

## STUDENT LAB INSTRUCTIONS

### INTRODUCTION

Mammals are believed to distinguish only five basic tastes: sweet, sour, bitter, salty, and umami (the taste of monosodium glutamate). Taste recognition is mediated by specialized taste cells that communicate with several brain regions through direct connections to sensory neurons. Taste perception is a two-step process. First, a taste molecule binds to a specific receptor on the surface of a taste cell. Then, the taste cell generates a nervous impulse, which is interpreted by the brain. For example, stimulation of “sweet cells” generates a perception of sweetness in the brain. Recent research has shown that taste sensation ultimately is determined by the wiring of a taste cell to the cortex, rather than the type of molecule bound by a receptor. So, for example, if a bitter taste receptor is expressed on the surface of a “sweet cell,” a bitter molecule is perceived as tasting sweet.

A serendipitous observation at DuPont, in the early 1930s, first showed a genetic basis to taste. Arthur Fox had synthesized some phenylthiocarbamide (PTC), and some of the PTC dust escaped into the air as he was transferring it into a bottle. Lab-mate C.R. Noller complained that the dust had a bitter taste, but Fox tasted nothing—even when he directly sampled the crystals. Subsequent studies by Albert Blakeslee, at the Carnegie Department of Genetics (the forerunner of Cold Spring Harbor Laboratory), showed that the inability to taste PTC is a recessive trait that varies in the human population.



Albert Blakeslee using a voting machine to tabulate results of taste tests at the AAAS Convention, 1938. (Courtesy Cold Spring Harbor Laboratory Research Archives)

Bitter-tasting compounds are recognized by receptor proteins on the surface of taste cells. There are approximately 30 genes for different bitter taste receptors in mammals. The gene for the PTC taste receptor, *TAS2R38*, was identified in 2003. Sequencing identified three nucleotide

positions that vary within the human population—each variable position is termed a single nucleotide polymorphism (SNP). One specific combination of the three SNPs, termed a haplotype, correlates most strongly with tasting ability.

Analogous changes in other cell-surface molecules influence the activity of many drugs. For example, SNPs in serotonin transporter and receptor genes predict adverse responses to anti-depression drugs, including PROZAC® and Paxil®.

In this experiment, a sample of human cells is obtained by saline mouthwash. DNA is extracted by boiling with Chelex resin, which binds contaminating metal ions. Polymerase chain reaction (PCR) is then used to amplify a short region of the *TAS2R38* gene. The amplified PCR product is digested with the restriction enzyme *HaeIII*, whose recognition sequence includes one of the SNPs. One allele is cut by the enzyme, and one is not—producing a restriction fragment length polymorphism (RFLP) that can be separated on a 2% agarose gel.

Each student scores his or her genotype, predicts their tasting ability, and then tastes PTC paper. Class results show how well PTC tasting actually conforms to classical Mendelian inheritance, and illustrates the modern concept of pharmacogenetics—where a SNP genotype is used to predict drug response.

Blakeslee, A.F. (1932). Genetics of Sensory Thresholds: Taste for Phenyl Thio Carbamide. *Proc. Natl. Acad. Sci. U.S.A.* **18**:120-130.

Fox, A.L. (1932). The Relationship Between Chemical Constitution and Taste. *Proc. Natl. Acad. Sci. U.S.A.* **18**:115-120.

Kim, U., Jorgenson, E., Coon, H., Leppert, M., Risch, N., and Drayna, D. (2003). Positional Cloning of the Human Quantitative Trait Locus Underlying Taste Sensitivity to Phenylthiocarbamide. *Science* **299**:1221-1225.

Mueller, K.L., Hoon, M.A., Erlenbach, I., Chandrashekar, J., Zuker, C.S., and Ryba, N.J.P. (2005). The Receptors and Coding Logic for Bitter Taste. *Nature* **434**:225-229.

Scott, K. (2004). The Sweet and the Bitter of Mammalian Taste. *Current Opin. Neurobiol.* **14**:423-427.

