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 *Hae*III, 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.

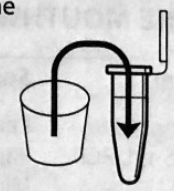
Scott, K. (2004). The Sweet and the Bitter of Mammalian Taste. *Current Opin. Neurobiol.* **14**:423-427.

LAB FLOW

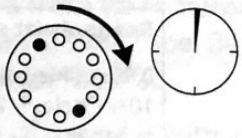
I. ISOLATE DNA BY SALINE MOUTHWASH

RINSE mouth with saline

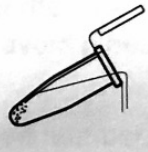
TRANSFER saline



CENTRIFUGE



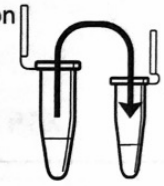
POUR OFF supernatant



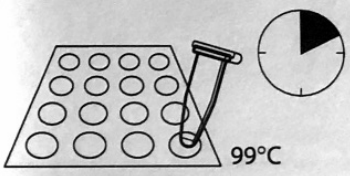
RESUSPEND

ADD Chelex

TRANSFER cell suspension

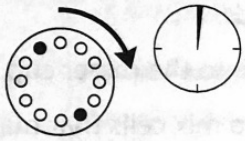


BOIL in thermal cycler

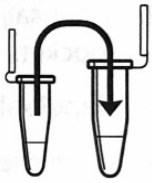


SHAKE vigorously

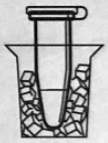
CENTRIFUGE



TRANSFER supernatant



STORE on ice



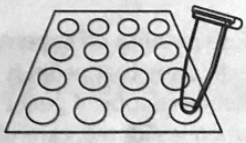
II. AMPLIFY DNA BY PCR

ADD primer/ loading dye mix

ADD DNA

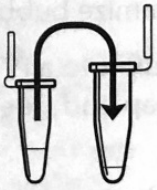
ADD mineral oil (if necessary)

AMPLIFY in thermal cycler



III. DIGEST PCR PRODUCTS WITH *Hae*III

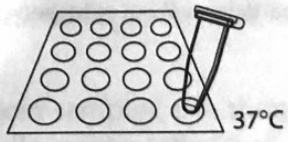
TRANSFER PCR product



ADD *Hae*III

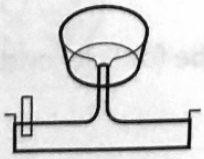
MIX

INCUBATE in thermal cycler



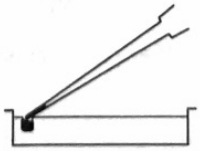
IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

POUR gel

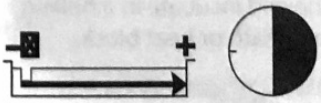


SET

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 10% Chelex®, 100 µL (in 0.2- or 0.5-mL PCR tube)	Permanent marker 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.

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

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.

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.

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.)

Food particles will not resuspend.

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.

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

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

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.

10. After boiling, vigorously shake the PCR tube for 5 seconds.

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 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 assigned number. 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.

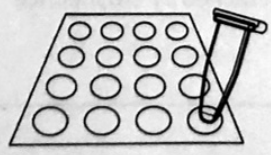
II. AMPLIFY DNA BY PCR

Reagents (at each student station)	**Supplies and Equipment**
*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 or 0.5-mL PCR tube)	Permanent marker Micropipet and tips (1–100 µL) Microcentrifuge tube rack Thermal cycler Container with cracked or crushed ice
Shared Reagent 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™ PCR Bead. Label with your assigned number.
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. 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 for 30 cycles of the following profile. The profile may be linked to a 4°C hold program after the 30 cycles are completed. Complete 35 cycles if you are staining with *Carolina*BLU™.

