CHAPTER 3:
Exploring Our DNA

INTRODUCTION

In this chapter, you will perform a process that medical laboratories use to collect, isolate, amplify, and examine a patient's DNA to determine which medication might be most effective for them.

ACTIVITY: Using Cheek Cells to Collect DNA

Your task is to extract genomic DNA from the nuclei of your cheek cells. The procedure described below and alternatively shown in a flowchart (**Figure 3.1**) will allow you to lyse (break open; related to the term *lysis* introduced in Chapter 2) the cheek cells, remove other cellular components, and isolate just the DNA from the cells.

MATERIALS (See **Figure 3.1** for illustration)

For each student:

- 1 pair of gloves
- 1 flat toothpick
- 100 µL of Chelex beads in a 0.2-mL microfuge tube
- 1 clean 0.2-mL microfuge tube

For each team:

- 1 P-20 micropipette and tips
- 1 fine-point permanent marker
- An ice bucket or a cup of crushed ice
- Microfuge tube rack

For the class:

- Microcentrifuge
- PCR machine (thermocycler) or heat block
- Waste container(s)
- Device with internet access and either USB or Bluetooth, such as a laptop computer or smartphone, with thermocycler software installed

PROCEDURE (see **Figure 3.2** for flowchart)

Step	Notes
1. Put on gloves.	
2. Turn on your thermocycler or heat block and set it to 99°C.	*This step can be done either as a class, using one large thermocycler, or with individual machines.*
3. Label the tube of Chelex beads with your initials.	*Record how you labeled your tube in your science notebook.*
4. Using a flat toothpick, gently swab the inside of your mouth for at least 30 seconds.	*Carefully scrape the inside of both cheeks. You do NOT need to apply a lot of pressure.*
5. Swirl the toothpick in the tube of the Chelex beads for at least 30 seconds to dislodge the cells.	*Ensure the toothpick touches the beads inside the tube. Swirling thoroughly will help dislodge as many cheek cells as possible.*
6. Dispose of the toothpick in the waste container.	
7. Incubate the Chelex bead tube containing cheek cells at 99°C for 10 minutes using the preheated thermocycler or the heat block you turned on in Step 2.	*Follow the steps in Programming the Thermocycler while samples are incubating at 99°C for 10 minutes.*
8. Spin the tube in the microcentrifuge at a minimum of 4,800 x *g* for 1 minute to pellet the cell debris and the Chelex beads.	*Insert the 0.2-mL tube into an adapter or an empty 0.5-mL tube so that it fits properly into the microcentrifuge.* *Make sure that you balance the microcentrifuge with other students' Chelex bead tubes or a balance tube of the same mass.*
9. Label a clean 0.2-mL microfuge tube with your initials and the letter "T."	*"T" stands for "template." This tube is the template tube, which will be used in the PCR.*
10. Set your P-20 pipette to 20 µL and add a clean tip.	

Step	Notes
11. Carefully transfer 20 µL of the supernatant (liquid above the beads) from the Chelex bead tube to your template tube ("T").	*Be very careful when removing the Chelex bead tube from the centrifuge and moving the tube to your station to not disrupt the Chelex bead bed.* ***DO NOT*** *transfer any Chelex beads to this new tube as it will interfere with the PCR and further analysis.*
12. Discard the used tip and tube in a designated waste container for proper disposal.	***Important:*** *Dispose of waste according to your teacher's instructions as the solution contains human body fluids and/or tissues.*
13. Optional stopping point: Store your template tube at –20°C to be used in the next lab.	