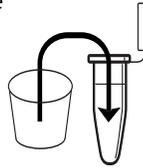
**LAB FLOW**

**I. ISOLATE DNA BY SALINE MOUTHWASH**

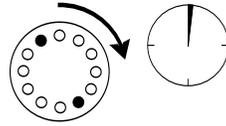
RINSE  
mouth  
with  
saline



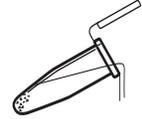
TRANSFER  
saline



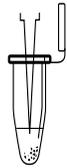
CENTRIFUGE



POUR OFF  
supernatant



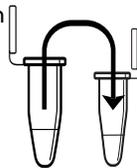
RESUSPEND



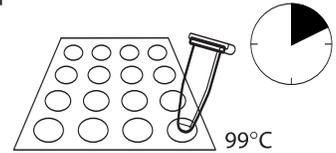
ADD  
Chelex



TRANSFER  
cell  
suspension



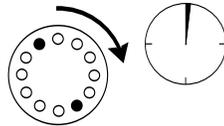
BOIL  
in thermal  
cycler



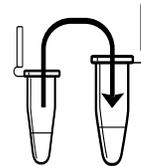
SHAKE  
vigorously



CENTRIFUGE



TRANSFER  
supernatant

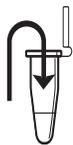


STORE  
on ice



**II. AMPLIFY DNA BY PCR**

ADD  
primer/  
loading  
dye mix



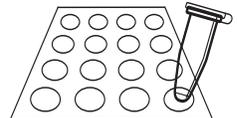
ADD  
DNA



ADD  
mineral oil  
(if necessary)

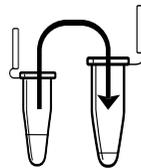


AMPLIFY  
in thermal  
cycler



**III. DIGEST PCR PRODUCTS WITH *HaeIII***

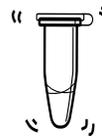
TRANSFER  
PCR  
product



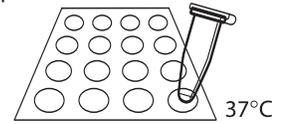
ADD  
*HaeIII*



MIX

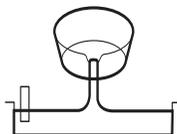


INCUBATE  
in thermal  
cycler



**IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS**

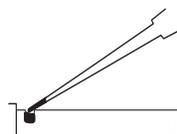
POUR gel



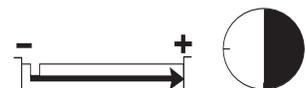
SET



LOAD gel



ELECTROPHORESE  
130V



## METHODS

### I. ISOLATE DNA BY SALINE MOUTHWASH

Reagents (at each student station)	Supplies and Equipment
0.9% saline solution, 10 mL	Permanent marker
10% Chelex®, 100 µL (in 0.2- or 0.5-mL PCR tube)	Paper cup
	Micropipets and tips (10–1000 µL)
	1.5-mL microcentrifuge tubes
	Microcentrifuge tube rack
	Microcentrifuge adapters
	Microcentrifuge
	Thermal cycler (or water bath or heat block)
	Container with cracked or crushed ice
	Vortexer (optional)

1. Use a permanent marker to label a 1.5-mL tube and paper cup with your assigned number.
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 in and out. 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 assigned number.
9. Place your PCR tube, along with other student samples, in a thermal cycler that has been programmed for one cycle of the following profile. The profile may be linked to a 4°C hold program. If you are using a 1.5-mL tube, use a heat block or boiling water bath.  
  
Boiling step:      99°C    10 minutes
10. After boiling, vigorously shake the PCR tube for 5 seconds.

Your teacher may instruct you to collect a small sample of cells to observe under a microscope.

Before pouring off supernatant, check to see that pellet is firmly attached to tube. If pellet is loose or unconsolidated, carefully use micropipet to remove as much saline solution as possible.

Food particles will not resuspend.

The near-boiling temperature lyses the cell membrane, releasing DNA and other cell contents.

Alternatively, you may add the cell suspension to Chelex in a 1.5-mL tube and incubate in a boiling water bath or heat block.

To use adapters, “nest” the sample tube within sequentially larger tubes: 0.2 mL within 0.5 mL within 1.5 mL. Remove caps from tubes used as adapters.

11. Place your tube, along with other student samples, in a balanced configuration in a microcentrifuge, and spin for 90 seconds at full speed. *If your sample is in a PCR tube, one or two adapters will be needed to spin the tube in a microcentrifuge designed for 1.5-mL tubes.*
12. Use a micropipet with a fresh tip to transfer 30  $\mu\text{L}$  of the clear supernatant into a clean 1.5-mL tube. Be careful to avoid pipetting any cell debris and Chelex<sup>®</sup> beads.
13. Label the cap and side of the tube with your assigned number. This sample will be used for setting up one or more PCR reactions.
14. Store your sample on ice or at  $-20^{\circ}\text{C}$  until you are ready to continue with Part II.