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

6. After cycling, store the amplified DNA on ice or at –20°C until you are ready to continue with Part III.

III. DIGEST PCR PRODUCTS WITH *Hae*III

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

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

1. Label a 1.5-mL tube with your assigned number and with a "U" (undigested).

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 *Hae*III enzyme in Step 3.

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 *Hae*III *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.

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.

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 µL	Micropipet and tips (1–20 µL)
**Hae*III-digested PCR product (from Part III), 16 µL	Microcentrifuge tube rack
	Gel electrophoresis chamber
Shared Reagents	Power supply
	Staining trays
*pBR322/*Bst*NI marker	Latex gloves
2% agarose in 1× TBE, 50 mL	UV transilluminator (for use with ethidium bromide)
1× TBE, 300 mL	White light transilluminator (for use with *Carolina*BLU™)
Ethidium bromide (1 µg/mL), 250 mL	Digital or instant camera (optional)
or	Water bath (60°C)
*Carolina*BLU™ Gel and Buffer Stain, 7 mL	Container with cracked or crushed ice
*Carolina*BLU™ Final Stain, 375 mL	
*Store on 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.

2. Pour 2% agarose solution to a depth that covers about 1/3 the height of the open teeth of the comb.
3. Allow the gel to solidify completely. This takes approximately 20 minutes.

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

4. Place the gel into the electrophoresis chamber, and add enough 1× TBE buffer to cover the surface of the gel.
5. Carefully remove the comb, and add additional 1× TBE buffer to just cover and fill in wells—creating a smooth buffer surface.

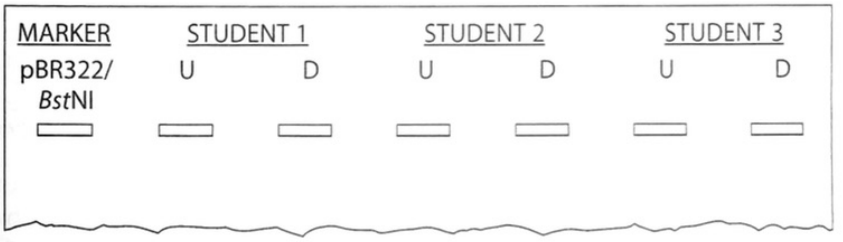
100-bp ladder may also be used as a marker.

6. Use a micropipet with a fresh tip to load 20 µL of pBR322/*Bst*NI size markers into the far left lane of the gel.

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.

7. 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.



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.

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.

9. Stain the gel using ethidium bromide or *Carolina*BLU™:
   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 *Carolina*BLU™, follow directions in the Instructor Planning section.

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

10. View the gel using transillumination, and photograph it using a digital or instant camera.

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 exercises were correct at the time of printing; however, links and labels within the NCBI Internet site change occasionally. When this occurs, please contact Carolina's Technical Support at 800-227-1150.

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

The following primer set was used in the experiment:

5'-CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG-3' (Forward Primer)
5'-AGGTTGGCTTGGTTTGCAATCATC-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.
   b. Click on the link to BLAST.
   c. Click on the link to nucleotide blast.
   d. Enter the sequences of the primers into the Search window. These are the query sequences.
   e. Omit any non-nucleotide characters. They are not 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 NCBI. 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 (from the forward primer) is missing. What does that 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 an 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. Find the hit to the *Homo sapiens* PTC-non-taster allele. Click on the hyperlink to move down to the *Alignments* section and see exactly where the two primers align with the sequence. Examine the alignment carefully. If you would like to, you can compare the alignment to the result with the PTC-taster allele.
   d. The lowest and highest nucleotide positions in the subject sequence should indicate the borders of the amplified sequence. Subtracting one from the other gives the difference between the two coordinates. However, take into account any observations you may have made in step 3c and make any necessary adjustments in your calculations.
   e. In addition, because the actual length of the fragment includes both ends, 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. Examine the header for the alignment between the primers and the taster allele.
2. Click on the *Accession* link after Sequence ID to open the sequence data sheet for this hit. Accession links are also found with the list of significant alignments.

