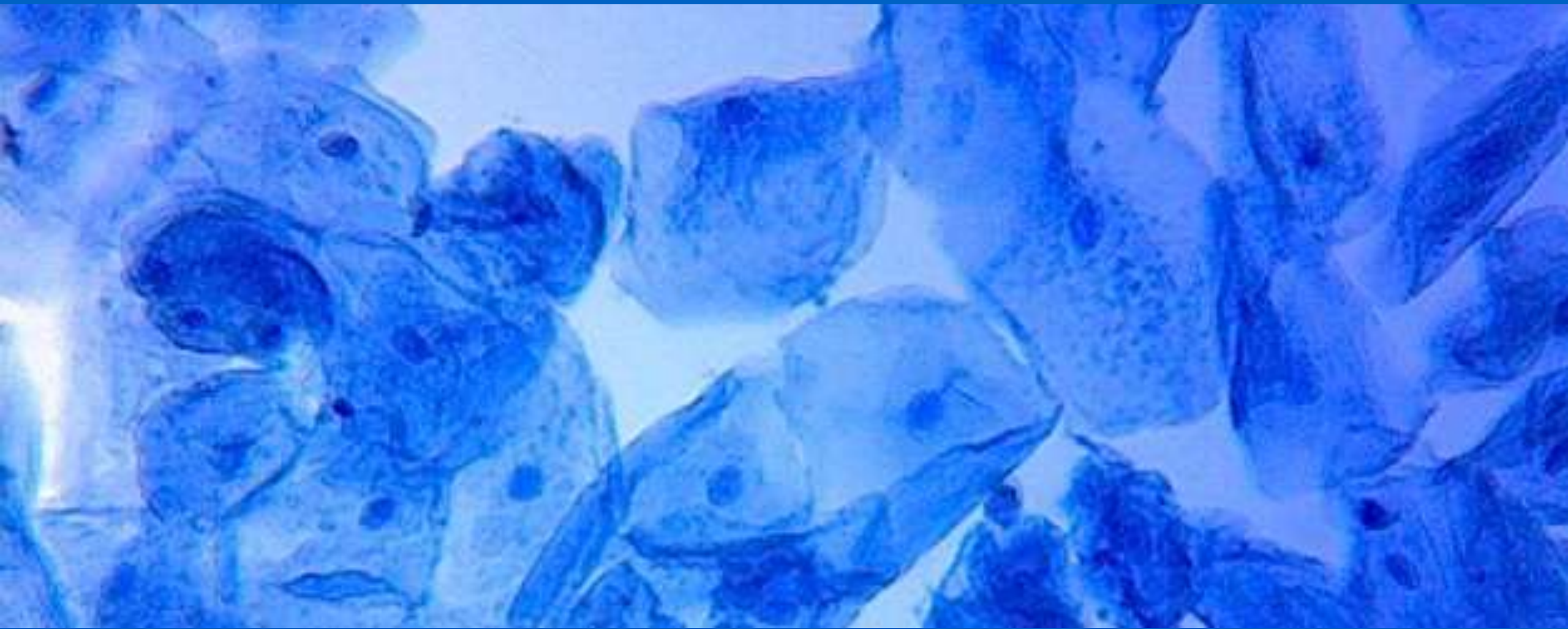
Exploring Precision Medicine

- Chapter 1: What's the Right Medicine?
- Chapter 2: Is My Sense of Taste Controlled by my Genes?
- Chapter 3: Exploring Our DNA
- Chapter 4: How Is DNA Sequenced, and What Can We Learn?
- Chapter 5: Restriction Enzyme Digestion of TAS2R38 PCR Products
- Chapter 6: Gel Electrophoresis and Genotyping
- Chapter 7: SNPs and Drug Metabolism

Chapter 3: Exploring our DNA



Video: How Does Your Body Process Medicine?

Why are we extracting our DNA and what are we looking for?

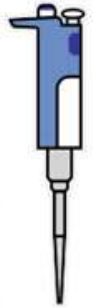
- Precision medicine studies variation in genes associated with particular phenotypes.
- We will look at a receptor gene associated with the ability to taste bitter foods.
- The bitter-taste phenotype is easy to test.
- This gene is a stand-in for medically relevant genes.

Exploring Precision Medicine: Activities

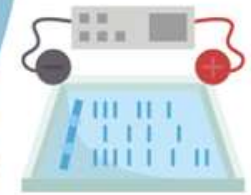
AMGEN Biotech Experience
Scientific Discovery for the Classroom

Sample Our Own DNA
Take a sample of cheek cells, isolate DNA, and amplify a short sequence of the bitter taste gene.

Sequence Analysis
Use bioinformatics software to explore the bitter taste gene and how genotypes can be distinguished.



Restriction Enzyme Digest
Use restriction enzyme digestion to prepare to determine our bitter taste genotypes.



Gel Electrophoresis
Use gel electrophoresis to determine our bitter taste genotypes.

Investigate Genetics of Drug Metabolism
Investigate the genetics of drug metabolism and consider how genotyping can aid medical treatment.

Medical Mystery
Explore the idea that people respond differently to medications and consider the possible reasons.

Is Taste Genetic?
Begin to explore a trait (taste) and investigate whether people experience it differently.

UNDERSTANDING PRECISION MEDICINE

In this module, we explore the genetics of the ability to taste bitter substances. It turns out that even small differences in our DNA—our genotype—can lead to major differences in traits—our phenotype. All of our genes have such individual differences, and some lead to changes in medically important traits. Advances in DNA sequencing and bioinformatics have made it much easier to discover these differences. Similarly, understanding how each of us metabolizes medications differently allows doctors to practice precision medicine—medicine based on each individual's genotype.

Read “Using Cheek Cells to Collect DNA” in your Student Guide

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 the flowchart that follows this table (**Figure 3.2**), will allow you to lyse (break open) the cheek cells, remove other cellular components, and isolate just the DNA from the cells.

MATERIALS

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 labeled with your initials and the letter "T"
  - This is the Chelex extraction from your cheek cells, which will provide the template DNA for your polymerase chain reaction.

For each team:

- 1 P-20 micropipette and tips
- 1 fine-point permanent marker
- Microfuge tube rack

For the class:

- Microcentrifuge
- PCR machine (thermocycler) or heat block
- Waste container(s)
- Device with internet access and thermocycler software installed

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FLOWCHART: Using Cheek Cells to Collect DNA

Figure 3.1: Materials for cheek cell DNA extraction

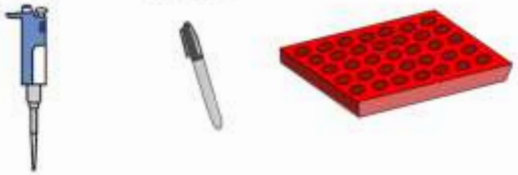
MATERIALS

For each student:

A pair of gloves | Flat toothpick | 0.2-mL microfuge tube with Chelex beads | 0.2-mL microfuge tube

For each team:

P-20 micropipette and tips | Permanent fine-point marker | Microfuge tube rack



For the class:

Microcentrifuge | PCR machine or heat block | Waste container

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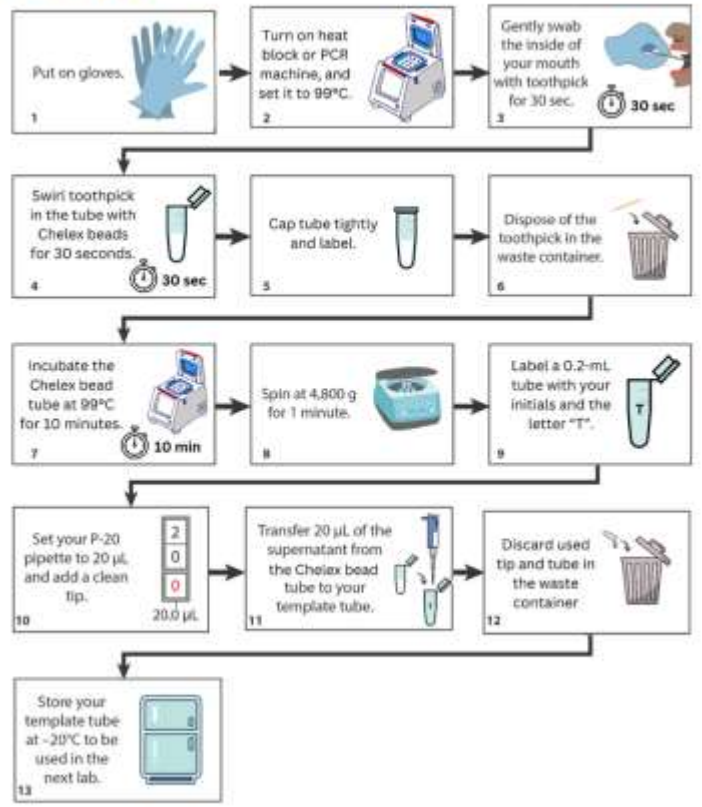
Figure 3.2: Flowchart for cheek cell DNA extraction

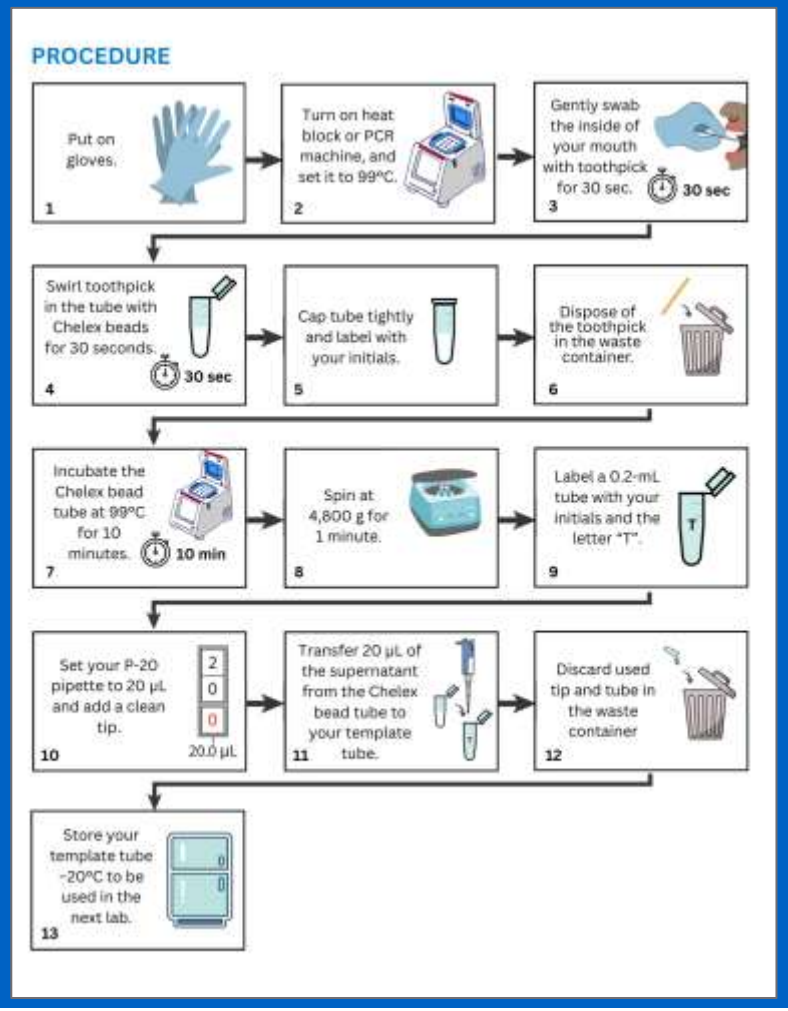
PROCEDURE



Discussion questions: “Using cheek cells to collect DNA”

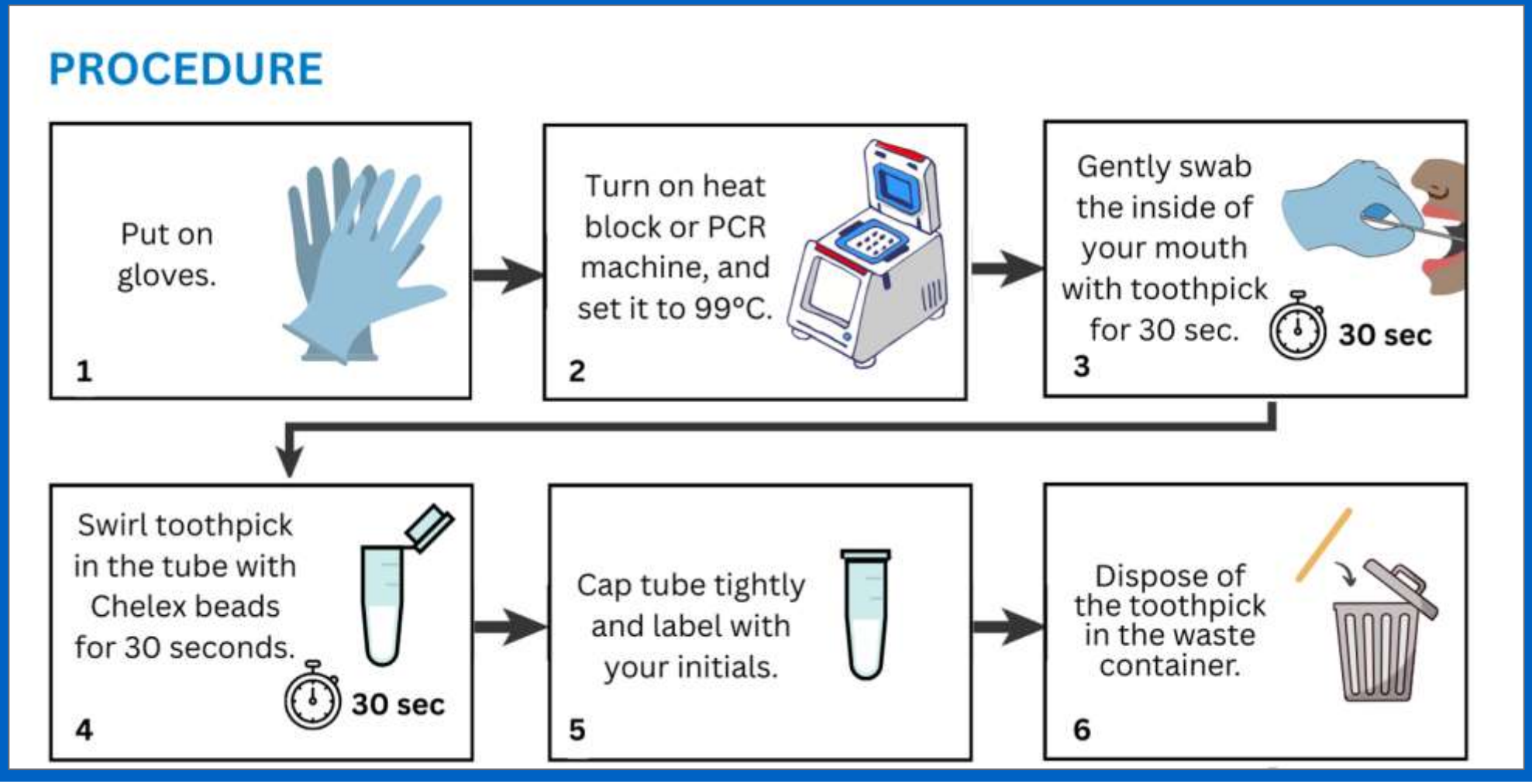
- **What happens when you heat up your sample?**
  - Heating the sample breaks open the cells’ nuclei and lets the DNA out
- **What does the Chelex do?**
  - The Chelex binds metal ions which allow DNAses to degrade damaged DNA. We want our DNA whole!
- **Why do you centrifuge the sample after incubating?**
  - Spinning the sample collects all the “garbage” at the bottom of the tube (broken-down cell components, etc.)

Collecting cheek cells: What you'll need

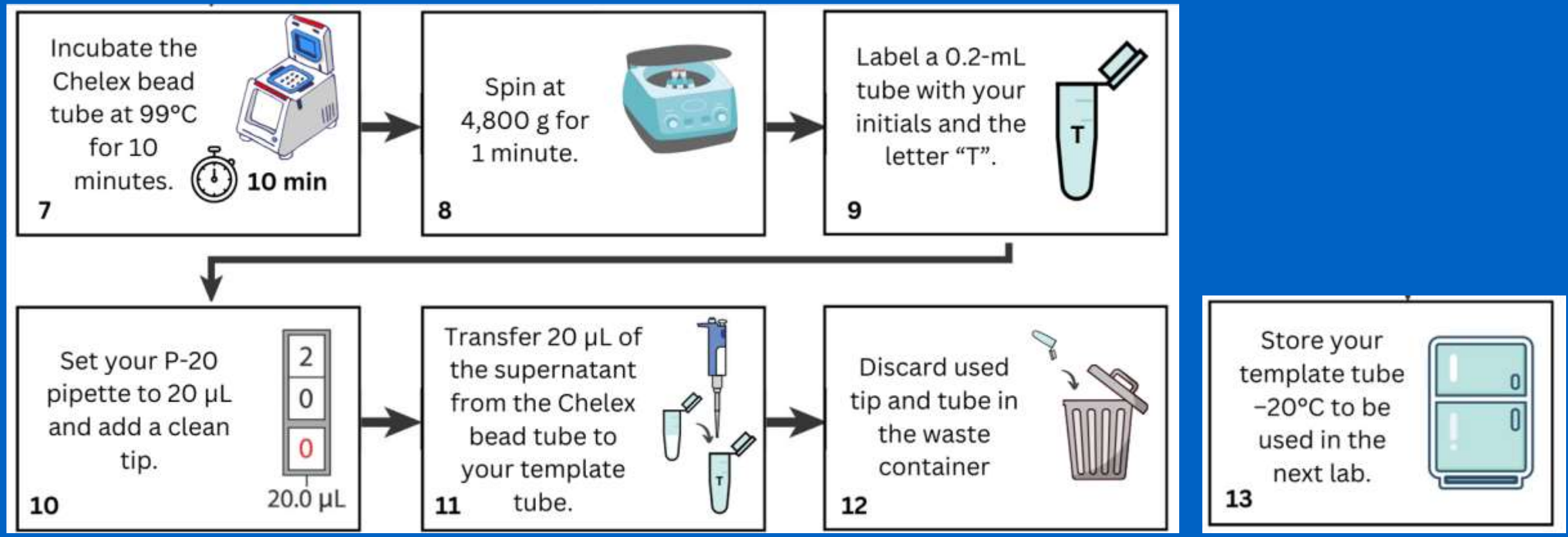


Video: Collecting your cheek cells

Collecting and Extracting DNA (Figure 3.2, Steps 1–6)



Collecting and Extracting DNA (Figure 3.2, Steps 7–13)



Make sure to pipette the supernatant without disturbing the Chelex beads at the bottom of the tube!

Program your thermocycler

“Copy That DNA” Program for thermocycler

Step	Number of cycles	Temperature	Time (s)
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

What you need to set up your PCR

COPY THAT DNA

MATERIALS

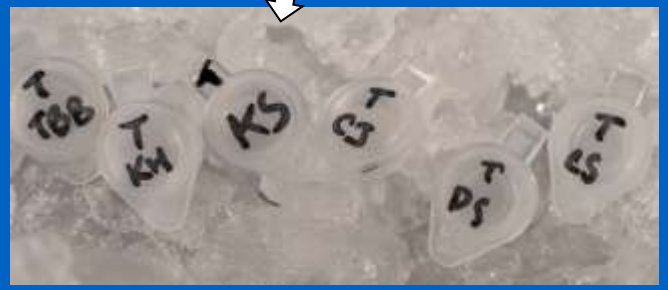
For each student:

A pair of gloves

0.2-mL template microfuge tube labeled "T"

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

0.2-mL microfuge tube containing 12 µL TAS2R38 primer mix, labeled "P" (must be kept on ice)



Your template DNA, labeled with your initials

PCR Master Mix

Primer mix

For each team:

Fine point permanent marker

P-20 micropipette and tips

Cup of crushed ice

For the class:

Microcentrifuge

Thermocycler

Computer, Chromebook, or tablet (if necessary)

0.2-mL microfuge tube containing 3 µL C+ control template sample

0.2-mL microfuge tube containing 3 µL ddH O

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

0.2-mL microfuge tube containing 12 µL TAS2R38 primer mix, labeled "P" (must be kept on ice)

In thermocycler, ready for PCR

Procedure for PCR amplification

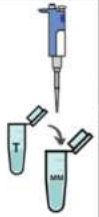
PROCEDURE

1 Put on gloves.

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

3 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.

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



5 Ensure that the tube is capped tightly.

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

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

Stop here and allow your teacher to complete the remaining 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 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.

While the PCR is running . . .

- **What reagents are required for PCR?**
  - Template DNA
  - Primers
  - Nucleotides
  - DNA Polymerase
  - Buffer
- **What are the 3 main stages of PCR?**
  - Denaturation
  - Annealing
  - Extension
- **Why is PCR necessary?**
  - To make numerous copies of a DNA sequence of interest

While the PCR is running . . . , continued

- **How many cells did you scrape from your mouth?**
  - Thousands
- **How can PCR "find" the right DNA sequence in a sample of genomic DNA?**
  - Primers hybridize only to that exact DNA sequence.
- **Can you use cells from just any human tissue to extract your template genomic DNA for PCR?**
  - The cells must have a nucleus (diploid).

While the PCR is running . . . , continued

- **Why did we use PCR controls C+ and C-?**
  - C+ contains template DNA. It shows that the PCR worked.
  - C- is only water. Nothing should show up except “primer dimers.” It demonstrates that the Master Mix is not contaminated with DNA.

Read “How Is DNA Sequenced?” in your Student Guide

- Knockout experiments (bullet points)
- Knockout experiments (table)
- Inheritance
- Location
- Relative size of amplified DNA on gel (“Small,” “Medium,” “Large,” “Extra Large,”) plus chromosomal band size/location on chromosome

NOTE: You may want to cross out the cells in the recording table where your gene is not located. This will help you eliminate possibilities and make it easier to identify the correct answer.

FOR HOMEWORK

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 determine the sequence of base pairs in our DNA. 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

Sequencing refers to the process of determining the order of nucleotides in a nucleic acid sample. DNA serves as a blueprint for making proteins and RNAs, such as mRNA and tRNA. For proteins, our cellular machinery deciphers the DNA code and uses them, along with RNAs, to manufacture 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 sophisticated software, we hope to unlock more of DNA’s secrets in the years to come.

Sanger sequencing is now used to quickly identify the order of nucleotides in a segment of genetic material. Sanger sequencing consists of three major phases: chain termination PCR, size separation by gel electrophoresis, and laser excitation and detection.

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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 feeds some 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 will now have a glowing nucleotide at its ***terminus*** (end), which can be detected by a computer. The process repeats, creating numerous fragments of varying lengths, each ending in a fluorescent nucleotide.

SIZE SEPARATION BY GEL ELECTROPHORESIS

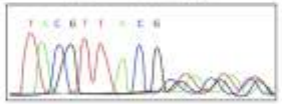
In this next phase of Sanger sequencing, the new fragments are sorted by size to help determine the order of the nucleotides. In the past this was done by hand using traditional gel electrophoresis, but gels can now also be run automatically within modern sequencing machines.

To sort the fragments by size, the DNA is loaded into a gel, and an electrical charge is run through the gel to separate the fragments. The smallest, most streamlined pieces will travel the farthest through the gel, whereas the largest, bulkiest pieces will travel the shortest distance. Because DNA polymerase only synthesizes in the 5′ to 3′ direction, the smallest fragment will correspond with the first nucleotide at the 5′ end of the target strand. The next smallest fragment will end 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 either analyzed by hand or within a sequencing machine.

Figure 3.7: A chromatogram



In automated machines, a laser excites the fluorescent nucleotides at the end of each fragment. Each nucleotide—A, T, C, and G—emits a unique type of light, which the computer can detect and interpret. The computer 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 ***chromatogram*** (see **Figure 3.7**). A chromatogram 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.

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