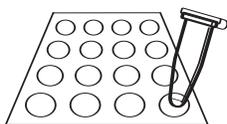
## II. AMPLIFY DNA BY PCR

Reagents (at each student station)	Supplies and Equipment
*Cheek cell DNA, 2.5 $\mu\text{L}$ (from Part I)	Permanent marker
*PTC primer/loading dye mix, 22.5 $\mu\text{L}$	Micropipet and tips (1–100 $\mu\text{L}$ )
Ready-To-Go <sup>™</sup> PCR beads (in 0.2-mL or 0.5-mL PCR tube)	Microcentrifuge tube rack
	Thermal cycler
	Container with cracked or crushed ice
<b>Shared Reagent</b>	
Mineral oil, 5 mL (depending on thermal cycler)	
*Store on ice	

The primer/loading dye mix will turn purple as the PCR bead dissolves.

If the reagents become splattered on the wall of the tube, pool them by pulsing in a microcentrifuge or by sharply tapping the tube bottom on the lab bench.

If your thermal cycler does not have a heated lid: Prior to thermal cycling, you must add a drop of mineral oil on top of your PCR reaction. Be careful not to touch the dropper tip to the tube or reaction, or the oil will be contaminated with your sample.



1. Obtain a PCR tube containing a Ready-To-Go<sup>™</sup> PCR Bead. Label with your assigned number.
2. Use a micropipet with a fresh tip to add 22.5  $\mu\text{L}$  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  $\mu\text{L}$  of your cheek cell DNA (from Part I) *directly into* the primer/loading dye mix. Insure that no cheek cell DNA remains in the tip after pipeting.
4. *Store your sample on ice until your class is ready to begin thermal cycling.*
5. Place your PCR tube, along with other student samples, in a thermal cycler that has been programmed to the following profile for 30 cycles if you use ethidium bromide or 35 cycles if you are using CarolinaBLU<sup>™</sup>. The profile may be linked to a 4 $^{\circ}\text{C}$  hold program after cycling is completed.
 

Denaturing step:	94 $^{\circ}\text{C}$	30 seconds
Annealing step:	64 $^{\circ}\text{C}$	45 seconds
Extending step:	72 $^{\circ}\text{C}$	45 seconds
6. After cycling, store the amplified DNA on ice or at  $-20^{\circ}\text{C}$  until you are ready to continue with Part III.

### III. DIGEST PCR PRODUCTS WITH *HaeIII*

Reagents (at each student station)	Supplies and Equipment
*PCR product (from Part II), 25 $\mu$ L	Permanent marker
<b>Shared Reagent</b>	1.5-mL microcentrifuge tubes
*Restriction enzyme <i>HaeIII</i> , 10 $\mu$ L	Microcentrifuge tube rack
	Micropipet and tips (1–20 $\mu$ L)
	Thermal cycler (or water bath or heat block)
*Store on ice	Container with cracked or crushed ice

The DNA in this tube will not be digested with the restriction enzyme *HaeIII*.

If you used mineral oil during PCR, pierce your pipet tip through the mineral oil layer to withdraw the PCR product in Step 2 and to add the *HaeIII* enzyme in Step 3.

Alternatively, you may incubate the reaction in a 37°C water bath or heat block. Thirty minutes is the minimum time needed for complete digestion. If time permits, incubate reactions for 1 or more hours.

1. Label a 1.5-mL tube with your assigned number and with a “U” (undigested).
2. Use a micropipet with a fresh tip to transfer 10  $\mu$ L 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  $\mu$ L of restriction enzyme *HaeIII* *directly into* the PCR product remaining in the PCR tube. Label this tube with a “D” (digested).
4. Mix and pool reagents by pulsing in a microcentrifuge or by sharply tapping the tube bottom on the lab bench.
5. Place your PCR tube, along with other student samples, in a thermal cycler that has been programmed for one cycle of the following profile. The profile may be linked to a 4°C hold program.  
 Digesting step:      37°C                      30 minutes
6. Store your sample on ice or in the freezer until you are ready to begin Part IV.

### IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

Reagents (at each student station)	Supplies and Equipment
*Undigested PCR product (from Part III), 10 $\mu$ L	Micropipet and tips (1–20 $\mu$ L)
* <i>HaeIII</i> -digested PCR product (from Part III), 16 $\mu$ L	Microcentrifuge tube rack
<b>Shared Reagents</b>	Gel electrophoresis chamber
*pBR322/ <i>Bst</i> NI marker	Power supply
2% agarose in 1 $\times$ TBE, 50 mL	Staining trays
1 $\times$ TBE, 300 mL	Latex gloves
Ethidium bromide (1 $\mu$ g/mL), 250 mL	UV transilluminator (for use with ethidium bromide)
or	White light transilluminator (for use with <i>CarolinaBLU</i> <sup>™</sup> )
<i>CarolinaBLU</i> <sup>™</sup> Gel and Buffer Stain, 7 mL	Digital or instant camera (optional)
<i>CarolinaBLU</i> <sup>™</sup> Final Stain, 375 mL	Water bath (60°C)
*Store on ice	Container with cracked or crushed ice

1. Seal the ends of the gel-casting tray with masking tape, and insert a well-forming comb.

Avoid pouring an overly thick gel, which is more difficult to visualize. The gel will become cloudy as it solidifies.

Do not add more buffer than necessary. Too much buffer above the gel channels electrical current over the gel, increasing running time.

100-bp ladder may also be used as a marker.

If you used mineral oil during PCR, pierce your pipet tip through the mineral oil layer to withdraw the PCR products. Do not pipet any mineral oil.

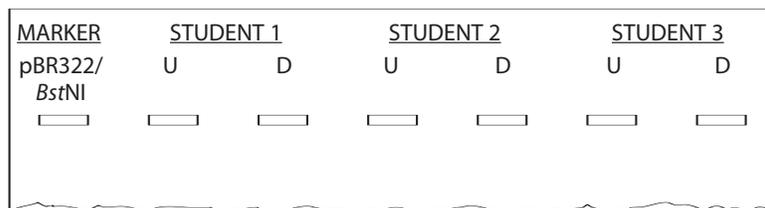
Expel any air from the tip before loading. Be careful not to push the tip of the pipet through the bottom of the sample well.



Destaining the gel for 5–10 minutes in tap water leeches unbound ethidium bromide from the gel, decreasing background and increasing contrast of the stained DNA.

Transillumination, where the light source is below the gel, increases brightness and contrast.

2. Pour 2% agarose solution to a depth that covers about  $\frac{1}{3}$  the height of the open teeth of the comb.
3. Allow the gel to solidify completely. This takes approximately 20 minutes.
4. Place the gel into the electrophoresis chamber, and add enough  $1\times$  TBE buffer to cover the surface of the gel.
5. Carefully remove the comb, and add additional  $1\times$  TBE buffer to just cover and fill in wells—creating a smooth buffer surface.
6. Use a micropipet with a fresh tip to load 20  $\mu\text{L}$  of pBR322/*Bst*NI size markers into the far left lane of the gel.
7. Use a micropipet with a fresh tip to add 10  $\mu\text{L}$  of the undigested (U) and 16  $\mu\text{L}$  of the digested (D) sample/loading dye mixture into different wells of a 2% agarose gel, according to the diagram below.



8. 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.
9. Stain the gel using ethidium bromide or *CarolinaBLU*<sup>™</sup>:
  - a. For ethidium bromide, stain 10–15 minutes. Decant stain back into the storage container for reuse, and rinse the gel in tap water. *Use gloves when handling ethidium bromide solution and stained gels or anything that has ethidium bromide on it. Ethidium bromide is a known mutagen, and care should be taken when using and disposing of it.*
  - b. For *CarolinaBLU*<sup>™</sup>, follow directions in the Instructor Planning section.
10. View the gel using transillumination, and photograph it using a digital or instant camera.

## BIOINFORMATICS

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*For a better understanding of the experiment, do the following bioinformatics exercises before you analyze your results.*

Biological information is encoded in the nucleotide sequence of DNA. Bioinformatics is the field that identifies biological information in DNA using computer-based tools. Some bioinformatics algorithms aid the identification of genes, promoters, and other functional elements of DNA. Other algorithms help determine the evolutionary relationships between DNA sequences.

Because of the large number of tools and DNA sequences available on the Internet, experiments done *in silico* (in silicon, or on the computer) now complement experiments done *in vitro* (in glass, or test tube). This movement between biochemistry and computation is a key feature of modern biological research.

In Part I, you will use the Basic Local Alignment Search Tool (BLAST) to identify sequences in biological databases and to make predictions about the outcome of your experiments. In Part II, you will find and copy the human PTC taster and non-taster alleles. In Part III, you will discover the chromosome location of the PTC tasting gene. In Part IV, you will explore the evolutionary history of the gene.

NOTE: The links in these bioinformatics exercises were correct at the time of printing. However, links and labels within the NCBI Internet site change occasionally. When this occurs, you can find updated exercises at <http://bioinformatics.dnalc.org>.

### I. Use BLAST to Find DNA Sequences in Databases (Electronic PCR)

The following primer set was used in the experiment:

5'-CCTTCGTTTTCTTGGTGAATTTTGGGATGTAGTGAAGAGCGG-3' (Forward Primer)  
5'-AGGTTGGCTTGGTTGCAATCATC-3' (Reverse Primer)

1. Initiate a BLAST search.
  - a. Open the Internet site of the National Center for Biotechnology Information (NCBI) [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov).
  - b. Click on *BLAST* in the top speed bar.
  - c. Click on the link *nucleotide BLAST* under the heading *Basic BLAST*.
  - d. Enter the sequences of the primers into the *Search* window. These are the query sequences.
  - e. Omit any non-nucleotide characters from the window, because they will not be recognized by the BLAST algorithm.
  - f. Under *Choose Search Set*, select the *Nucleotide collection (nr/nt) database* from the drop-down menu.

- g. Under *Program Selection*, optimize for somewhat similar sequences by selecting *blastn*.
    - h. Click on *BLAST!* and the query sequences are sent to a server at the National Center for Biotechnology Information in Bethesda, Maryland. There, the BLAST algorithm will attempt to match the primer sequences to the millions of DNA sequences stored in its database. While searching, a page showing the status of your search will be displayed until your results are available. This may take only a few seconds, or more than a minute if a lot of other searches are queued at the server.
2. The results of the BLAST search are displayed in three ways as you scroll down the page:
  - a. First, a graphical overview illustrates how significant matches, or hits, align with the query sequence. Matches of differing lengths are coded by color.
  - b. This is followed by a list of *significant alignments*, or hits, with links to *Accession* information.
  - c. Next, is a detailed view of each primer sequence (*query*) aligned to the nucleotide sequence of the search hit (*subject*). Notice that a match to the forward primer (nucleotides 1–42), and a match to the reverse primer (nucleotides 44–68) are within the same *Accession*. Also notice that position 43 of the forward primer is missing. What does this mean?
3. Determine the predicted length of the product that the primer set would amplify in a PCR reaction (in vitro):
  - a. In the list of *significant alignments*, notice the *E-values* in the column on the right. The *Expectation* or *E-value* is the number of alignments with the query sequence that would be expected to occur by chance in the database. The lower the *E-value*, the higher the probability that the hit is related to the query. What does the *E-value* of  $6e-12$  mean?
  - b. Note the names of any *significant alignments* that have *E-values* less than 0.1. Do they make sense? What do they have in common?
  - c. Scroll down to the *Alignments* section to see exactly where the two primers have landed in a subject sequence.
  - d. The lowest and highest nucleotide positions in the subject sequence indicate the borders of the amplified sequence. Subtracting one from the other gives the difference between the two coordinates.
  - e. However, the actual length of the fragment *includes* both ends, so add 1 nucleotide to the result to determine the exact length of the PCR product amplified by the two primers.

## II. Find and Copy the Human (*Homo sapiens*) PTC Taster and Non-taster Alleles

1. In the list of *significant alignments*, select the hit containing the human taster allele from among those with the lowest *E-values*.
2. Click on the *Accession* link at the left to open the sequence datasheet for this hit.
3. At the top of the report, note basic information about the sequence, including its basepair length, database accession number, source, and references.
4. In the middle section of the report, note annotations of gene and regulatory features, with their beginning and ending nucleotide positions (xx .. xx). Identify the feature(s) contained between the nucleotide positions identified by the primers, as determined in 3.d above.
5. The bottom section of the report lists the entire nucleotide sequence of the gene or DNA sequence that contains the PCR product. Highlight all the nucleotides between the beginning of the forward primer and end of reverse primer. Paste this sequence into a text document. Then, delete all non-nucleotide characters and spaces. This is the amplicon or amplified product.
6. Repeat Steps 1–5 to copy the human non-taster allele.

## III. Use *Map Viewer* to Determine the Chromosome Location of the *TAS2R38* Gene

1. Return to the NCBI home page, then click on *Map Viewer* located in the *Hot Spots* column on the right.
2. Find *Homo sapiens (humans)* in the table to the right and click on the “B” icon under the *Tools* header. If more than one build is displayed, select the one with the highest number, as this will be the most recent version.
3. Enter the primer sequences into the search window. Omit any non-nucleotide characters from the window, because they will not be recognized by the BLAST algorithm.
4. Select *BLASTN* from the drop-down menu under *Program* and click on *Begin Search*.
5. Click on *View report* to retrieve the results.
6. Click on [*Human genome view*] in the list of *Other reports* at the top of the page to see the chromosome location of the BLAST hit. On what chromosome have you landed?
7. Click on the marked chromosome number to move to the *TAS2R38* locus.
8. Click on the small blue arrow labeled *Genes seq* to display genes. The *TAS2R38* gene occupies the whole field of the default view, which displays 1/10,000 of the chromosome. Move the *zoom out* toggle on the

left to  $1/1000$  to see the chromosome region surrounding *TAS2R38* and its nearest gene “neighbors.” What genes are found on either side of *TAS2R38*? How do their structures differ from *TAS2R38*? Click on their names and follow links for more information about them.

9. Click on the *blue arrow* at the top of the chromosome image to scroll up the chromosome. Look at each of the genes. Scroll up one more screen, and look at those genes. What do most of these genes have in common with *TAS2R38*, and what can you conclude?
10. Zoom out to view  $1/100$  of the chromosome for a better view of this region.

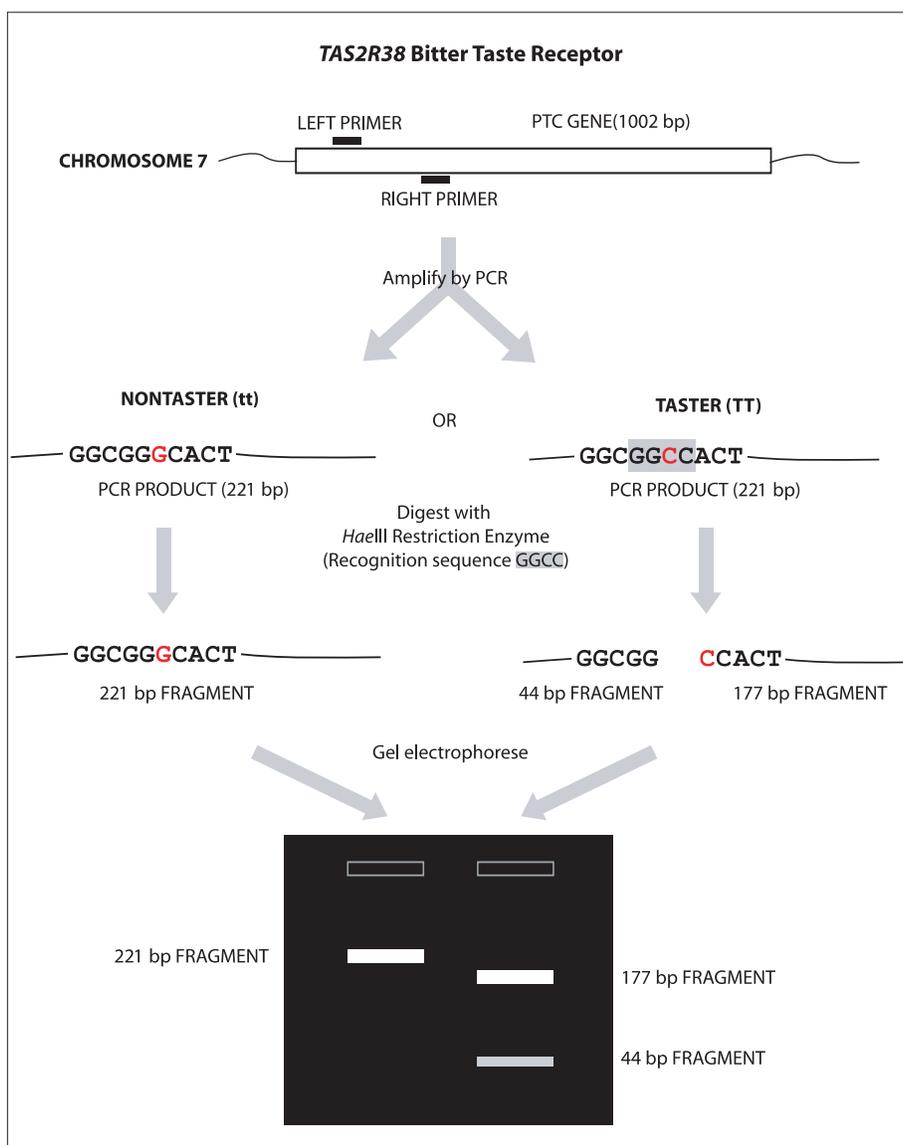
#### IV. Use Multiple Sequence Alignment to Explore the Evolution of *TAS2R38* Gene

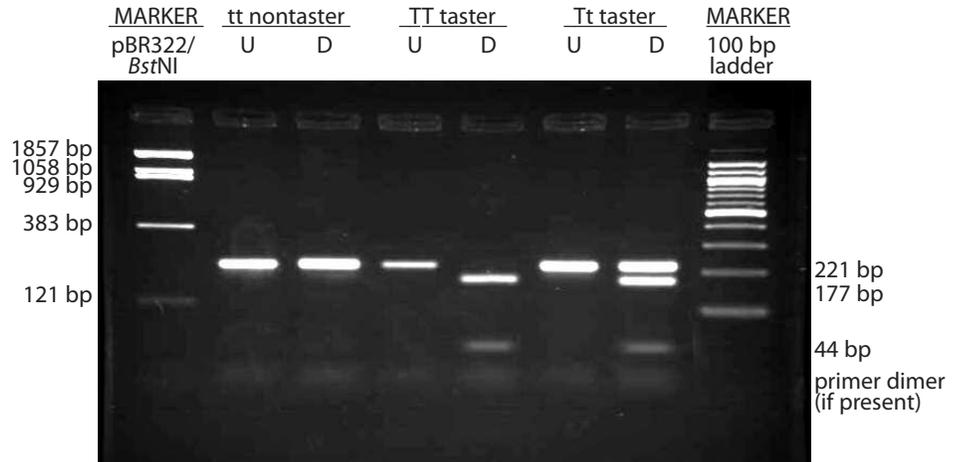
1. Return to your original BLAST results, or repeat Part I above to obtain a list of *significant alignments*.
2. Find sequences of the *TAS2R38* gene from chimpanzee (*Pan troglodytes*), bonobo (*Pan paniscus*), and gorilla. Use only entries listed as “complete cds” (coding sequence). For each, open its *Accession* link, copy its complete nucleotide sequence from the bottom of the datasheet, and paste the sequence into a text document.
3. Open the *BioServers* Internet site at the Dolan DNA Learning Center [www.bioservers.org](http://www.bioservers.org).
4. Enter *Sequence Server* using the button in the left-hand column. (You can register if you want to save your work for future reference.)
5. Create PTC gene sequences for comparison:
  - a. Click on *Create Sequence* at the top of the page.
  - b. Copy one of the *TAS2R38* sequences (from Step 2 above), and paste it into the *Sequence* window. Enter a name for the sequence, and click OK. Your new sequence will appear in the workspace at the bottom half of the page.
  - c. Repeat Steps a. and b. for each of the human and primate sequences from Step 2. Also create a sequence for the forward primer used in your PCR amplification, and for the amplicon.
6. Compare each of the following sets of sequences:
  - Human PTC taster vs. human PTC non-taster vs. 221 basepair amplicon.
  - Human PTC taster vs. human PTC non-taster.
  - Human PCT taster vs. human PTC non-taster vs. chimpanzee vs. bonobo vs. gorilla.
  - Forward primer vs. human PTC taster vs. human PTC non-taster.
  - a. Click on the *Check Box* in the left-hand column to compare two or more sequences.

- b. Click on *Compare* in the grey bar. (The default operation is a multiple sequence alignment, using the *CLUSTAL W* algorithm.) The *checked* sequences are sent to a server at Cold Spring Harbor Laboratory, where the *CLUSTAL W* algorithm will attempt to align each nucleotide position.
- c. The results will appear in a new window. This may take only a few seconds, or more than a minute if a lot of other searches are queued at the server.
- d. The sequences are displayed in rows of 25 nucleotides. Yellow highlighting denotes mismatches between sequences or regions where only one sequence begins or ends before another.
- e. To view the entire gene, enter *1100* as the number of nucleotides to display per page, then click *Redraw*.
- f. Repeat Steps a–e for each of the four sets of sequences to be aligned.
- g. *Human PTC taster vs. human PTC non-taster vs. 221 basepair amplicon*. What does the initial stretch of highlighted sequences mean? Where does the amplicon track along with the two human alleles? At what position in the gene is the SNP examined in the experiment, and what is the difference between taster and non-taster alleles?
- h. *Human PTC taster vs. human PTC non-taster*. List the nucleotide position(s) and nucleotide differences of any additional SNP(s). Count triplets of nucleotides from the initial ATG start codon to determine codon(s) affected by SNP(s). Use a standard genetic code chart to determine if an amino acid is changed by each SNP.
- i. *Human PTC taster vs. human PTC non-taster vs. chimpanzee vs. bonobo vs. gorilla*. What is the ancestral (original) state of this gene at nucleotide positions 145, 785, and 886? Are other primates tasters or non-tasters, and what does this suggest about the function of bitter taste receptors? What patterns do you notice in SNPs at other locations in the gene?
- j. *Forward primer vs. human PTC taster vs. human PTC non-taster*. Where does the primer bind? What discrepancy do you notice between the primer sequence and the *TAS2R38* gene sequence? Of what importance is this to the experiment?

## RESULTS AND DISCUSSION

The following diagram shows how PCR amplification and restriction digestion identifies the G-C polymorphism in the *TAS2R38* gene. The “C” allele, on the right, is digested by *HaeIII* and correlates with PTC tasting.





1. **Determine your PTC genotype.** Observe the photograph of the stained gel containing your PCR digest and those from other students. Orient the photograph with the sample wells at the top. Use the sample gel shown above to help interpret the band(s) in each lane of the gel.
  - a. Scan across the photograph to get an impression of what you see in each lane. You should notice that virtually all student lanes contain one to three prominent bands.
  - b. Locate the lane containing the pBR322/*Bst*NI markers on the left side of the sample gel. Working from the well, locate the bands corresponding to each restriction fragment: 1857 bp, 1058 bp, 929 bp, 383 bp, and 121 bp. The 1058-bp and 929-bp fragments will be very close together or may appear as a single large band. The 121-bp band may be very faint or not visible. (Alternatively, use a 100-bp ladder as shown on the right-hand side of the sample gel. These DNA markers increase in size in 100-bp increments starting with the fastest migrating band of 100 bp.)
  - c. 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/*Bst*NI lane. Confirm that the undigested PCR product corresponds with a size of about 221 bp.
  - d. 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 ahead 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.)

- e. It is common to see a diffuse (fuzzy) band that runs just ahead of the 44-bp fragment. This is “primer dimer,” an artifact of the PCR reaction that results from the primers overlapping one another and amplifying themselves. The presence of primer dimer, in the absence of other bands, confirms that the reaction contained all components necessary for amplification.
  - f. Additional faint bands at other positions occur when the primers bind to chromosomal loci other than the PTC gene and give rise to “nonspecific” amplification products.
2. **Determine your PTC phenotype.** First, place one strip of control taste paper in the center of your tongue for several seconds. Note the taste. Then, 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: strongly bitter, weakly bitter, or no taste other than paper?
  3. Correlate PTC genotype with phenotype. Record class results in the table below.

Genotype	Phenotype		
	Strong taster	Weak taster	Nontaster
<b>TT</b> (homozygous)			
<b>Tt</b> (heterozygous)			
<b>tt</b> (homozygous)			

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

4. How does the *HaeIII* enzyme discriminate between the C-G polymorphism in the *TAS2R38* gene?
5. The forward primer used in this experiment incorporates part of the *HaeIII* recognition site, GGCC. How is this different from the sequence of the human *TAS2R38* gene? What characteristic of the PCR reaction allows the primer sequence to “override” the natural gene sequence? Draw a diagram to support your contention.
6. 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?
7. Research other mutations in the *TAS2R38* gene and how they may influence bitter taste perception.

8. The frequency of PTC nontasting is higher than would be expected if bitter-tasting ability were the only trait upon which natural selection had acted. In 1939, the geneticist R.A. Fisher suggested that the PTC gene is under “balancing” selection—meaning that a possible negative effect of losing this tasting ability is balanced by some positive effect. Under some circumstances, balancing selection can produce heterozygote advantage, where heterozygotes are fitter than homozygous dominant or recessive individuals. What advantage might this be in the case of PTC?
9. Research how the methods of DNA typing used in this experiment differ from those used in forensic crime labs. Focus on: a) type(s) of polymorphism used, b) method for separating alleles, and c) methods for insuring that samples are not mixed up.
10. What ethical issues are raised by human DNA typing experiments?