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 you identified in 3.d in Part I as matching the ends of the primers.
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 the reverse primer. Paste this sequence into a text document and note that it is the taster allele. This is the amplicon or amplified product.
6. Repeat Steps 1–5 to copy the human non-taster allele. You will use the sequences in step IV.

III. Use the Genome Data Viewer to Determine the Chromosome Location of the TAS2R38 Gene.

1. Search for "NCBI Genome Data Viewer" in your favorite search engine, then follow links to the Viewer at https://www.ncbi.nlm.nih.gov/genome/gdv/.
2. Under *Select organism*, enter *Homo sapiens* (human).
3. Under *Search* in genome, enter *TAS2R38*.
4. Under *Assembly*, select the one with the highest number, as this will be the most recent version.
5. Click on the magnifying glass next to where you entered TAS2R38 to launch the *Genome Data Viewer* view of the gene location.
6. On what chromosome have you landed?
7. Coding regions are in dark green, and untranslated regions are in light green, while arrows indicate the direction of the coding region for the gene. In what direction does the gene code on the chromosome?
8. Explore the variants in TAS2R38.
   a. Under the graphic of the gene, find the line labeled *Cited Variants*… . That line indicates published gene variants using blue boxes containing a "1." How many variants are shown that are present in the coding region (i.e., that line up with the coding region indicated by the dark green)?
   b. Hover the mouse over the blue box for the variant farthest to the right to open a pop-up window with a link to information about the variant. Click on the link listed as "Variation ID (rs713598)" to open up details about the variant you are currently hovering over. This opens a record in a database for genetic variations called dbSNP. Click on the direction "Switch to classic site."
   c. In the new window, data about that variant is summarized. There is a great deal of information, some of which may be hard to understand. Focus on the section labeled *GeneView*.

d. Within that section, find the area labeled "Gene Model(s)," which summarizes the type of mutation and its effect on the protein. What nucleotide in the mRNA is affected? What are the two versions of this position? What is the effect on the protein sequence? Do you think that the change would make the receptor coded for by the gene completely nonfunctional?

e. Go back to the view of the gene and explore the other variants. What sorts of polymorphisms are these? Do you think they will affect the receptor's function? Why or why not?

9. While in the genome data viewer, click on the "-" magnifying glass above the gene display to zoom out from the gene. 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.
10. Zoom out further 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. In the table of BLAST results, click on the box to the left of the TAS2R38 entries for the *Homo sapiens* taster and non-taster alleles, then select complete "cds" (coding sequence) entries for chimpanzee (*Pan troglodytes*), bonobo (*Pan paniscus*), and gorilla. Once they are all selected, click on *Download* at the top of the table of significant alignments, then select *FASTA* (complete sequence). Click "Continue" and open the resulting *seqdump.txt* file. This file contains the FASTA-formatted sequence information for the selected hits. The sequences may appear as a continuous string of characters with the labels separating each sequence. To make analyses easier to follow, simplify the labels for each sequence. For instance, change ">AY677143.1 *Pan paniscus* taste receptor T2R38 (TAS2R) gene, complete cds" to ">*Pan_paniscus*_cds" and ">AY258597.1 *Homo sapiens* PTC bitter taste receptor (PTC) gene, PTC-taster allele, complete cds" to ">Taster_cds." Note the use of underscores, as DNA Subway, the program you will use next, does not accept spaces within sequence names.
3. Open the DNA Subway site, https://dnasubway.cyverse.org/.
4. Register or Enter as Guest: Click "Register" to create a profile to save and share your work. This process requires time, as registration is curated. Once registered, click "Log In" and enter your password. Alternatively, click "Enter as Guest." Guest accounts are temporary and work is not saved.
6. Click the blue square to create a new project.
7. Select "DNA" under phylogenetics for the project type.
8. