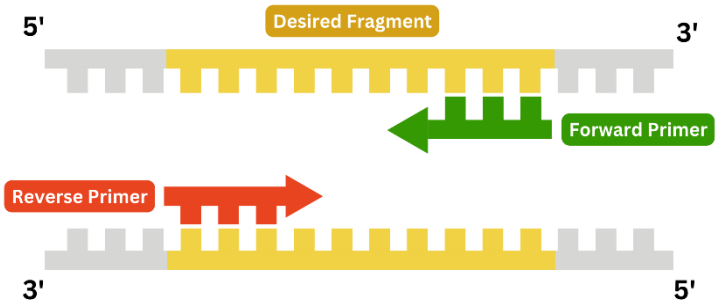
Overview

Now that you have a sample of your DNA, it is time to focus specifically on your bitter taste gene, TAS2R38. Thanks to genetics research, we know there is a minor DNA coding difference between two common variants of this gene (**alleles**) that results in a difference in people's abilities to taste PTC. You can use **polymerase chain reaction** (or **PCR**), to make multiple copies of one portion of your TAS2R38 DNA. Also, you or your teacher will perform some extra tests, called **positive and negative controls**, to make sure the PCR ran properly and was not contaminated with random DNA.

How it works

Living things use a specialized protein called **DNA polymerase** to copy their DNA so that dividing cells can each end up with an entire genome. Researchers discovered a way to use a form of DNA polymerase from the bacterium ***Thermus aquaticus (Taq)***, which thrives in hot springs, to make multiple copies of any target DNA sequence. Because DNA separates ("melts") into two single strands at high temperatures, PCR is carried out in a programmable heat block called a **thermocycler**.

PCR involves multiple rounds of DNA replication, resulting in the production of over 1 billion copies of a specific segment of DNA. To set up PCR, you must add five ingredients:



1. **Template DNA**—The pool of genomic DNA from which you will make copies
2. **Forward and reverse primers**—Short stretches of DNA designed to match the beginning and end of the section of genomic DNA that you want to copy; think of these as the start of a zipper
3. **DNA nucleotide bases (dNTPs)**—Loose "building bricks" used to build the new copies of DNA
4. ***Taq* polymerase enzyme**—The helper protein that connects loose DNA bases to build the new DNA strands
5. **A buffer**—A chemical solution that helps the reaction run

The DNA sequence of the forward and reverse primers, as well as the temperature conditions for the PCR, are based on published scientific research.

What you will explore today

Using micropipettes, you will combine the DNA you extracted with primers and with a mix containing DNA nucleotide bases, *Taq* polymerase, and a buffer. Then you will place your reaction tube into the thermocycler that the teacher has programmed for you.

Put on gloves.

1

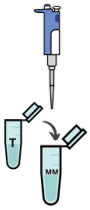
Label the top of the master mix (MM) tube with your initials or assigned number.

2

Keeping the MM tube on ice, add 10 µL of the primer mix into the MM tube. Gently pipette up and down 3X to mix.

3

Transfer 2.5 µL from your template tube (T) into your MM tube. Gently pipette up and down 3X to mix.



4

Ensure that the tube is capped tightly.

5

Centrifuge tubes for 5–10 seconds. Place tubes on ice until all samples are ready.

6

Add all sample tubes to the thermocycler(s). Run the “Copy That DNA” program.

7

Stop here and allow your teacher to complete the remaining step of the procedure.

Once the “Copy That DNA” program is finished, open the lid of the thermocycler and leave it for 15–30 seconds to cool before removing the tubes from the PCR machine. If you will be proceeding with restriction digestion within a few days, store the samples at 4°C; otherwise, store sample tubes in the freezer at –20°C.

8