

# ***PTC PCR LAB RESOURCES***

**ATTENTION TEACHERS:**

**Please have your students know how to use a pipette before proceeding to do this lab!**

PTC PCR LAB KIT ITEMS	LABELS	VOLUMES: Amounts per 1 Group (minimum 4 students)
Sterile toothpicks		<b>20 TOOTHPICKS</b> in each 15mL conical vials
Thermocycler		1
Mini-PCR		--
Large Micro-centrifuge		1
Mini centrifuge		1
P---20 micropipette		12
P---20 pipette tips		--
P---10 micropipette		12
P---10 pipette tips		--
P---200 micropipette		12
P---200 pipette tips		--
P---1000 micropipette		12
P---1000 pipette tips		--
Light box/ amber filter		1
0.2mL <b>Green</b> Microfuge tubes (PCR Tubes) used for <b>Chelex</b>		--
0.2mL <b>Clear</b> Microfuge tubes (PCR Tubes) used for Master Mix		--
Fine-tip Permanent Marker		12
Waste cups		6
Gel Electrophoresis system; <b>(includes trays, combs, and boxes)</b>		6-boxes and trays 12-combs
Power supply		3
Biohazard Bags and stands		2
Capless microfuge tubes used as adapters for centrifuge and to hold tubes		--
Staining trays		6
Spatula		1
Microfuge floats		6
<b>FREEZER BOX ITEMS:</b>		
DNA (100bp) ladder- <b>TEACHER MUST ALIQUOT</b>	<b>M</b>	10uL per gel
PCR Master Mix: <b>(TEACHER MUST ALIQUOT: 135µL of Master Mix per group of 4 students)</b>	<b>MM</b>	60uL of Primer and 75uL of One Taq quick load <b>TEACHER MUST PREPARE DAY OF LAB</b>
One Taq Quick Load	<b>TAQ</b>	75uL per group (12.5 uL per student)
PTC Primer	<b>Primer</b>	60uL per group (10 uL per student)

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HaeIII	HaeIII	10uL per group (2uL per student)
<b>REFRIGERATE ITEMS:</b>		
Chelex- <b>TEACHER MUST ALIQUOT</b>	X	100uL in a 0.2mL tube per student
5x Orange G loading dye- <b>TEACHER MUST ALIQUOT</b>	LD	10uL per group (2uL per student)
<b>ROOM TEMPERATURE</b>		
1X Sodium Borate- <b>TEACHER MUST PREPARE</b>		20mL of 20X SB to 380mL of DI water
Distilled or deionized water-(used to dilute the 20X SB buffer provided into 1X SB.) <b>NOT SUPPLIED!</b>		380mL of DI water to 20 mL of 20x SB to make 1x SB
Agarose- (4g agarose to 120mL of 1X SB)		~30-40mL per casting tray-(1gram per 40mL of 1x SB Buffer
<b>KEEP AWAY FROM LIGHT</b>		
PTC PCR Strips		1 Vial
Sybr Safe (gel stain)		12uL per 120mL of SB Buffer + 4g of agarose
Sybr Safe DNA Stain for post staining (1 bottle is provided)		Cover gel with post stain and leave for 30 minutes.

**Notes:**

**-15mL conical tubes containing sterile toothpicks:** When passing out the toothpicks, we recommend that the teacher wear gloves to preserve sterility.

**-We recommend when pipetting the Chelex:** cut the p- 200 pipette tip and then pipette up and down to retrieve an appropriate amount of Chelex beads.

**-Please remember that the GREEN microfuge tubes are used for CHELEX aliquots only.**

**-When centrifuging samples:** Please do not spin cap-less microfuge tubes at the highest speed, press the **QUICK** button only when spinning: they will crack if spun at the highest speed.

**-P-20, P-200, and P-1000 pipettes may contain locks on them:** Please **UNLOCK** the pipette when adjusting the measurement.

**\* We recommend that groups should consist of at least 4 students.**

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This is an addition to the kit for the PTC PCR lab. This is used to keep important stocks cold and can also be used to aliquot samples. **This must be returned in your kit when your rental is up.**

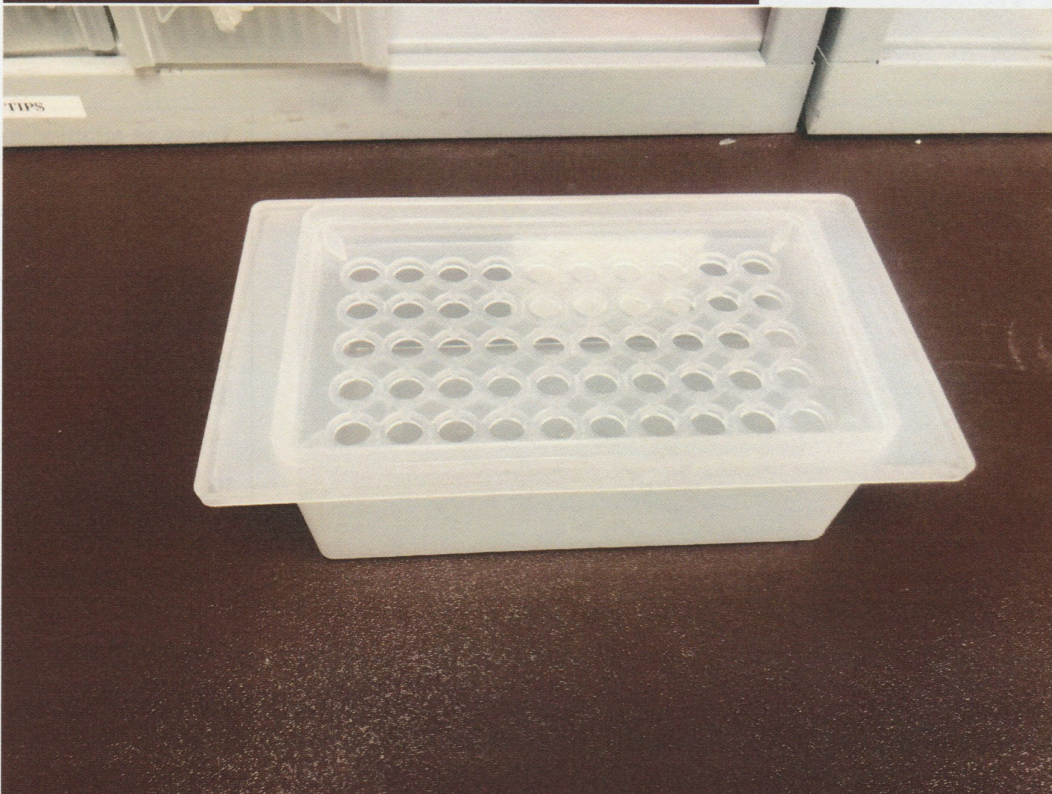
\*This will also be provided to teachers who do any lab with frozen reagents.

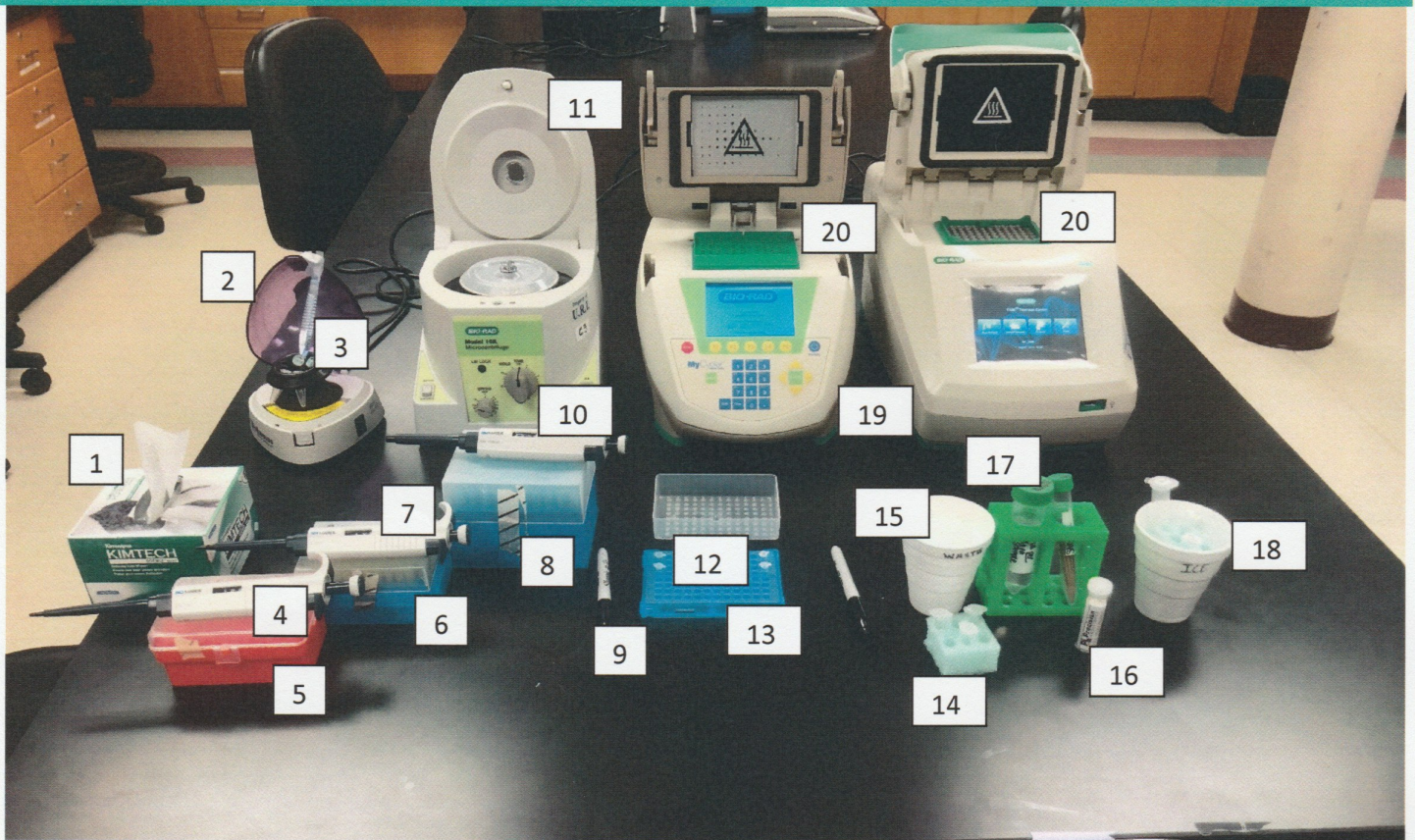
\*\*We do also provide a bigger size of this grey aliquot holder. **(Please return both!)**



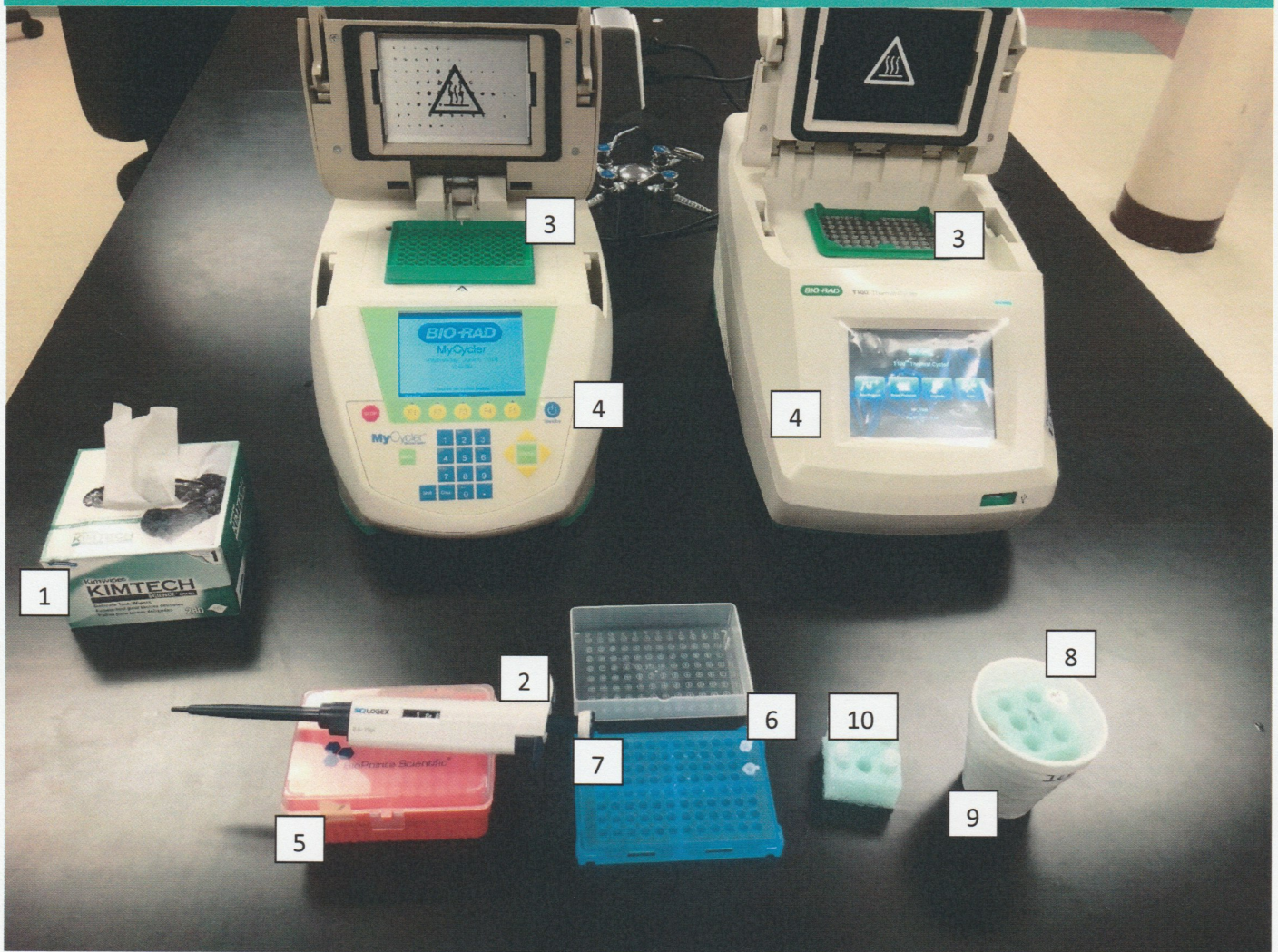
We are currently providing teacher a PTC PCR Ice tray that will give teachers the ability to aliquot multiple samples on ice at the same time. **This is only for teacher use and must be returned to us with the kit you have rented.**

\*This will also be provided to teachers who do any lab with frozen reagents.

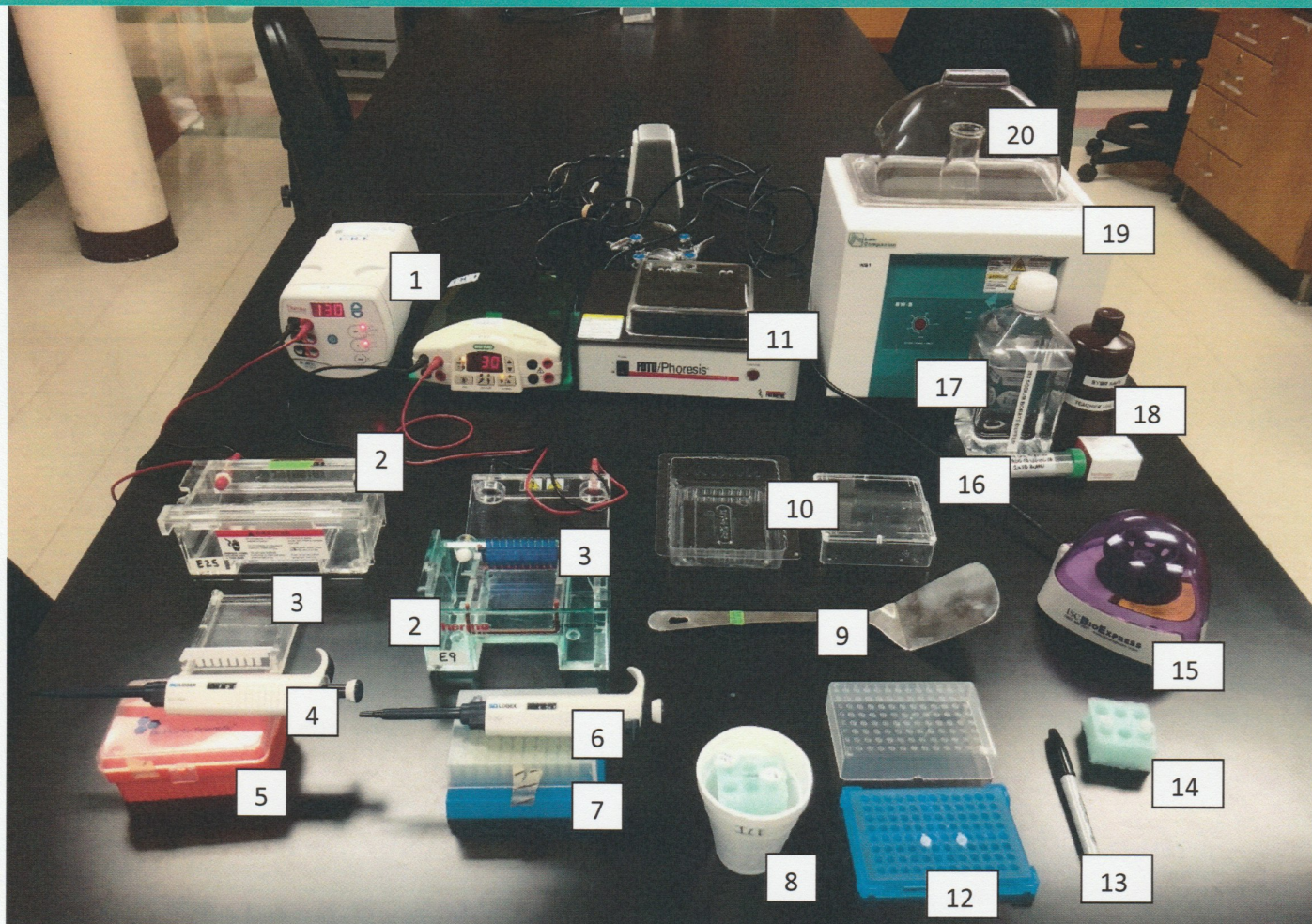




1. Kimwipes
2. Mini centrifuge
3. Cap-less tubes & forceps: **cap-less tubes** should be used to keep small PCR tubes safe when centrifuging, **forceps** should be used to help retrieve PCR tubes and cap-less tubes from either the mini centrifuge or the large centrifuge.
4. P0.5-10 pipette
5. P10 pipette tips
6. P20-200 pipette tips
7. P20-200 micropipette
8. P100-1000 pipette tip
9. Sharpie marker
10. P100-1000 pipette
11. Large centrifuge
12. **Green** flat cap tube (used **only** for Chelex) & **Clear** flat cap tube (used **only** for master mix)
13. PCR tube holder
14. Green float- holds **saline** that has been aliquoted by **teacher**
15. Waste cup
16. PTC taste strips (will usually be wrapped in aluminum foil to protect it from the light- **keep out of light!**)
17. Microfuge tube rack holder: should be used by teacher to pass out toothpicks & aliquot the saline.
18. Ice cup: should hold tubes labeled: Chelex, Primer & Taq.
19. Thermocycler (PCR)- both types that may be provided in kits are being displayed
20. PCR green adapter- both types that may be provided in kits are being displayed



1. Kim wipes
2. P0.5-10 pipette
3. PCR green adapter- both types that may be provided in kits are being displayed
4. Thermocycler (PCR)- both types that may be provided in kits are being displayed
5. P-10 pipette tips
6. Clear flat cap PCR tubes that should contain amplified DNA.
7. PCR tube holder
8. Haelll tube should be on ice
9. Ice cup
10. Green float: should be used to hold tubes in cap-less adapters.



1. Electrophoresis power packs (both types that may be provided in kits are being displayed)
2. Electrophoresis chambers (both types that may be provided in kits are being displayed)
3. Trays and combs (both types that may be provided in kits are being displayed)
4. P0.5-10 pipette
5. P-10 pipette tips
6. P20-200 micropipette
7. P20-200 pipette tips
8. Ice cup: holding the 100bp ladder: (M) and orange G loading dye: (LD)
9. Spatula
10. Gel trays (both types that may be provided in kits are being displayed)
11. UV trans illuminator
12. PCR tube holder
13. Sharpie marker
14. Green float
15. Mini centrifuge
16. 4g of agarose tube
17. 20x SB buffer
18. Sybrsafe in bottle & tube (choose either)
19. Water bath (used to cool down the agarose- set temp at 55-60°C)
20. Flask containing the hot agarose should be placed in the water bath to cool down before being poured.



# AMGEN<sup>®</sup> Biotech Experience

## Scientific Discovery for the Classroom

### ABE 2.0: PTC Lab Protocol

#### Overview

This teacher guide is intended to help instructors navigate the set-up and execution of the PTC laboratory protocol. For your convenience, the student protocol has been annotated to include helpful text and optional stopping points with storage conditions, in red. Additional resources for PCR, gel loading, and sequencing can be found at the end of this document.

The teacher guide includes:

- Pre-PTC Laboratory Prep Work
- Annotated PTC Student Manual
- Sequencing Guide
- Teacher Resource: Optional Pre-Laboratory Vocab Review
- Teacher Resource: What is Chelex®?
- Teacher Resource: What is PCR?
  - Guidelines to creating your own PCR-book analogy
- Teacher Resource: Why do we use the PTC reverse primer for sequencing?
- Teacher Resource: Template Sign-Up Sheet for Gel Loading

**TEACHER  
GUIDE**

Reagent	Per 1 Student	Per 25 Students	Per 4 Students
TAS2R38 Primer	10µL	30 X 10µL = 300µL	6 X 10µL = 60µL
30 X 12.5µL of Standard Master Mix	12.5µL	30 X 12.5µL = 375µL	6 X 12.5µL = 75µL
Total	22.5µL	675µL	135µL

## Teacher Pre-Laboratory Prep Work

- PCR Primer Master Mix
  - You will receive a tube of TAS2R38 (PTC) Primers, which is a mixture of both forward and reverse primers. You will also receive a tube of Standard Master Mix, which may or may not include loading dye. If it does, you may still want to add additional loading dye in part IV step 1 (page 11 in the student manual). You will need to combine these two reagents to make the TAS2R38 Primer Master Mix used in part II, step 1. Each PCR reaction needs the following:
    - 10 $\mu$ L of TAS2R38 Primer
    - 12.5 $\mu$ L of Standard Master Mix
  - We suggest calculating enough for your number of students plus 5 extra reactions for a class of 25. For example, if you are running this lab with 25 students, you would do the following below. You can also prepare reagents for groups of 4 students with 1 extra reaction per group. Label MM on the microcentrifuge tube.

Reagent	Per 1 Student	Per 25 Students	Per 4 students
TAS2R38 Primer	10 $\mu$ L	30 X 10 $\mu$ L = 300 $\mu$ L	6X10 $\mu$ L=60 $\mu$ L
30 X 12.5 $\mu$ L of Standard Master Mix	12.5 $\mu$ L	30 X 12.5 $\mu$ L = 375 $\mu$ L	6 X 12.5 $\mu$ L = 75 $\mu$ L
<b>Total</b>	<b>22.5<math>\mu</math>L</b>	<b>675<math>\mu</math>L</b>	<b>135 <math>\mu</math>L</b>

- In this example, you will pipette 300 $\mu$ L of the TAS2R38 Primers into a new tube. Add 375 $\mu$ L of Standard Master Mix to this tube and pipette up and down to thoroughly mix. Aliquot 22.5 $\mu$ L of this mixture into clean, clear 0.2mL PCR tubes. One tube will be needed for one student. Put on ice in adapter tubes.
- You can prepare this TAS2R38 Primer Master Mix up to one hour before use.
- Gel loading
  - Prepare gels before part IV. First, prepare 1X Sodium Borate Buffer. This is the same buffer for labs 1.2 and 4A. A 20X stock is provided in your kit. Dilute with deionize water. Make sure you add the appropriate amount of SyberSafe® to your molten agarose/buffer mix with a ratio of 1 part SyberSafe to 10,000 part 1X Sodium Borate Buffer. For example, if your gel tray takes 30mL of this molten agarose/buffer mix, you must add  $\mu$ L of

SyberSafe®. For 120 mL of molten agarose, add 12µL SyberSafe®. Remember to mix thoroughly before pouring into your gel tray. Once poured, cover or keep gels out of light until ready to use.

- You will need one lane per student, one lane of undigested control, and one lane for ladder in each gel.
  - It is up to you whether you allow students to assign themselves or you pre-assign each student to a gel and a lane before students load samples in part IV step 3 (keep track of this list). We have included a sign-up template at the end of this document.
- Before students load their samples, ensure that you have loaded 10µL of 100bp ladder into the first lane and 20µL of your undigested control in the second lane.
- Chelex Preparation:
  - Obtain a 5mL tube of Chelex beads (labeled **X**) . Pipet 100uL of beads to 0.2mL green PCR tube for each student. Re-suspend beads by pipetting them gently in and out of the pipettor repeatedly between samples.
- 5x Orange G loading dye Preparation: Label **LD** on microcentrifuge tube
  - 12µL per group of 4 students (2uL per student)
- DNA ( 100bp) ladder: Label **M** on microcentrifuge tube
  - 12µL per gel set up (10uL per gel)
  - The teacher may decide to load the ladder in which case it may not be necessary to aliquot.
- HaeIII: Label **HaeIII** on the microcentrifuge tube
  - 12 µL per group (2µL per student)

## Annotated Student Manual

### Overview

In this laboratory you will explore the connection between your genetics (**genotype**) and your observed characteristics (**phenotype**) using taste perception. During a subsequent activity, you will be given an opportunity to taste the compound **phenylthiocarbamide (PTC)**. Upon tasting PTC you will experience one of these three phenotypic reactions: a strong bitter taste, a mild bitter taste, or nothing at all.

The population studies conducted by chemist Arthur L. Fox in the 1930s revealed that the ability to taste PTC is an **inherited dominant trait** that varies in the human population and influences how we taste PTC. The **gene** that encodes for the PTC taste receptor, **TAS2R38**, was identified in 2003. **Taste receptors** are found on the surface of some of the cells on our tongue and we know today that TAS2R38 encodes for one of the thirty taste receptors that allow detection of bitter-tasting compounds.

Metabolizing our foods begins with breaking them down as we chew. **Chemical compounds** within the foods will either **bind or not bind** to taste receptors on the cells of our tongue. If a compound binds to a receptor, this results in **activation** of the receptor, in which a signal is transmitted to our brains, allowing us to perceive taste.

The TAS2R38 gene has a **dominant** and a **recessive** version (**allele**). Each allele encodes a different version of the PTC taste receptor, and differences in perception of taste are based on which alleles an individual carries. The goal of this laboratory is to determine your genotype using multiple molecular biology techniques. These steps are outlined in Figure 1 (page 2) and described in Table 1 (page 3).

First, you will use **Polymerase Chain Reaction (PCR)** to amplify (make many copies of) a short region of the TAS2R38 gene. Figure 3 (page 7) outlines the steps of PCR. In order to differentiate between the TAS2R38 alleles, the amplified PCR product will be digested (cut) with the **restriction enzyme HaeIII**. Restriction enzymes cut at specific DNA sequences, termed **recognition sequences**. The HaeIII recognition sequence will allow us to distinguish the **single-nucleotide polymorphism (SNP)** that differentiates the PCR products of the dominant and recessive TAS2R38 alleles. The PCR product of the dominant allele will be cut by the enzyme, while the PCR product of the recessive allele will not, as shown in Figure 1 (page 2). This means that differently sized fragments will be produced for **homozygous dominant**, **homozygous recessive**, and **heterozygous** individuals. Therefore, after the restriction digest with HaeIII, you will use **gel electrophoresis** to visualize your resulting fragments in order to conclude your genotype.

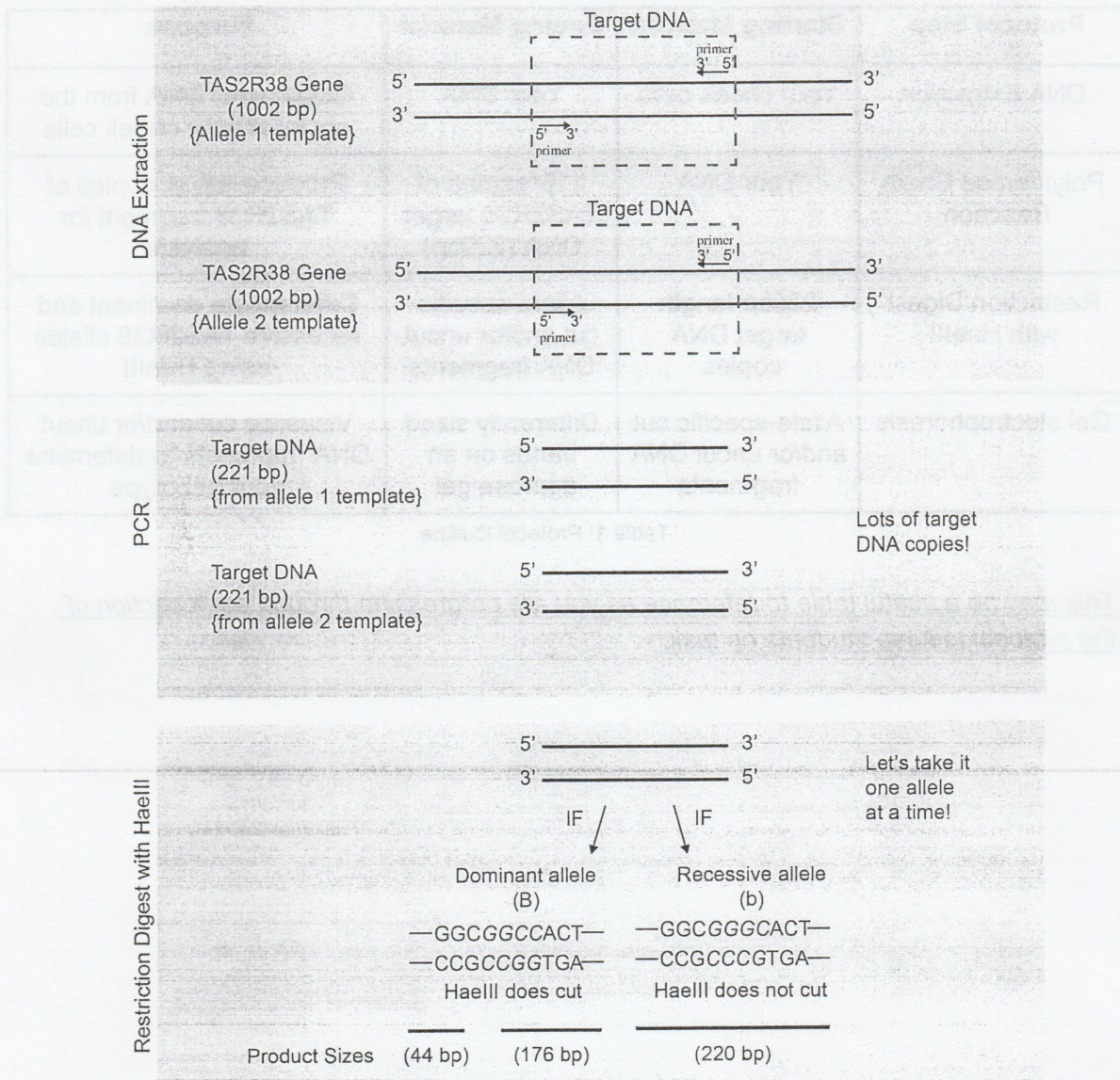


Figure 1: Overview of Laboratory Procedure

Figure 1. In this laboratory procedure, you will first isolate your own DNA from your cheek cells. We are interested in determining your genotype at the TAS2R38 locus. Therefore, we will use primers that have been designed to specifically amplify a portion of the TAS2R38 gene using PCR. Figure 3 (page 7) outlines the three key steps in PCR that allow us to make billions of copies of our target DNA. We need this many copies in order to first perform a restriction digest using HaeIII to differentiate between the dominant and recessive alleles, and then finally to visualize these resulting fragments using gel electrophoresis.

Protocol Step	Starting Material	Ending Material	Purpose
DNA Extraction	Your cheek cells	Your DNA	Obtain your DNA from the nuclei of your cheek cells
Polymerase Chain Reaction	Your DNA	>10 <sup>6</sup> copies of TAS2R38 target DNA (220bp)	Produce lots of copies of TAS2R38 fragment for analysis
Restriction Digest with HaeIII	220bp-length target DNA copies	Allele-specific cut and/or uncut DNA fragments	Differentiate dominant and recessive TAS2R38 alleles using HaeIII
Gel electrophoresis	Allele-specific cut and/or uncut DNA fragments	Differently sized bands on an agarose gel	Visualize cut and/or uncut DNA fragments to determine your genotype

Table 1: Protocol Outline

*This may be a useful table to reference as you are progressing through each section of the protocol to keep students on task.*

## Protocol

### I. Collecting and Isolating DNA from Your Cheek Cells

Extract your own DNA as you demonstrate how to swab from your cheek cells. Use your DNA sample to also model how to set up the PCR reaction in part II. Include your reaction in the miniPCR™ machine. Do not digest your sample with HaeIII in part III. This can be used as an undigested control sample in the gel electrophoresis in part IV.

You may want to store your students DNA samples in case of PCR failure. The supernatant from their DNA extractions can be transferred to clean 0.2mL tubes and be stored at 4°C.

You will be extracting DNA found in the nuclei of your cheek cells. These cells can be easily collected by gently swabbing the inside of your mouth with a toothpick. You will transfer your cheek cells by twirling the toothpick in a tube containing Chelex® beads. Chelex® is a **chelating** (binding) agent that is often used for DNA extraction. In order to isolate your DNA, we must break down the **cellular and nuclear membranes**. We will do this by boiling your cheek cell samples. Once these membranes are broken down, components normally in the cytoplasm have access to the DNA. These components include enzymes called **DNases** that break down DNA. DNases require metal co-factors for activity. The Chelex® resin will bind these metal co-factors away from the DNases, thereby **inhibiting** DNase activity.

1. You will receive 100µL of Chelex® beads in a small, green 0.2mL tube. Clearly label the top and side of this tube with your initials using a permanent waterproof marker. You labeled your tube as: \_\_\_\_\_

The 0.2mL green tube containing the Chelex® beads may be sitting in an adapter. Please remind students to label the top and the side of smallest tube (the 0.2mL tube with the Chelex® beads) and **not** the adapter.

2. Using the broad or flat end of a sterile toothpick, gently swab the inside of your mouth for at least 30 seconds.

This is the first make or break step in the protocol. Make sure that students scrape the inside of their cheeks thoroughly with the tip of the wide end of the toothpick.

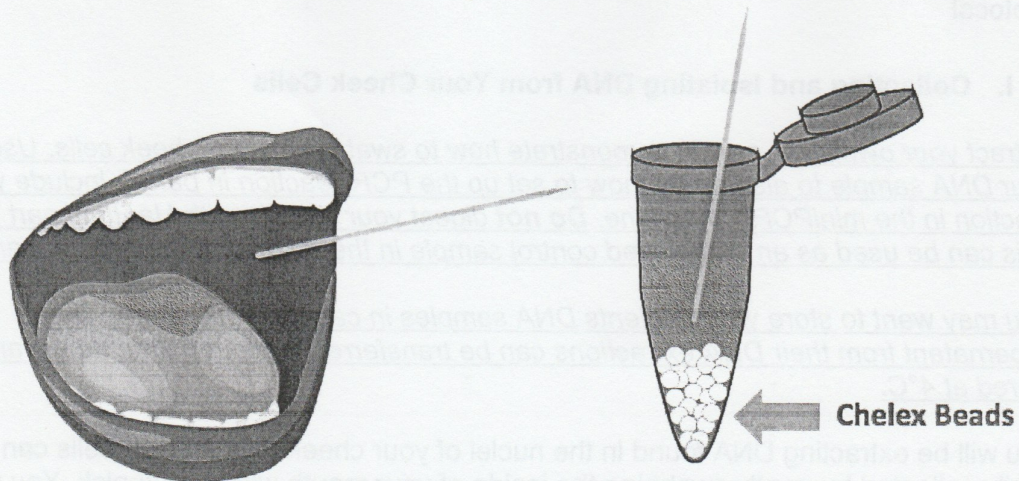


Figure 2: DNA collection and extraction procedure

3. Swirl the toothpick in the tube of the Chelex® beads for at least 30 seconds to dislodge the cells and cap the tube tightly. Place used toothpick in waste container.
4. Create a program on the thermocycler to hold a temperature of 99°C for 10 minutes. Name this “**DNA Extraction Heat Block**”.

Can either program a subsequent 4°C cooling step or remove samples immediately after these 10 minutes and place on ice.

OPTIONAL STEP: In case of PCR failure you may want to store your students’ DNA extraction samples. This will allow you to simply re-run the PCR reactions without repeating the DNA extraction. To do this, pipette supernatant (careful to avoid beads) into a clean, labeled 0.2mL tube and store in the fridge at 4°C.

5. Place your 0.2mL green tube into the thermocycler. Close the lid and run “**DNA Extraction Heat Block**”.
6. Once the **DNA Extraction Heat Block** protocol is finished, allow your teacher to remove your tubes from the thermocycler carefully so that the Chelex® beads at the bottom are not disturbed.

Emphasize to your students that the thermocycler is being used as a heat block and that this step is NOT the running of their PCR reactions.



## II. Amplifying PTC DNA by PCR

In human cells, DNA replication results in two identical copies of the entire human genome. PCR is a molecular biology lab technique that allows us to replicate a small portion of DNA that we are interested in (the “target DNA”). As you may imagine, it would be terribly difficult to find and work with only two tiny fragments of DNA isolated from one individual’s DNA. Instead, we can use PCR to locate, isolate, and make (“*amplify*”) millions of copies of a gene from one person. In this case, you will use PCR to isolate and amplify your own PTC gene.

### A PCR reaction contains:

- Source DNA from one person (from your cheek cells!)
  - Provides a template for DNA polymerase to read
- *Taq* DNA polymerase
  - Bacterial enzyme that allows DNA replication at high temperatures
- Primers
  - Molecular “post-it notes” that define target DNA
    - One per each strand of DNA (forward and reverse)
  - Short DNA fragments (18-22 base pairs) that we design
  - Bind to target DNA through complementary base pairing
- dNTPs
  - Deoxynucleotide building blocks (A, T, C, G) used to make new strains of DNA
- Salts and buffers
  - Ensure correct chemical conditions for reaction
  - Includes  $MgCl_2$  which is a co-factor required for *Taq* DNA polymerase activity

### Steps of a PCR reaction (refer to Figure 3, page 7 for diagrams of each step)

The “chain” reaction of PCR is composed of three steps: denaturation, annealing and extension.

1. Denaturation (94°C)
  - Hydrogen bonds that hold DNA together are broken, therefore DNA is separated (“denatured”) into single strands
2. Annealing (64°C)
  - Primers bind to single strands of template DNA
3. Extension (72°C)
  - *Taq* DNA polymerase creates a new strand of DNA starting at the end of each primer

These three steps constitute one synthesis cycle during which the number of copies of DNA doubles. A reaction with 35 cycles will therefore produce over a billion copies.

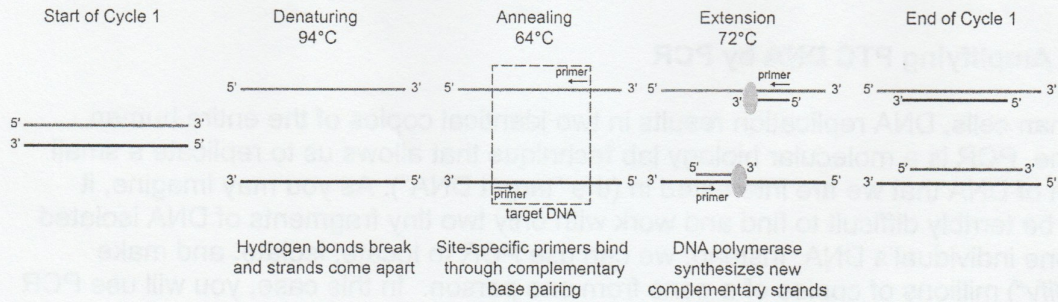
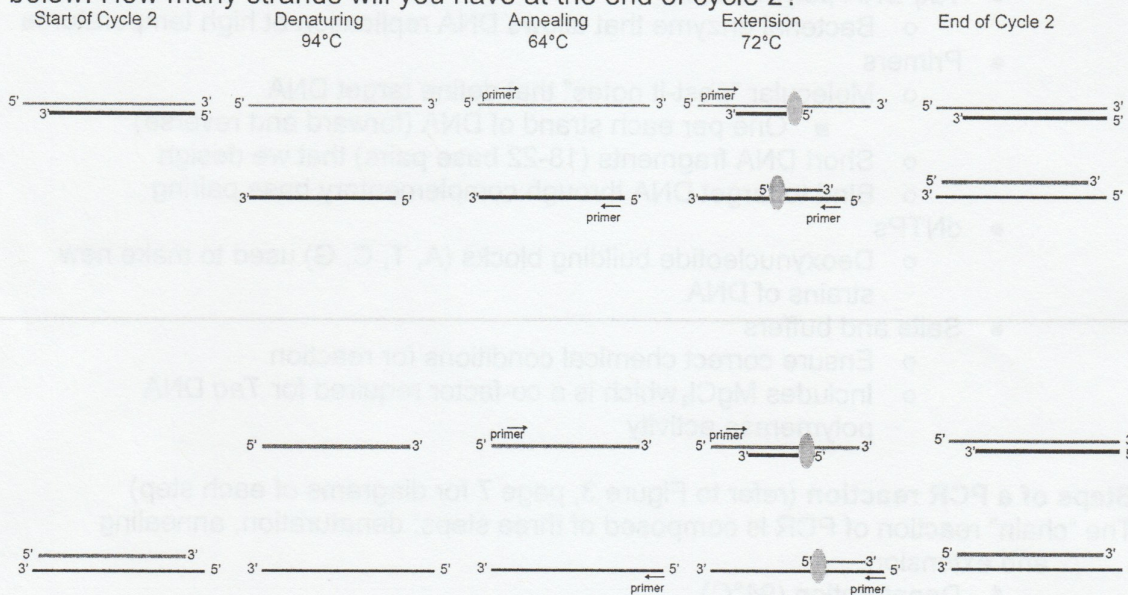


Figure 3: Steps of a PCR reaction

At the end of the first cycle of PCR you will have 4 strands (2 original template strands, 2 newly synthesized strands). Draw out the steps that occur during the second cycle below. How many strands will you have at the end of cycle 2?



**Stop and Think**

At which cycle of the PCR reaction will you have amplified a fragment of **just** the target sequence? 3

In order to ensure DNA sample is added, have your students pipette to the side of the 0.2mL clear tube that contains the Master Mix. They should see a small droplet of their DNA on the side of the tube. Once they confirm this, have them close their tubes and tap them down to make the DNA droplet pool at the bottom with the Master Mix.

If you have a centrifuge, we recommend having your student pulse down their samples before starting the PCR reaction. If you are planning to centrifuge your samples, make sure the 0.2mL clear PCR tubes are in the appropriate adapters before placing them in the centrifuge.

1. Carefully pipette 2.5µL of the supernatant, which contains your DNA, to the 0.2mL clear PCR tube that already contains the **TAS2R38 Primer Master Mix**. Label the tube with an asterisk\* and your initials. This tube now contains everything you need in order to run a successful PCR reaction (template DNA, TAS2R38 primers, and a standard master mix). Centrifuge your sample for 30 seconds in the adapters to pool reagents and store it on ice until ready to begin the reaction.

The **PCR Master Mix** includes:

- OneTaq® DNA Polymerase
- TAS2R38 specific primers
- dNTPs (A, T, C, G)
- Salts (Tris-HCl, KCl, NH<sub>4</sub>Cl, MgCl<sub>2</sub>) pH 8.9

Remind students of the PCR analogy detailed at the end of this teacher's guide and in the slides. The purpose of salts is for optimal OneTaq® DNA polymerase efficiency.

2. Program your thermocycler by creating a new PCR protocol, following the temperature and time specifications below. Name this protocol "**PTC PCR Program**".

	Step	Temperature	Time
	Initial Denaturation	94°C	300 seconds
Repeat 35 times	Denaturation	94°C	30 seconds
	Annealing	64°C	30 seconds
	Extension	72°C	30 seconds
	Final Extension	72°C	60 seconds

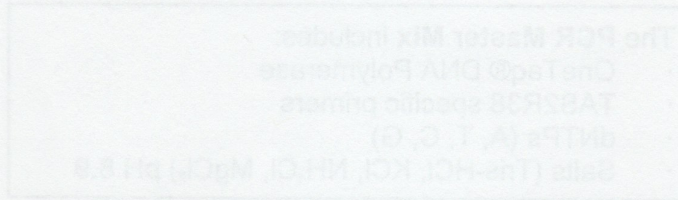
Table 2: PCR Cycle details

3. When the program is finished, allow your teacher to remove your labeled tube from the thermocycler as it will be hot.

Can either program a subsequent 4°C cooling step or remove samples immediately after place the 0.2mL clear tubes on ice.

OPTIONAL STOPPING POINT: Once PCR reactions have finished, can store PCR reactions in fridge at 4°C until ready to proceed or leave them in the thermocycler at 4°C (not recommended for longer than overnight due to wear on machine itself).

4. Store the rest of your samples on ice or at 4°C until ready to begin the next part.



Step	Temperature	Time
Initial Denaturation	94°C	300 seconds
Denaturation	94°C	30 seconds
Repeat 35 times	64°C	30 seconds
Annealing	64°C	30 seconds
Extension	72°C	30 seconds
Final Extension	72°C	60 seconds



1. Using a p10 or p20 micropipette, add 2 $\mu$ L of HaeIII restriction enzyme from the pink tube your teacher handed out into your PCR sample. You will be digesting your entire sample.

As you will be demonstrating the steps for your students, we suggest that you create your own undigested control as well. Make sure to store your own PCR reaction (and **not digest it**) in order to have an undigested control sample for the gel. You will need to do multiple samples for the PCR reaction in order to have a control DNA sample for each gel.

We have tested that 2 $\mu$ L keeps HaeIII in sufficient excess to DNA to ensure full digestion of PCR products.

2. Gently pipette up and down 3-4 times to ensure that the enzyme mixes with your sample.
3. Centrifuge the PCR tubes for 30 seconds in the adapters to pool reagents.

If centrifuging, make sure that clear PCR tubes are placed in adapters. Alternatively, students can tap tubes down to pool reagents at the bottom.

4. Create a program on the thermocycler to hold a temperature of 37°C for 5 minutes. Name this “**PTC Digest**”.

Can optionally program a subsequent heat-inactivation step at 80°C for 20 minutes. This will inactivate the enzyme and definitively prevent further digestion.

OPTIONAL STOPPING POINT: Can store PTC Digest samples in fridge at 4°C until ready to run gel electrophoresis.

5. Place your labeled clear tube back into the thermocycler for digestion. Close the lid run “**PTC Digest**”.

#### IV. Gel Electrophoresis

- Gel loading
  - Prepare gels before part IV. Make sure you add the appropriate amount of SyberSafe® to your molten agarose/buffer mix. For example, if your gel

tray takes 30mL of this molten agarose/buffer mix, you must add 3 $\mu$ L of SyberSafe®. Remember to mix thoroughly before pouring into your gel tray. Cover or keep your gels out of light while they dry.

- You will need one lane per student sample, one lane for undigested control sample, and one lane for ladder in each gel.
  - It is up to you whether students sign themselves up for a lane or you pre-assign each student and keep track of the list. We have included a sample sign-up template on page 23 of this manual.
- Before students load their samples, ensure that you have loaded 10 $\mu$ L of 100bp ladder into the first lane and 20 $\mu$ L of your undigested control in the second lane.

After the restriction digest with HaeIII, the resulting DNA fragments will be separated on an agarose gel using gel electrophoresis.

Gel electrophoresis is a technique that uses an electrical current to separate biomolecules based on size and charge. Since all DNA is negatively charged (due to the phosphate groups in the sugar-phosphate backbone), gel electrophoresis will separate DNA fragments based on size. DNA is loaded into the wells of the agarose gel at the negative end (anode). Electricity is applied and as electrical current passes through the gel, the negatively charged DNA fragments move towards the positive end (cathode). An agarose gel is a porous matrix. Therefore, smaller DNA fragments will be able to move through the pores easier and travel faster. Larger DNA fragments will have more difficulty moving through the gel and therefore travel slower. As the sample progresses through the gel, the fragments sort into distinct bands based on their size.

Since we amplified billion of copies of our TAS2R28 gene in the PCR reaction, we should have sufficient DNA to visualize. The bands of DNA can be visualized by illuminating the gel with the UV light. The gels have been prepared with SyberSafe®, a dye that attaches to DNA and fluoresces under UV light.

1. Add 2 $\mu$ L of colored loading dye to your PCR sample before loading it on the gel.

It is possible that your master mix already included a green loading dye. If it did, you may still want to add additional loading dye to ensure sample settles in well.

2. Your teacher will direct you to the gel sign up sheet. Sign up for a specific lane for which you will load your sample. Your teacher will load the 100bp ladder and the undigested control into the specific lanes that are noted on the signup sheet.

On page 23, you will find an example gel sign up sheet. You may want students to signup in whichever lane they'd like, or you can pre-assign students to specific lanes to avoid confusion. As the teacher, you are responsible for loading the

100bp ladder and the undigested control. Use these samples to model for students how to load into a gel.

3. Load 20 $\mu$ L of your PCR sample into your assigned well. Gently depress the pipette button to the *first stop* to slowly expel the sample without introducing bubbles.

Consult the gel system user manual (or your ABE technician) to find out what the volume range is for the comb you are using. For example, many combs have two size options for the wells they create. You do not want to overflow the well with too much sample.

4. Run the gel as instructed (voltage and time). Depending upon your gel system, the gel will end on its own or you will need to stop the gel and notify your teacher that you have finished.

There are many different types of gel electrophoresis chambers available for teachers to use. It is important to remember that certain lab protocol specifications can change, depending on your gel electrophoresis system. These may include:

- o Volume of sample loaded into a gel (dependent on tray and comb size)
- o Volume of buffer loaded into the chamber (dependent on chamber size)
- o Voltage (some gel boxes give users free range to choose a voltage, others have three pre set voltages to choose from, and finally others do not let the user change the voltage)
- o Time (dependent on the voltage of the machine)

If you are using the Fotodyne or Thermal Fisher gel system, we suggest you run the gel for 30 minutes at 120-130 volts.

We suggest follow their recommendations making sure to not melt the gel (which will happen at too high of voltage) and watching the loading dye to ensure that samples do not run off the gel (which will happen if run at too high of voltage or for too much time).

5. In Table 3 (page 10) you deduced for each genotype whether the alleles present would be cut or uncut and you further noted the fragment sizes. Using this information, for each genotype draw where the resulting fragments would be predicted to run on the gel and draw the bands on Figure 4 (page 13).



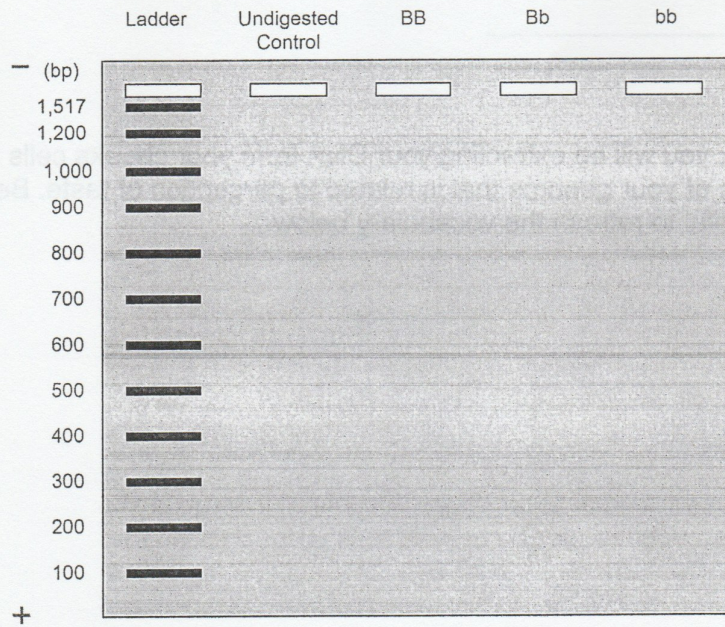


Figure 4: Predicted Gel Results for each tasting genotype

6. After your gel has finished running, you will visualize your bands. Draw your actual results in Figure 5 (page 13) and then compare with classmates for the other corresponding genotypes to complete the figure.

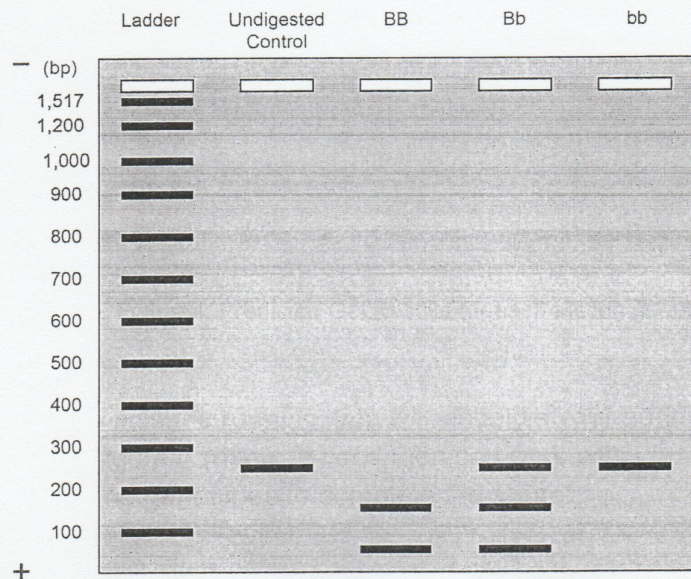


Figure 5: Actual Gel Results for each tasting genotype

## Teacher Resource: Optional Pre-Laboratory Vocab Review

Name: \_\_\_\_\_

Class/Period: \_\_\_\_\_

In the laboratory, you will be extracting your DNA from your cheek cells and analyzing a specific portion of your genome that is related to perception of taste. Before doing this lab, it will be helpful to refresh the vocabulary below.

Phenotype:

Genotype:

DNA:

Allele:

Dominant:

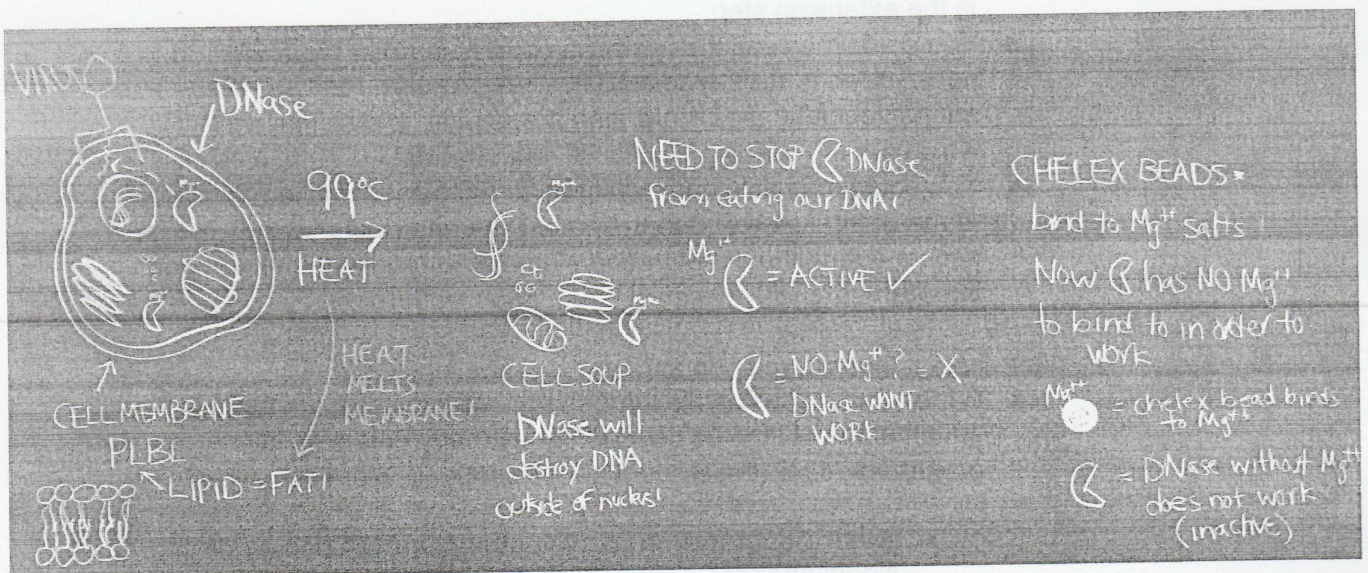
Recessive:

Homozygous:

Heterozygous:

## Teacher Resource: What is Chelex®?

- Chelex is a chelating agent from Bio-Rad that binds transition metal ions such as  $Mg^{2+}$
- Why is it needed? What happens when cells are lysed?
  - When lysing the cells, DNA is released from the nucleus and mitochondria and mixed with the contents of the cytoplasm
  - The cytoplasm contains DNases, which degrade DNA. While we do boil at  $99^{\circ}C$  for 10 minutes, some DNases may still have residual activity.
  - These DNases require  $Mg^{2+}$  for activity
  - By removing  $Mg^{2+}$  using Chelex®, the DNases are prevented from degrading the DNA. This ensures the DNA is kept intact for the subsequent PCR reaction.



## Teacher Resource: PCR

### I. What is PCR?

Polymerase chain reaction (PCR) is used to amplify a specific region of DNA.

- Why is it necessary to amplify DNA?
  - When DNA is isolated all of the DNA is isolated, not just the region of interest. PCR allows us to 1) make sufficient copies of DNA to analyze via gel electrophoresis and 2) copy just our region of interest.
- What happens during the PCR reaction?
  - Students may know that PCR is about copying DNA, point out this is the extension step.
  - You could then ask them what is needed to allow the DNA polymerase to start synthesizing DNA (Answer: Primers, which anneal to the target DNA during the annealing step and dNTPs, which are the building blocks for new strands).
  - You could then ask them what has to happen for primers to anneal to the DNA (Answer: Separation of DNA into single strands, which occurs during the denaturation step due to breakage of hydrogen bonds).
- What does a thermocycler do and how it useful for PCR?
  - A thermocycler will cycle through pre-set temperatures for pre-set amounts of time as needed, for as many cycles as it is programmed for (the amount of DNA doubles with each cycle).
    - 94°C: **Denaturation** (double-stranded DNA denatures into single strands)
    - 64°C: **Annealing** (primers anneal to single-stranded DNA)
    - 72 °C: **Extension** (*Taq* polymerase synthesizes new DNA strands complementary to the existing strands using the primers as starting points)
  - *Taq* is a specific polymerase that has been isolated from a thermophilic (high temperature-loving) organism and thus can withstand high temperatures
  - The discovery of this enzyme was the breakthrough in PCR technology, because it meant a thermocycler could be used and scientists did not have to add new enzyme for each PCR cycle

## II. How do primers work?

- Two primers (one of each strand) will together act like bookmarks to determine which region of DNA will be amplified (the “target DNA”). They themselves are short pieces of DNA that anneal to the beginning and end of the target DNA through complementary base pairing to the forward and reverse strands, respectively.

## III. Guidelines to creating your own PCR-Book Analogy

- Introducing PCR through an analogy can help students understand the overall concept of PCR, as well as the individual components of a PCR reaction.
- PCR allows users to generate lots (over one billion) of copies of a specific target area of DNA. **It’s quite similar to making copies of a specific quote in any book!**
- Let’s take a look at the components necessary to make copies of a specific quote in a book
  - The book
  - The quote that you are interested in copying
  - Post-its showing where the quote starts and where it stops
  - Pen to write out the quote
  - Ink: You need ink in order for your pen to work
  - ABCs: You need the letters of the alphabet
- Let’s take a look at the components of a successful PCR reaction
  - Template DNA
  - Specific region of DNA (“target DNA”) you want to copy
  - Specific primers
  - DNA polymerase
  - Salts
  - dNTPs
- How do these items link together?
  - See our table below
- How should you introduce this analogy to your students?
  - Depending on the level of your students, we recommend that you decide which components to introduce first: either the PCR components or the book analogy components. You can then ask them to deduce how the components are connected.
  - Where to start?
    - a) You may want to write the book-analogy items on the board first (book, Post-its, quote, etcl).
    - b) Students are then asked what biological components correlate to these words.
    - c) It may work best by writing the simple words on the board first rather than writing template DNA, primers, polymerase,

dNTPs, gene or DNA of interest, as your students may not be familiar with these terms

- d) If introducing the book analogy components, students can then utilize the PCR components table in the student manual to begin making connections.

Book Analogy	PCR	Explanation
Book	Template DNA	Extracted from our cheek cells, template DNA is our entire genome, much like the book is the entire story.
Quote	Specific region of DNA	We are interested in analyzing only a small portion of our genome ("target DNA"), much like specific quotes from a book can be analyzed.
Post-its	Specific Primers	We use primers to designate the region we're interested in, much like post-its can be used to mark where a quote ends and begins to quickly find it in a book.
Pen	DNA Polymerase	The DNA polymerase synthesizes or makes new copies of this target region, much like a pen is used to copy down a quote.
Ink	Salts	Salts are used to create the unique and optimal environment for the DNA polymerase to work, much like the ink in a pen. If the pen has no ink, no copying will take place.
ABCs	dNTPs	dNTPs, the building blocks of DNA, are required by DNA polymerase in order to synthesize new strands, much like a pen will depend on letters of the alphabet to put together the words of the quote.

### Teacher Resource: Template Sign-Up Sheet for Gel Loading

Adjust the number of lanes to reflect the gels that you will be using in class. You may want students to sign up in whichever lane they'd like, or you can pre-assign students to specific lanes to avoid confusion. As the teacher, you are responsible for loading the 100bp ladder and the undigested control. Use these samples as demos to remind students how to load into a gel.

#### Gel Loading Scheme for PTC PCR

Class Period: \_\_\_\_\_

Gel Number: \_\_\_\_\_

Lane	1	2	3	4	5	6	7	8	9	10
Initials	LAD	UCS								

Label your the initials on your tube in the diagram above and write your name in the sign-up below.

Lane 1: 100bp DNA Ladder

Lane 2: Undigested Control Sample

Lane 3: \_\_\_\_\_

Lane 4: \_\_\_\_\_

Lane 5: \_\_\_\_\_

Lane 6: \_\_\_\_\_

Lane 7: \_\_\_\_\_

Lane 8: \_\_\_\_\_

Lane 9: \_\_\_\_\_

Lane 10: \_\_\_\_\_

# AMGEN<sup>®</sup> Biotech Experience

## Scientific Discovery for the Classroom

### ABE 2.0: PTC Lab Protocol

## STUDENT GUIDE

#### Overview

In this laboratory you will explore the connection between your genetics (**genotype**) and your observed characteristics (**phenotype**) using taste perception. During a subsequent activity, you will be given an opportunity to taste the compound **phenylthiocarbamide (PTC)**. Upon tasting PTC you will experience one of these three phenotypic reactions: a strong bitter taste, a mild bitter taste, or nothing at all.

The population studies conducted by chemist Arthur L. Fox in the 1930s revealed that the ability to taste PTC is an **inherited dominant trait** that varies in the human population and influences how we taste PTC. The **gene** that encodes for the PTC taste receptor, **TAS2R38**, was identified in 2003. **Taste receptors** are found on the surface of some of the cells on our tongue and we know today that TAS2R38 encodes for one of the thirty taste receptors that allow detection of bitter-tasting compounds.

Metabolizing our foods begins with breaking them down as we chew. **Chemical compounds** within the foods will either **bind or not bind** to taste receptors on the cells of our tongue. If a compound binds to a receptor, this results in **activation** of the receptor, in which a signal is transmitted to our brains, allowing us to perceive taste.

The TAS2R38 gene has a **dominant** and a **recessive** version (**allele**). Each allele encodes a different version of the PTC taste receptor, and differences in perception of taste are based on which alleles an individual carries. The goal of this laboratory is to determine your genotype using multiple molecular biology techniques. These steps are outlined in Figure 1 (page 2) and described in Table 1 (page 3).

First, you will use **Polymerase Chain Reaction (PCR)** to amplify (make many copies of) a short region of the TAS2R38 gene. Figure 3 (page 7) outlines the steps of PCR. In order to differentiate between the TAS2R38 alleles, the amplified PCR product will be digested (cut) with the **restriction enzyme HaeIII**. Restriction enzymes cut at specific DNA sequences, termed **recognition sequences**. The HaeIII recognition sequence will allow us to distinguish the **single-nucleotide polymorphism (SNP)** that differentiates the PCR products of the dominant and recessive TAS2R38 alleles. The PCR product of the dominant allele will be cut by the enzyme, while the PCR product of the recessive allele will not, as shown in Figure 1 (page 2). This means that differently sized fragments will be produced for **homozygous dominant**, **homozygous recessive**, and **heterozygous** individuals. Therefore, after the restriction digest with HaeIII, you will use **gel electrophoresis** to visualize your resulting fragments in order to conclude your genotype.



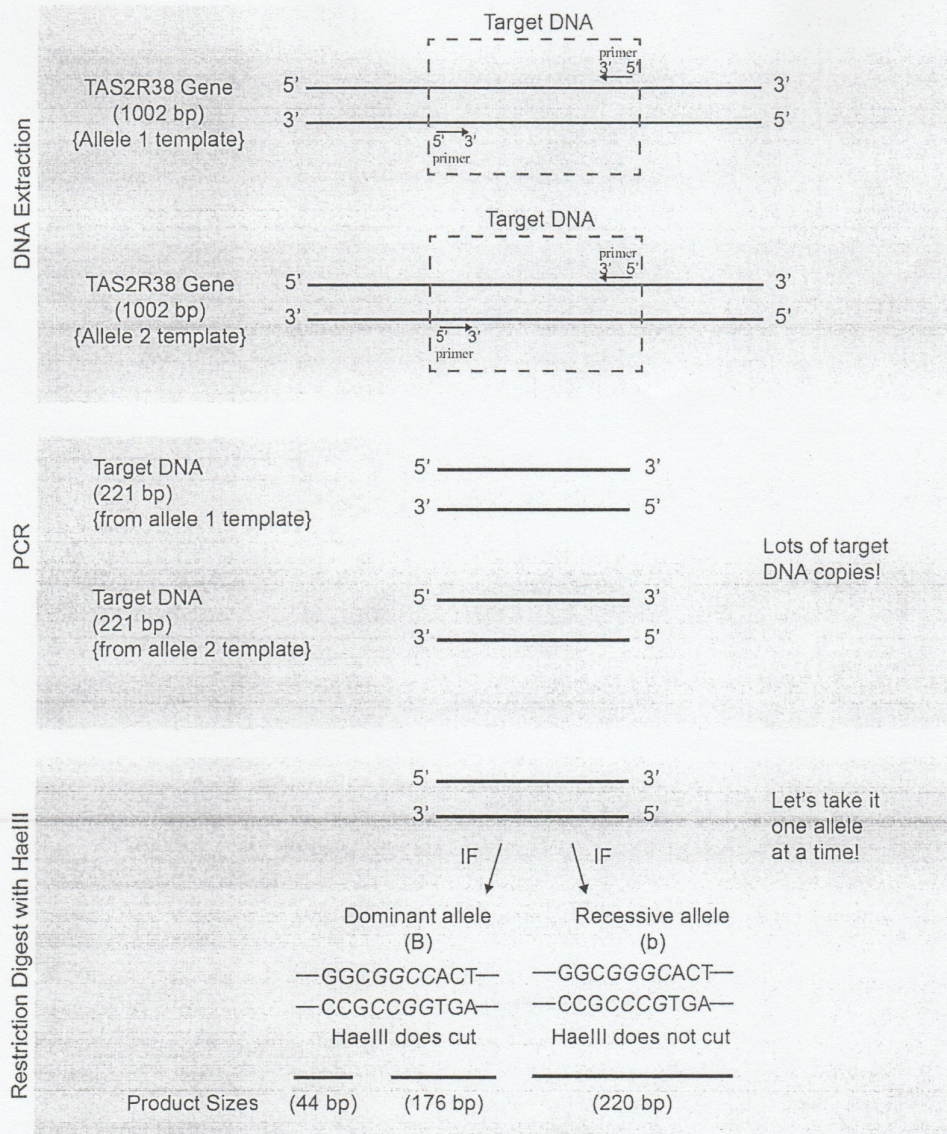


Figure 1: Overview of Laboratory Procedure

Figure 1. In this laboratory procedure, you will first isolate your own DNA from your cheek cells. We are interested in determining your genotype at the TAS2R38 locus. Therefore, we will use primers that have been designed to specifically amplify a portion of the TAS2R38 gene using PCR. Figure 3 (page 7) outlines the three key steps in PCR that allow us to make billions of copies of our target DNA. We need this many copies in order to first perform a restriction digest using HaeIII to differentiate between the dominant and recessive alleles, and then finally to visualize these resulting fragments using gel electrophoresis.

<b>Protocol Step</b>	<b>Starting Material</b>	<b>Ending Material</b>	<b>Purpose</b>
DNA Extraction	Your cheek cells	Your DNA	Obtain your DNA from the nuclei of your cheek cells
Polymerase Chain Reaction	Your DNA	>10 <sup>6</sup> copies of TAS2R38 target DNA (220bp)	Produce lots of copies of TAS2R38 fragment for analysis
Restriction Digest with HaeIII	220bp-length target DNA copies	Allele-specific cut and/or uncut DNA fragments	Differentiate dominant and recessive TAS2R38 alleles using HaeIII
Gel electrophoresis	Allele-specific cut and/or uncut DNA fragments	Differently sized bands on an agarose gel	Visualize cut and/or uncut DNA fragments to determine your genotype

Table 1: Protocol Outline

## Protocol

### I. Collecting and Isolating DNA from Your Cheek Cells

You will be extracting DNA found in the nuclei of your cheek cells. These cells can be easily collected by gently swabbing the inside of your mouth with a toothpick. You will transfer your cheek cells by twirling the toothpick in a tube containing Chelex® beads. Chelex® is a **chelating** (binding) agent that is often used for DNA extraction. In order to isolate your DNA, we must break down the **cellular and nuclear membranes**. We will do this by boiling your cheek cell samples. Once these membranes are broken down, components normally in the cytoplasm have access to the DNA. These components include enzymes called **DNases** that break down DNA. DNases require metal co-factors for activity. The Chelex® resin will bind these metal co-factors away from the DNases, thereby **inhibiting** DNase activity.

1. You will receive 100 $\mu$ L of Chelex® beads in a small, green 0.2mL tube. Clearly label the top and side of this tube with your initials using a permanent waterproof marker. You labeled your tube as: \_\_\_\_\_
2. Using the broad or flat end of a sterile toothpick, gently swab the inside of your mouth for at least 30 seconds.

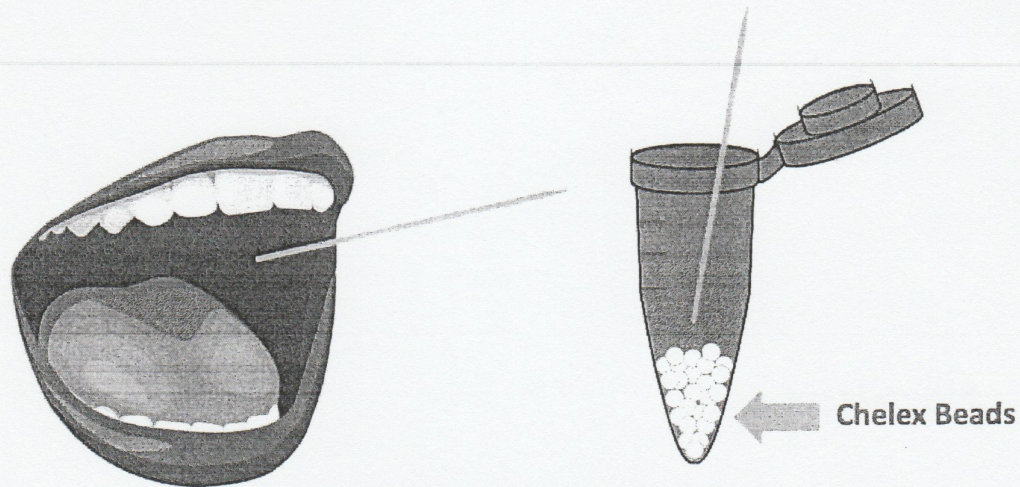


Figure 2: DNA collection and extraction procedure

3. Swirl the toothpick in the tube of the Chelex® beads for at least 30 seconds to dislodge the cells and cap the tube tightly. Place used toothpick in waste container.

4. Create a program on the thermocycler to hold a temperature of 99°C for 10 minutes. Name this "**DNA Extraction Heat Block**".
5. Place your 0.2mL green tube into the thermocycler. Close the lid and run "**DNA Extraction Heat Block**".
6. Once the **DNA Extraction Heat Block** protocol is finished, allow your teacher to remove your tubes from the thermocycler carefully so that the Chelex® beads at the bottom are not disturbed.

## II. Amplifying PTC DNA by PCR

In human cells, DNA replication results in two identical copies of the entire human genome. PCR is a molecular biology lab technique that allows us to replicate a small portion of DNA that we are interested in (the “target DNA”). As you may imagine, it would be terribly difficult to find and work with only two tiny fragments of DNA isolated from one individual’s DNA. Instead, we can use PCR to locate, isolate, and make (“*amplify*”) millions of copies of a gene from one person. In this case, you will use PCR to isolate and amplify your own PTC gene.

### A PCR reaction contains:

- Source DNA from one person (from your cheek cells!)
  - Provides a template for DNA polymerase to read
- *Taq* DNA polymerase
  - Bacterial enzyme that allows DNA replication at high temperatures
- Primers
  - Molecular “post-it notes” that define target DNA
    - One per each strand of DNA (forward and reverse)
  - Short DNA fragments (18-22 base pairs) that we design
  - Bind to target DNA through complementary base pairing
- dNTPs
  - Deoxynucleotide building blocks (A, T, C, G) used to make new strands of DNA
- Salts and buffers
  - Ensure correct chemical conditions for reaction
  - Includes  $MgCl_2$  which is a co-factor required for *Taq* DNA polymerase activity

### Steps of a PCR reaction (refer to Figure 3, page 7 for diagrams of each step)

The “chain” reaction of PCR is composed of three steps: denaturation, annealing and extension.

1. Denaturation (94°C)
  - Hydrogen bonds that hold DNA together are broken, therefore DNA is separated (“denatured”) into single strands
2. Annealing (64°C)
  - Primers bind to single strands of template DNA
3. Extension (72°C)
  - *Taq* DNA polymerase creates a new strand of DNA starting at the end of each primer

These three steps constitute one synthesis cycle during which the number of copies of DNA doubles. A reaction with 35 cycles will therefore produce over a billion copies.

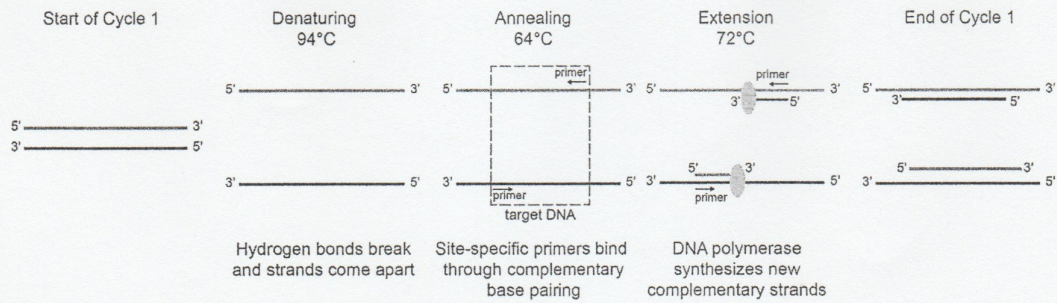
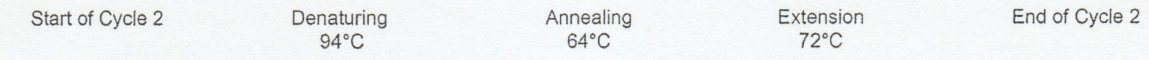


Figure 3: Steps of a PCR reaction

At the end of the first cycle of PCR you will have 4 strands (2 original template strands, 2 newly synthesized strands). Draw out the steps that occur during the second cycle below. How many strands will you have at the end of cycle 2?



**Stop and Think**

At which cycle of the PCR reaction will you have amplified a fragment of **just** the target sequence?

- Carefully pipette 2.5 $\mu$ L of the supernatant, which contains your DNA, to the 0.2mL clear PCR tube that already contains the **TAS2R38 Primer Master Mix**. Label the tube with an asterisk\* and your initials. This tube now contains everything you need in order to run a successful PCR reaction (template DNA, TAS2R38 primers, and a standard master mix). Centrifuge your sample for 30 seconds in the adapters to pool reagents and store it on ice until ready to begin the reaction.

The **PCR Master Mix** includes:

- OneTaq<sup>®</sup> DNA Polymerase
- TAS2R38 specific primers
- dNTPs (A, T, C, G)
- Salts (Tris-HCl, KCl, NH<sub>4</sub>Cl, MgCl<sub>2</sub>) pH 8.9

- Program your thermocycler by creating a new PCR protocol, following the temperature and time specifications below. Name this protocol "**PTC PCR Program**".

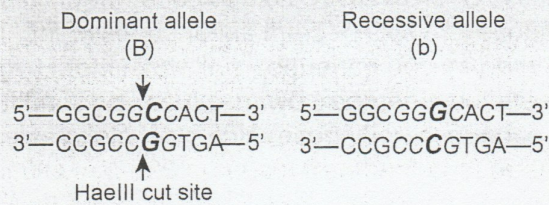
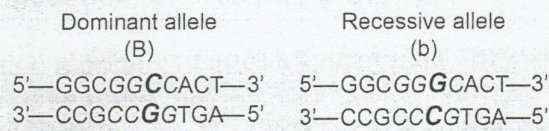
	Step	Temperature	Time
	Initial Denaturation	94°C	300 seconds
	Denaturation	94°C	30 seconds
Repeat 35 times	Annealing	64°C	30 seconds
	Extension	72°C	30 seconds
	Final Extension	72°C	60 seconds

Table 2: PCR Cycle details

- When the program is finished, allow your teacher to remove your labeled tube from the thermocycler as it will be hot.
- Store your samples on ice or at 4°C until ready to begin the next part.

### III. Restriction digest of PCR products with HaeIII

You have now amplified a portion of the TAS2R38 gene. PCR amplification will result in a DNA fragment 220 **base pairs** (bp) long. Recall that the TAS2R38 gene has a **dominant** and a **recessive** version, and that the two **alleles** can be differentiated by a single-nucleotide polymorphism (SNP). In order to differentiate between a dominant and a recessive allele, we will perform a **restriction digest** on the PCR products using the HaeIII restriction enzyme. **Restriction enzymes** cut at specific DNA sequences termed recognition sequences. HaeIII recognizes a **5' GGCC 3'** DNA sequence (refer to Figure 1, page 2). The dominant allele contains the 5' GGCC 3' recognition sequence and therefore will be cut by HaeIII. Note this sequence occurs on both strands when read from the 5' to 3' direction. This results in two fragments post restriction digest: 176 bp and 44 bp. The recessive allele lacks this recognition sequence and therefore will not be cut by HaeIII. This results in a 220 bp uncut fragment post restriction digest.



Based on this information, fill out Table 3 below and compare with your classmates.

Genotype	Allele 1: cut, uncut?	Allele 2: cut, uncut?	DNA fragment sizes
BB			
Bb			
bb			

Table 3: Results of Restriction digest with HaeIII



1. Using a p10 or p20 micropipette, add 2 $\mu$ L of HaeIII restriction enzyme from the pink tube your teacher handed out into your PCR sample. You will be digesting your entire sample.
2. Gently pipette up and down 3-4 times to ensure that the enzyme mixes with your sample.
3. Centrifuge the PCR tubes for 30 seconds in the adapters to pool reagents.
4. Create a program on the thermocycler to hold a temperature of 37°C for 5 minutes. Name this "**PTC Digest**".
5. Place your labeled clear tube back into the thermocycler for digestion. Close the lid run "**PTC Digest**".

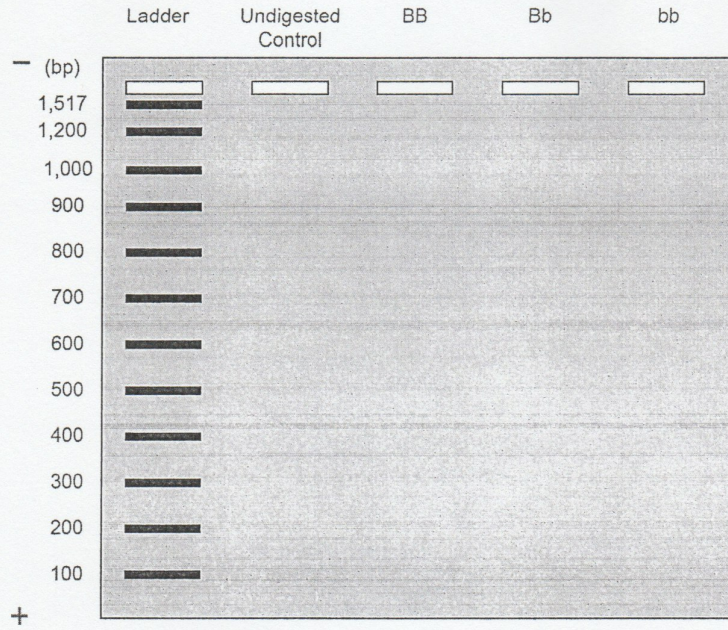
#### IV. Gel Electrophoresis

After the restriction digest with HaeIII, the resulting DNA fragments will be separated on an agarose gel using gel electrophoresis.

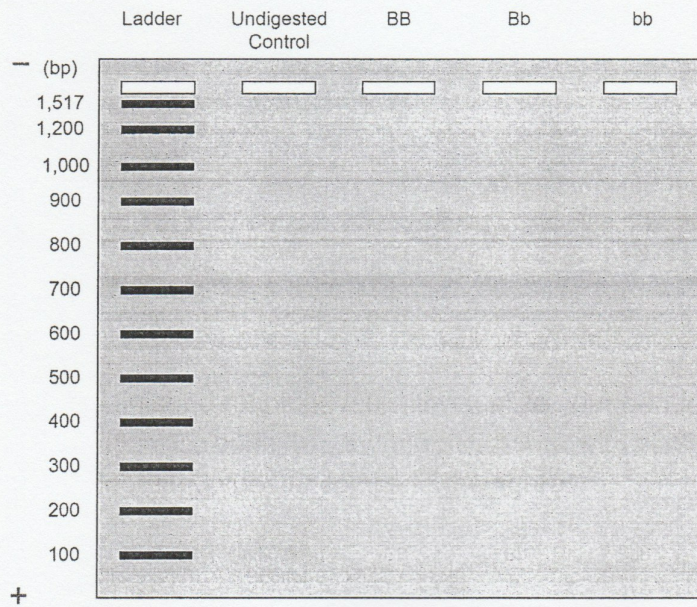
Gel electrophoresis is a technique that uses an electrical current to separate biomolecules based on size and charge. Since all DNA is negatively charged (due to the phosphate groups in the sugar-phosphate backbone), gel electrophoresis will separate DNA fragments based on size. DNA is loaded into the wells of the agarose gel at the negative end (anode). Electricity is applied and as electrical current passes through the gel, the negatively charged DNA fragments move towards the positive end (cathode). An agarose gel is a porous matrix. Therefore, smaller DNA fragments will be able to move through the pores easier and travel faster. Larger DNA fragments will have more difficulty moving through the gel and therefore travel slower. As the sample progresses through the gel, the fragments sort into distinct bands based on their size.

Since we amplified billion of copies of our TAS2R28 gene in the PCR reaction, we should have sufficient DNA to visualize. The bands of DNA can be visualized by illuminating the gel with blue light. The gels have been prepared with SyberSafe®, a dye that attaches to DNA and fluoresces under UV light.

1. Add 2 $\mu$ L of colored loading dye to your PCR sample before loading it on the gel.
2. Your teacher will direct you to the gel sign up sheet. Sign up for a specific lane for which you will load your sample. Your teacher will load the 100bp ladder and the undigested control into the specific lanes that are noted on the signup sheet.
3. Load 20 $\mu$ L per your teacher's instructions of your PCR sample into your assigned well. Gently depress the pipette button to the *first stop* to slowly expel the sample without introducing bubbles.
4. Run the gel as instructed (voltage and time). Depending upon your gel system, the gel will end on its own or you will need to stop the gel and notify your teacher that you have finished.
5. In Table 3 (page 9) you deduced for each genotype whether the alleles present would be cut or uncut and you further noted the fragment sizes. Using this information, for each genotype draw where the resulting fragments would be predicted to run on the gel and draw the bands on Figure 4 (page 12).



6. After your gel has finished running, you will visualize your bands. Draw your actual results in Figure 5 (page 13) and then compare with classmates for the other corresponding genotypes to complete the figure.



# PTC PCR FLOW CHART

## I. ISOLATE DNA

**SCRAPE** inside of cheek cells with flat end of toothpick for 30 sec



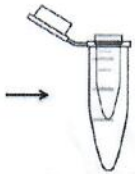
Directly place tooth pick with cheek cells into chelex; mix for 30 sec



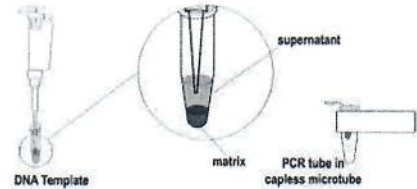
**Boil:** 99° C for 10min in thermal cycler.



**\*CENTRIFUGE;** Full speed; 90 sec  
Use capless microtube adapters



Keep tube on **ICE**; DNA in supernatant; if not proceeding to Part II. Aliquot supernatant and refrigerate

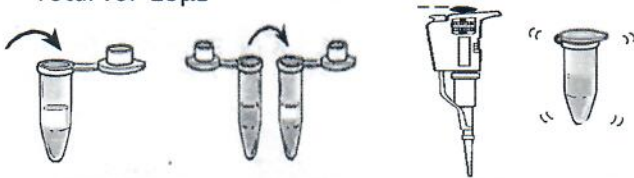


## II. AMPLIFY DNA BY PCR - in PCR tube (keep on ice)

Add **22.5 µL** of master mix (MM) to labeled (your initials) clear PCR tube. Pipet **2.5µL** of your DNA in the supernatant to this tube with the master mix in it. **Mix with Pipet.** Total vol=25µL

**AMPLIFY** in thermal cycler

*PCR will run through 35cycles cycle of denaturation, annealing and extension. (~ 1hr 25 min). Refrigerate for next day.*



Add **2µL** Hae III to tube from thermal cycler

## III. Restriction Digest with HAEIII

Incubate b@37°C for 5 min in thermal cycler.

**MIX**

Add **2µL** of Loading Dye (LD) to your sample. Mix with pipet.



## IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

**POUR** gel (3% agarose with 1;10,000 dilution of Sybersafe dye in 1X Sodium Borate buffer)

**Load** 20 µL of your sample into the gel (1 sample per well) Include 10 µL of 100bp DNA ladder and 20µL undigested control.)

Let gel set

Run 120-130 V for 30 min