Figure 3.1: Materials for cheek cell DNA extraction

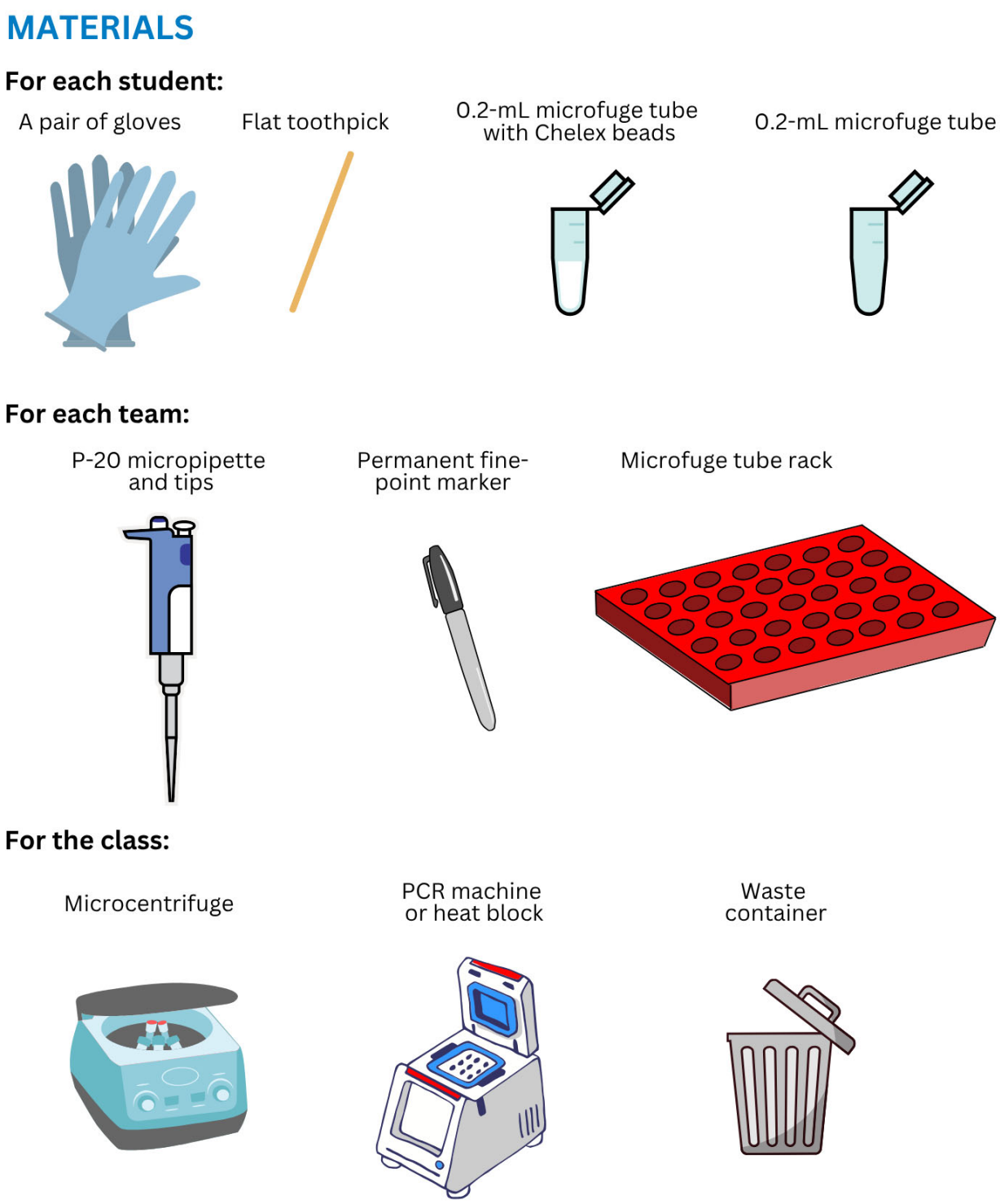
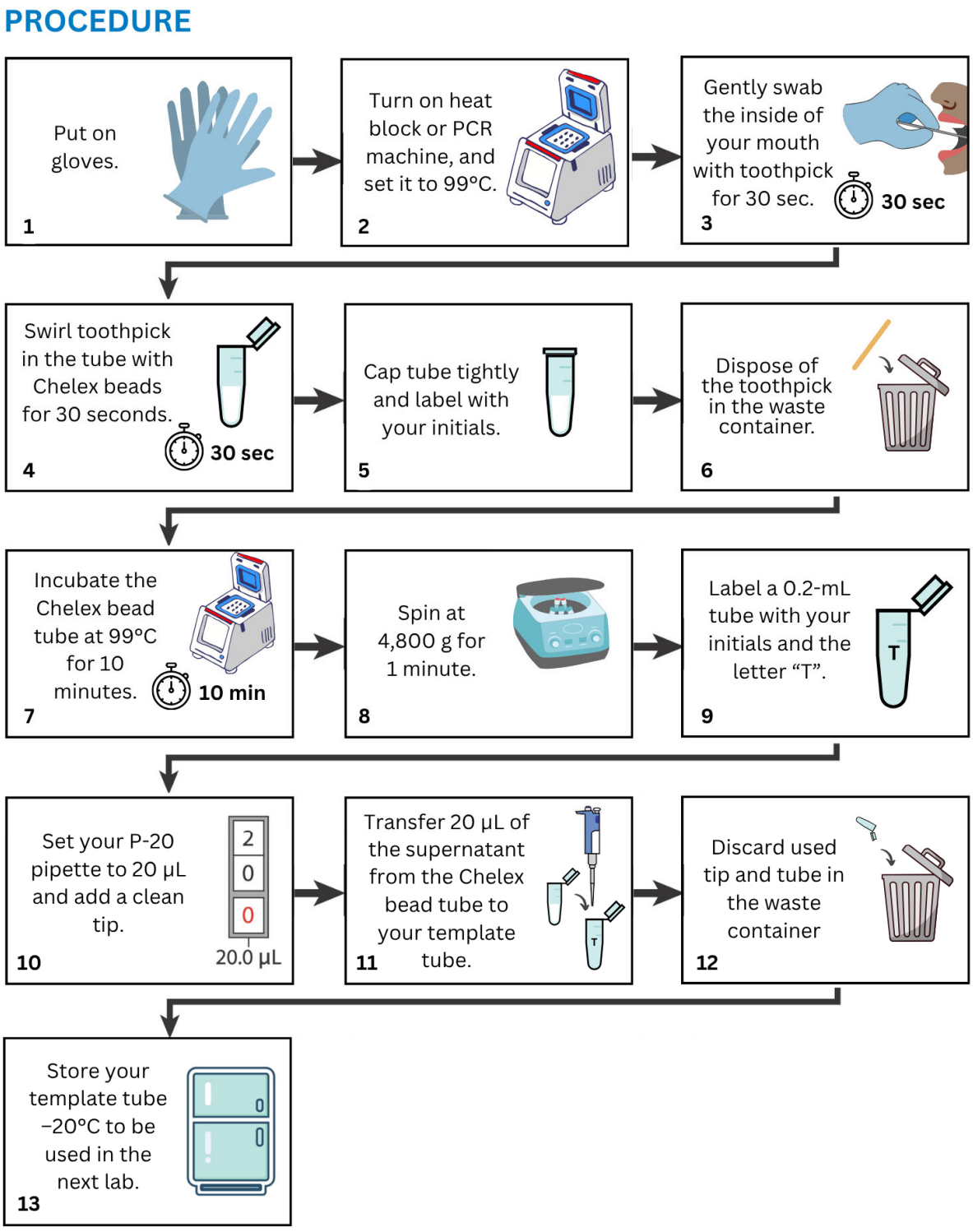


Figure 3.2: Flowchart for cheek cell DNA extraction



ACTIVITY: Programming the Thermocycler

Your next task is to amplify a specific section of your DNA using PCR. The process of PCR is controlled precisely by software. With your team, you'll create a program for your thermocycler that will use PCR to copy the desired DNA sequence.

MATERIALS

- Device with internet access and either USB or Bluetooth, such as a laptop computer or smartphone
- Software that controls your thermocycler

PROCEDURE

1. Using the software that controls your thermocycler, name the program that you are creating "Copy That DNA."
2. Set up the thermocycler program as shown in **Table 3.1**.

Table 3.1: "Copy That DNA" program for the thermocycler

Step	Number of cycles	Temperature	Time (seconds)
Initial Denaturation	1	94°C	300
Denaturation	35	94°C	30
Annealing		64°C	30
Extension		72°C	30
Final Extension	1	72°C	60

LABORATORY: Copy That DNA!

In this lab, you will use PCR to amplify (copy) a small fragment of DNA from your cheek cells. The area you wish to copy is called the ***template DNA***. In your body, every ***somatic cell*** (non-reproductive cell) that has a nucleus contains your full genome, but we're only interested in the gene associated with the ability to taste PTC. This gene is called TAS2R38, and it is located on chromosome 7. Rather than amplify your entire genome, which has over 3 billion base pairs, you will amplify only this small portion of your DNA—fewer than 300 base pairs. When you complete the lab, your sample will contain over a million copies of that small fragment of DNA!

In a medical context, a patient's DNA would be amplified in a very similar way to ensure that there is sufficient DNA for testing and sequencing. Technicians might amplify a patient's entire genome or only a small portion of particular interest. The patient's DNA sample would then be analyzed for the presence or absence of a specific allele (one of two or more alternative forms of a gene) or changes (mutations) to the DNA in question.

MATERIALS (see **Figure 3.3** for illustration**)**

For each student:

- 1 pair of gloves
- 1 0.2-mL template microfuge tube labeled "T"
- 1 0.2-mL microfuge tube containing 12.5 µL One*Taq* Hot Start 2X Master Mix (MM), labeled "MM" on the side of the tube (must be kept on ice)
- Access to an ice bucket or a cup of crushed ice

For each pair of students:

- 1 0.2-mL microfuge tube containing 11 µL TAS2R38 forward primer, labeled "FWD" (must be kept on ice)
- 1 0.2-mL microfuge tube containing 11 µL TAS2R38 reverse primer, labeled "REV" (must be kept on ice)

For each team:

- 1 fine-point permanent marker
- 1 P-20 micropipette and tips
- Access to an ice bucket or a cup of crushed ice

For the class:

- Microcentrifuge
- Thermocycler
- Laptop or desktop computer with internet access (if necessary)
- 1 0.2-mL microfuge tube containing 3 µL PCR positive control plasmid ("C+")
- 1 0.2-mL microfuge tube containing 3 µL ddH_2O ("C–")

- 1 0.2-mL microfuge tube containing 12.5 µL One*Taq* Hot Start 2X MM, labeled "MM" on the side of the tube (must be kept on ice)
- 1 0.2-mL microfuge tube containing 11 µL TAS2R38 forward primer, labeled "FWD" (must be kept on ice)
- 1 0.2-mL microfuge tube containing 11 µL TAS2R38 Reverse primer, labeled "REV" (must be kept on ice)

Note: Your teacher will designate one gel per class to run the negative control PCR. Depending on your class size and the number of gels your teacher has prepared, your teacher may opt to run several additional positive control PCRs so that each gel can have a positive control lane.

PROCEDURE (see **Figure 3.4** for flowchart)

Step	Notes
1. Put on gloves.	
2. Label the top of the MM tube with your initials or assigned number with a fine-point permanent marker.	*Master mix ("MM") contains Taq polymerase, cofactors, dNTPs and buffer.*
3. While keeping the MM tube on ice, add 5 µL of the forward primer into the MM tube. Using a new tip, add 5 µL of the reverse primer into the MM tube. Gently pipette up and down 3X to mix the solutions.	*It is OK if there is extra primer left in either tube after you and a partner have removed a total of 10 µL.*
4. Carefully transfer 2.5 µL from your template tube (T) into your labeled MM tube. Gently pipette up and down 3X to mix the solutions.	*Now this is your reaction tube, as it has the five components needed (listed earlier) for a PCR in addition to template DNA.*
5. Ensure that the tube is capped tightly.	*Store your sample on ice until you are ready to begin the reaction.*
6. Centrifuge the tubes for 5–10 seconds to bring the PCR solution to the bottom of the tubes. Place the tubes on ice until all samples are ready.	**NOTE:** *Your teacher has the materials for positive and negative control PCRs. They will either set these up or instruct you to do so.*
7. Add all sample tubes to the thermocycler(s). Run the "Copy That DNA" program.	*You created this program when you programmed your thermocycler (see Programming the Thermocycler).*

Step	Notes
 **Stop here! Your teacher will carry out the next step of the procedure.**	
8. 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 thermocycler and storing them at 4°C. (For longer storage, your teacher will store your samples at –20°C). **NOTE:** It would be OK to leave the tubes in the thermocycler at room temperature overnight if the PCR will not be complete before the end of the day.	*The thermocycler interior and/or tubes might be hot, so make sure to let them cool slightly before removing.*

Figure 3.3: Materials for PCR

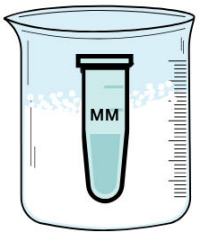
MATERIALS

For each student:

A pair of gloves

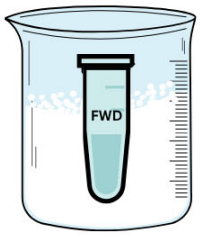
0.2-mL template microfuge tube ("T")

0.2-mL microfuge tube containing 12.5 µL PCR master mix ("MM") (must be kept on ice)



For each pair of students:

1 0.2-mL microfuge tube containing 11 µL TAS2R38 forward primer ("FWD"). (must be kept on ice)

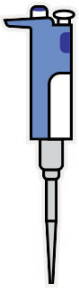


1 0.2-mL microfuge tube containing 11 µL TAS2R38 reverse primer ("REV") (must be kept on ice)

For each team:

Permanent fine-point marker

P-20 micropipette and tips



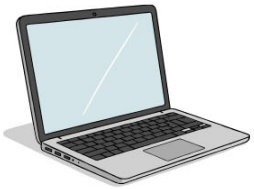
Cup of crushed ice

For the class:

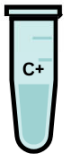
Microcentrifuge

Thermocycler

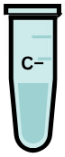
Computer or laptop (if necessary)



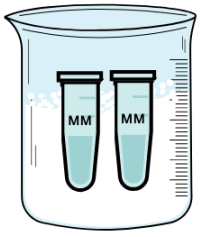
For every additional classroom gels, 1 0.2-mL microfuge tube containing 3 µL C+ control template DNA (add extras as needed)



0.2-mL microfuge tube containing 3 µL ddH_2O



2 0.2-mL microfuge tubes containing 12.5 µL PCR master mix (“MM”) (must be kept on ice)



2 0.2-mL microfuge tubes, one containing 11 µL TAS2R38 forward primer ("FWD") and one containing 11 µL TAS2R38 reverse primer (“REV”) (must be kept on ice)

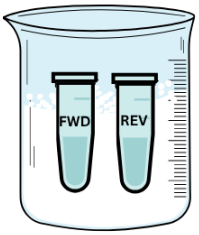
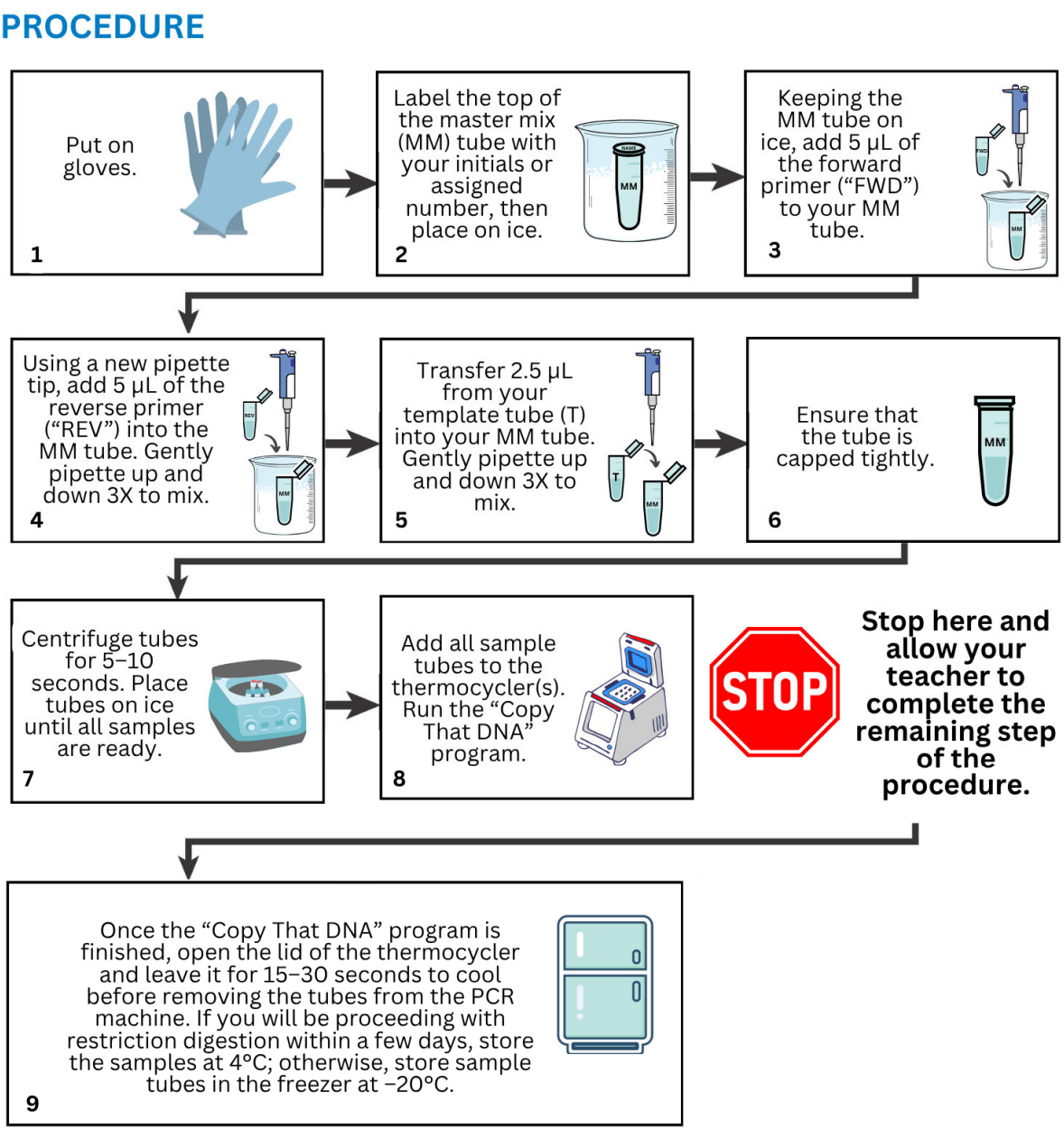


Figure 3.4: Flowchart for PCR



READING: How Is DNA Sequenced?

The human genome contains approximately 3 billion base pairs in our 23 pairs of chromosomes! How do we know this? Technology has allowed scientists to use ***sequencing*** (the process of determining the order of nucleotides in a DNA sample) to decode DNA from any organism. Although there are now multiple technologies used to sequence DNA, one of the early methods was ***Sanger sequencing*** (named after Frederick Sanger, who developed the method with his colleagues in 1977). The ***Human Genome Project*** (1990–2003)—a large, collaborative, international project that generated the first full sequence of the human genome—used Sanger sequencing.

SANGER SEQUENCING

Decoding DNA is essential because DNA serves as a blueprint for making RNAs, including mRNA, which our cellular machinery translates into the proteins we need. Since 2003, when the results of the Human Genome Project were published, DNA sequencing technology has become much faster, cheaper, and more readily accessible. However, scientists are still working to understand precisely which regions of the human genome do what and under what circumstances. By analyzing and comparing thousands of genomes with powerful software, researchers will continue to unlock more of DNA's secrets in the years to come.

Sanger sequencing permits the rapid identification of the order of nucleotides in a segment of DNA. Sanger sequencing consists of three major phases: chain-termination PCR, size separation by gel electrophoresis or capillary electrophoresis, and laser excitation and detection.

CHAIN TERMINATION PCR

Sanger sequencing begins by harnessing the power of PCR. In a standard polymerase chain reaction, DNA polymerase adds nucleotides in an order determined by a template DNA strand. Sanger sequencing introduces specially modified nucleotides into the reaction at random, in addition to normal nucleotides. These modified nucleotides have two special properties: they lack an oxygen group, and they are also able to fluoresce.

When one of these fluorescent modified nucleotides is added to a strand of template DNA, the chemical difference jams the polymerase and stops it from replicating the remainder of the strand, much like a stuck zipper. The fragment now has a glowing nucleotide at its ***terminus*** (end), which can be detected by a laser, then saved to analytic software. The process repeats, creating numerous fragments of varying lengths, each ending in a fluorescent nucleotide.

SIZE SEPARATION BY GEL ELECTROPHORESIS OR CAPILLARY ELECTROPHORESIS

In the next phase of Sanger sequencing, the new fragments are sorted by size using an electric field (electrophoresis) to help determine the order of the nucleotides. In the past, this was done by hand, using an agarose gel in traditional gel electrophoresis. Nowadays, automated sequencing machines typically use ***capillary electrophoresis sequencing***, in which nucleic acid fragments are loaded into a polymer-filled capillary tube to which an electric field is applied for size separation.

In both gel electrophoresis and capillary electrophoresis sequencing, an electrical charge is run through a polymer to separate DNA fragments. The smallest, most streamlined pieces travel the

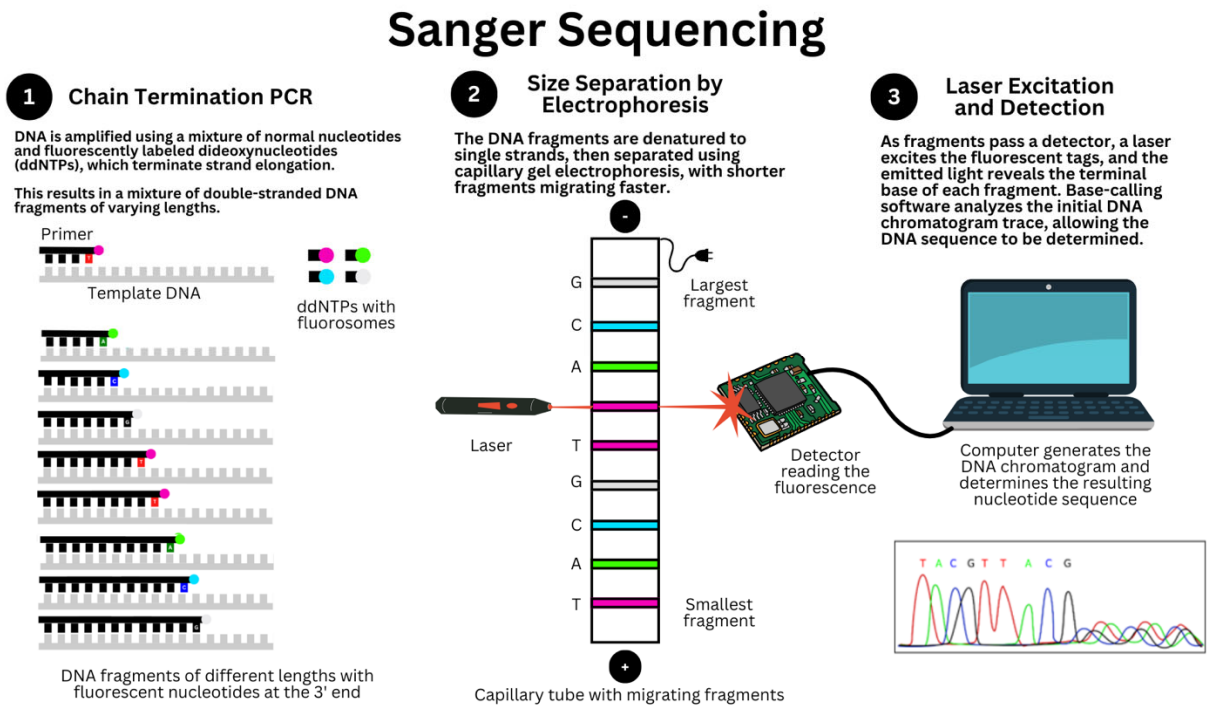
farthest through the polymer, whereas the largest, bulkiest pieces travel the shortest distance. Because DNA polymerase only synthesizes in the 5’ to 3’ direction, the smallest fragment corresponds with the first nucleotide at the 5’ end of the target strand. The next smallest fragment ends with the second nucleotide from the 5’ end, the third smallest with the third nucleotide, and so on.

LASER EXCITATION AND DETECTION

In the final phase, the gel results are analyzed within an automated sequencing machine. In the past, scientists read sequencing gels by eye, which was laborious and error prone.

In automated machines, a laser excites the fluorescent nucleotides at the end of each fragment as they flow through the capillary. Each nucleotide—A, T, C, and G—emits a unique type of light, which a camera detects. The resulting fluorescence data are sent to automated ***base-calling software***, which compiles the information about the nucleotides at the end of each fragment, sorts the fragments into the correct order, and generates an output called a ***DNA chromatogram*** (see Figure 3.5).

Figure 3.5: The phases of Sanger sequencing by capillary electrophoresis



A DNA chromatogram (see Figure 3.6) displays the nucleotides at each position and indicates the level of fluorescence emitted by each nucleotide in the sequence. Higher peaks (on the left in the image) indicate stronger signals, whereas lower peaks (on the right) indicate weaker signals and less certainty.

Figure 3.6: A DNA chromatogram