Biology 196 Laboratory
Using Polymerase Chain Reaction to Detect Bitter-Tasting

Laboratory Objectives

After completing this lab you should be able to:

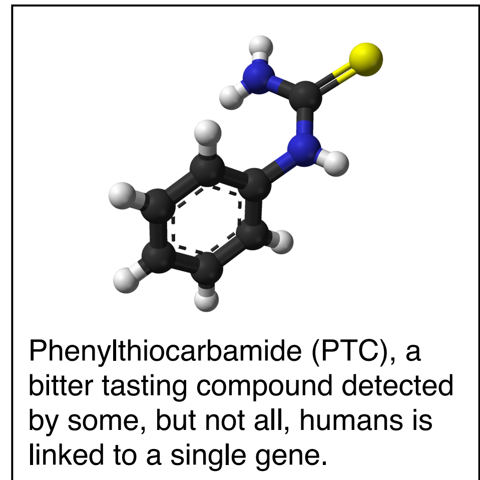
- Define single-nucleotide-polymorphisms and explain their relationship to the phenotype of bitter tasting.
- Describe the PCR mechanism and its components.
- Describe the mechanism of DNA extraction from human cheek cells.
- Define restriction endonuclease function.
- Utilize restriction endonucleases and gel electrophoresis.
- Make bioinformatic predictions from phenotypes.

Bitter Tasting Genetics[1]

The tongue is covered with small bumps (papillae) that have protein **receptors** on them. If a molecule binds tightly to the receptor it can signal its presence to the brain as one of the five essential tastes (sweet, bitter, salty, sour, and umami) . In humans the base of the tongue contains many receptor proteins for bitter compounds. A powerful bitter taste is often an evolved response to toxic substances. Some plants produce toxic alkaloids to deter predators.

> What would you say is your response to common bitter tasting compounds (e.g. coffee, tobacco, spinach, etc.)? Very powerful, moderate or very weak?
>
> Think about how natural selection would effect the presence of these “taster genes”.

Like all other proteins, the protein receptors are the product of **gene expression**, and differences in the genes can exist within a population of individuals. In the early 1930s, Arthur Fox, a researcher, noticed a colleague complaining about the bitter taste of **phenylthiocarbamide (PTC)**, while he could taste nothing at all. Geneticists determined that there is an inherited **gene** (*TAS2R38*) that controls the tasting of PTC. The shape of the tasting receptor (dictated by the gene sequence) determines how well it binds to PTC, and therefore whether a bitter taste is detected.



Phenylthiocarbamide (PTC), a bitter tasting compound detected by some, but not all, humans is linked to a single gene.

There are several variations of the gene and, since humans have two copies of every gene, different combinations of the gene determine whether an individual detects a powerful bitter taste, slightly bitter, or no taste at all. Two of the most common variations of *TAS2R38* differ at only one nucleotide, referred to as a **single-nucleotide polymorphism (SNP)**. We will be isolating some DNA from your cheek cells to determine your **genotype** by **PCR**, **restriction endonuclease** digestion, and **gel electrophoresis**. We will also be tasting papers coated with small amounts of PTC to analyze the relationship between your genotype and your **phenotype**.

[1] Phenylthiocarbamide: A 75-Year Adventure in Genetics and Natural Selection. *Genetics* April 2006 vol.172 no.4 pp. 2015-2023

Procedure (Week #1):

Part 1- Isolation of cheek cell DNA[2]:

Supplies needed at your workstation	
Paper cup	Microcentrifuge
0.9% saline solution, 10 mL	Boiling water bath
10% Chelex®, 100 µL	Crushed ice
Micropipets and tips (10–1000 µL)	Vortexer (optional)
1.5-mL microcentrifuge tubes	
Microcentrifuge tube rack	

1. Use a permanent marker to label a 1.5-mL tube and paper cup with your name or initials.
2. Pour saline solution into your mouth, and vigorously rinse your cheek pockets for 30 seconds.
3. Expel saline solution into the paper cup.
4. Swirl the cup gently to mix cells that may have settled to the bottom. Use a micropipet with a fresh tip to transfer 1000 µL of the solution into your labeled 1.5-mL microcentrifuge tube.
5. Place your sample tube, along with other student samples, in a balanced configuration in a microcentrifuge, and spin for 90 seconds at full speed.
6. Carefully pour off supernatant into the paper cup. Try to remove most of the supernatant, but be careful not to disturb the cell pellet at the bottom of the tube. (The remaining volume will reach approximately the 0.1 mark of a graduated tube.)
7. Set a micropipet to 30 µL. Resuspend cells in the remaining saline by pipetting up and down. Work carefully to minimize bubbles.
8. Withdraw 30 µL of cell suspension, and add it to a PCR tube containing 100 µL of **Chelex®**. Label the cap and side of the tube with your initials.
9. Place your PCR tube, along with other student samples, in a boiling water bath.

   Boiling step: 99°C 10 minutes

10. After boiling, vigorously shake or vortex the PCR tube for 5 seconds.
11. Place your tube, along with other student samples, in a balanced configuration in a microcentrifuge, and spin for 90 seconds at full speed.
12. Use a micropipet with a fresh tip to transfer 30 µL of the clear supernatant into a clean 1.5-mL tube. Be careful to avoid pipetting any cell debris and Chelex® beads.
13. Label the cap and side of the tube with your initials. This sample will be used for setting up one or more PCR reactions.
14. Store your sample on ice or at –20°C until you are ready to continue with Part II.

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[2] Copyright © 2006, Dolan DNA Learning Center, Cold Spring Harbor Laboratory.

Part 2- Amplify DNA by PCR:

Supplies needed at your workstation
Cheek cell DNA, 2.5 µL (from Part I)
PTC primer/loading dye mix, 22.5 µL
Ready-To-Go™ PCR beads (in 0.2-mL PCR tube)
Micropipets and tips (10–1000 µL)
Crushed ice

1. Obtain a PCR tube containing a Ready-To-Go™ PCR Bead. Label with your initials on both the side and top.
2. Use a micropipet with a fresh tip to add 22.5 µL of PTC primer/loading dye mix to the tube. Allow the bead to dissolve for a minute or so.
3. Use a micropipet with a fresh tip to add 2.5 µL of your cheek cell DNA (from Part I) *directly into* the primer/loading dye mix. Ensure that no cheek cell DNA remains in the tip after pipeting.
4. Place your PCR tube, along with other student samples, in a thermal cycler that has been programmed to the following profile for 30 cycles. Your instructor will run the thermal cycler.

Denaturing step: 94°C	30 seconds
Annealing step: 64°C	45 seconds
Extending step: 72°C	45 seconds

The end of week #1

Procedure (Week #2):

Part 3- Digest PCR Products with *HaeIII*

Supplies needed at your workstation
PCR product (from Part 2), 25 µL
Restriction enzyme *HaeIII*
1.5-mL microcentrifuge tubes
Micropipets and tips (10–1000 µL)

1. Label a 1.5-mL tube with your initials and with a “U” (undigested). This will be a control reaction.
2. Use a micropipet with a fresh tip to transfer 10 µL of your PCR product to the “U” tube. Store this sample on ice until you are ready to begin Part IV.
3. Use a micropipet with a fresh tip to add 1 µL of **restriction enzyme** *HaeIII* directly into the PCR product remaining in the PCR tube. Label this tube with a “D” (digested). Use your pipet tip to gently mix the solution.
4. Place your PCR tube, along with the other student samples, back in the **thermocylcer** that has been programmed to maintain 37°C. Let the reaction proceed for 30 minutes.

While your PCR samples are incubating perform the PTC tasting exercise (Part 4)

5. When your reaction has completed, store your sample on ice or in the freezer until you are ready to begin Part 5.

Part 4- PTC Tasting Phenotype and Genotype Predictions:

Supplies needed at your workstation
Control test strips
PTC strips

1. Place one strip of control taste paper in the center of your tongue for several seconds. Note the taste.

2. Remove the control paper, and place one strip of PTC taste paper in the center of your tongue for several seconds. How would you describe the taste of the PTC paper, as compared to the control: A) strongly bitter, B) weakly bitter, or C) no taste other than paper (same as the control test paper)? Record your observations:

   ____

   What do you predict your genotype will be based upon your phenotype, homozygous dominant taster (TT), heterozygous taster (Tt), or homozygous recessive non taster (tt)? Record your prediction below:

   ____

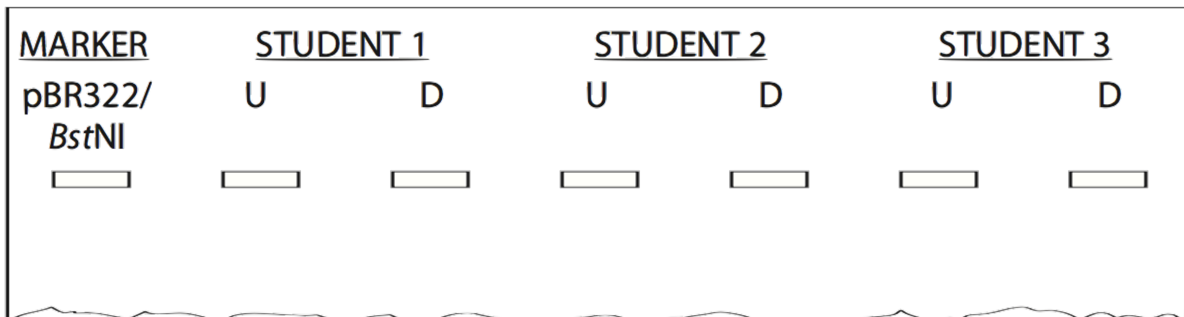
Part 5- Separate PCR Products by Gel Electrophoresis

Supplies needed at your workstation
Undigested PCR product from part 3
HaeIII digested PCR product, 16 μl
1.5-mL microcentrifuge tubes
Micropipets and tips (10–1000 µL)

1. Your instructor will place an **electrophoresis gel** into the gel chamber and cover it with buffer.

2. You or another student volunteer needs to load 20 µL of pBR322/BstNI size markers into the far left lane of the gel.

3. Use a micropipet with a fresh tip to add 10 µL of the undigested (U) and 16 µL of the digested (D) sample/ loading dye mixture into different wells of a 2% agarose gel, according to the diagram below



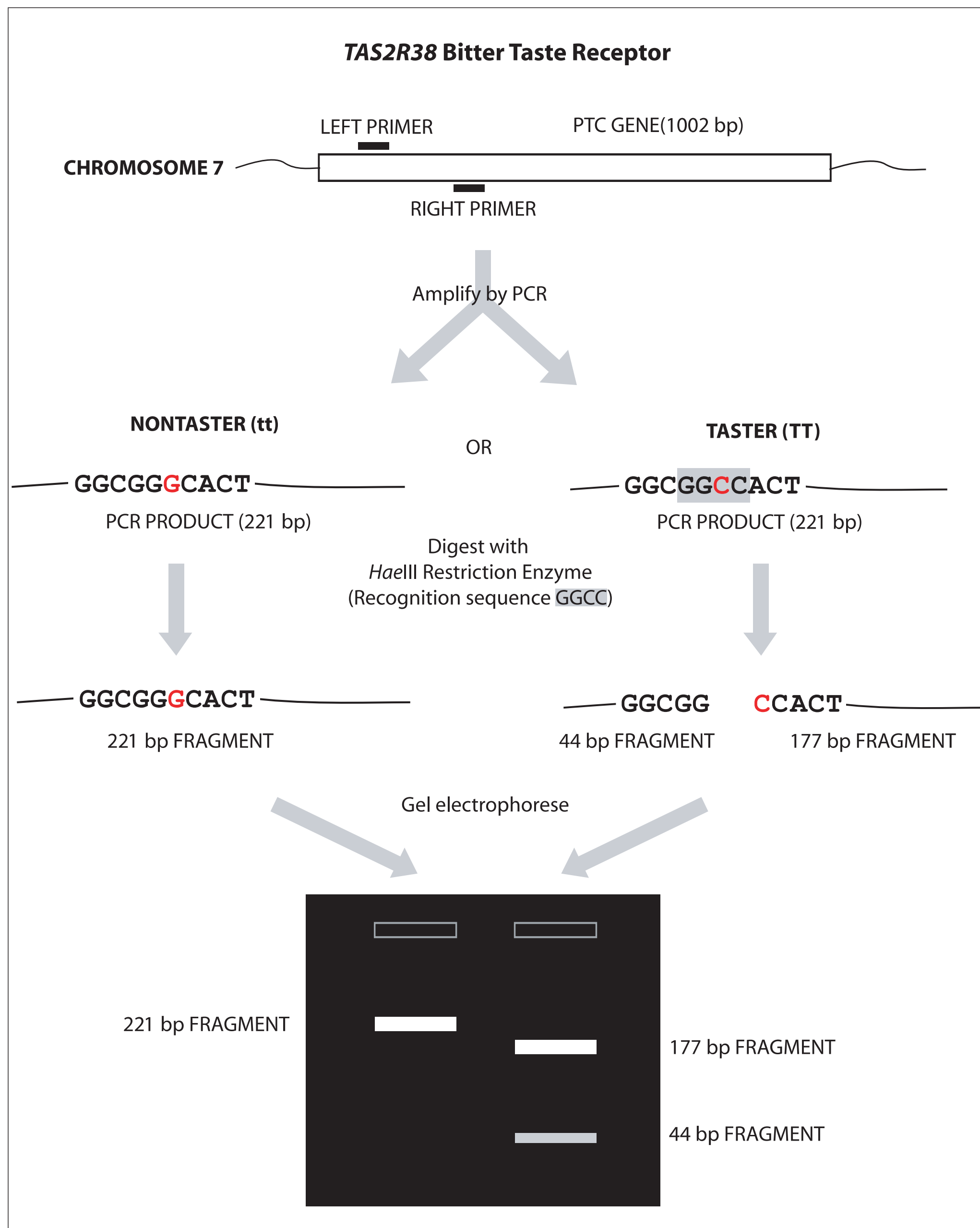
4. Run the gel at 130 V for approximately 30 minutes. Adequate separation will have occurred when the cresol red dye front has moved at least 50 mm from the wells.

While the gel is running perform the technique of DNA extraction from the banana.

5. View the gel using transillumination, and photograph it using a digital camera or cell phone camera.

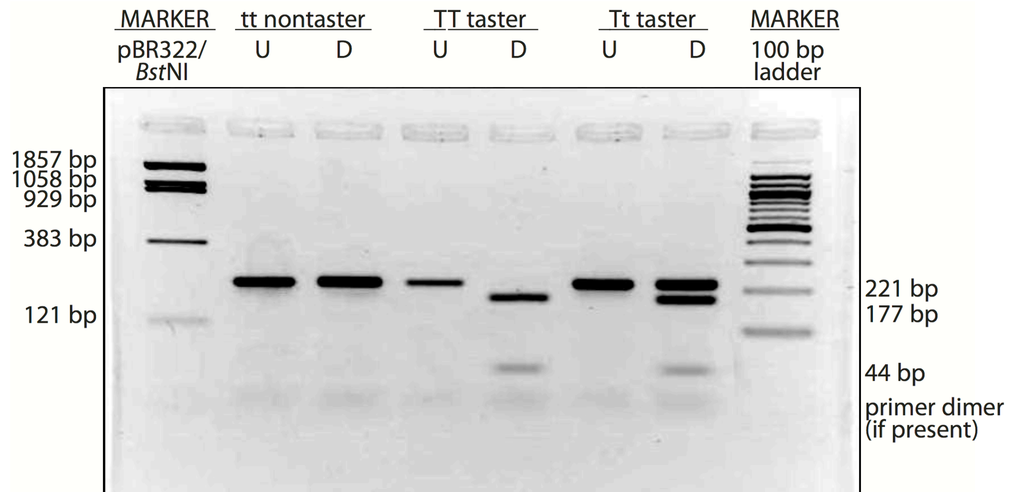
Part 6- Analysis of PTC Genotype

The restriction endonuclease *HaeIII* cuts DNA at the sequence of "GGCC" only. Look at the diagram below. For each of the two homologous chromosome VII molecules you carry there is a chance that they will contain the *taster* allele for PTC. This sequence has the *HaeIII* recognition sequence, which will cut the PCR fragment into two pieces (177 nucleotides and 44 nucleotides). Alternatively, one or both may contain the *nontaster* polymorphism, which does not have the cut sequence. In this case you would see only one PCR fragment at 221 nucleotides.



1. Look at your gel and locate the lane containing the undigested PCR product (U). There should be one prominent band in this lane. Compare the migration of the undigested PCR product in this lane with that of the 383-bp and 121-bp bands in the pBR322/BstNI lane. Confirm that the undigested PCR product corresponds with a size of about 221 bp.

   An example of a gel is shown below.



2. To “score” your alleles, compare your digested PCR product (D) with the uncut control. You will be one of three genotypes:

   tt nontaster (homozygous recessive) shows a single band in the same position as the uncut control.

   TT taster (homozygous dominant) shows two bands of 177 bp and 44 bp. The 177-bp band migrates just above of the uncut control; the 44-bp band may be faint. (Incomplete digestion may leave a small amount of uncut product at the 221-bp position, but this band should be clearly fainter than the 177-bp band.)

   Tt taster (heterozygous) shows three bands that represent both alleles—221 bp, 177 bp, and 44 bp. The 221-bp band must be stronger than the 177-bp band. (If the 221-bp band is fainter, it is an incomplete digest of TT.)

   Your instructor will draw a table on the board in the front of the class similar to the one below. Look at the table below and figure out where you fit. Place a mark on the board in the appropriate box.

   When all the students have finished, fill in the table below with the numbers from your class.

	Phenotype		
Genotype	Strong taster	Weak taster	Nontaster
TT (homozygous)			
Tt (heterozygous)			
tt (homozygous)			

Questions to consider:

A. According to your class results, how well does *TAS2R38* genotype predict PTC-tasting phenotype? What does this tell you about classical dominant/recessive inheritance?

B. How does the *HaeIII* enzyme discriminate between the C-G polymorphism in the *TAS2R38* gene?

C Research the terms **synonymous** and **nonsynonymous mutation**. Which sort of mutation is the G-C polymorphism in the *TAS2R38* gene? By what mechanism does this influence bitter taste perception?

Study Checklist

To perform well on the first quiz, you need to have a thorough knowledge of the following:

- You should know and understand all of the terms which appear in **boldface** type.
- Understand the function of each of the steps in the cheek cell DNA extraction (i.e. Chelex® beads, centrifugation, boiling).
- Define what a restriction endonuclease is and how they are used as a research tool.
- Describe the purpose of each of the components of the PCR reaction (i.e. PTC primer/loading dye mix, Ready-To-Go™ PCR beads, cheek cell DNA, etc.).
- Describe what is happening at each of the three steps of a typical PCR cycle.
- Determine the genotype of the PTC gene from an electrophoresis gel.
- Describe the relationship between genotype and phenotype of the PTC gene for your class.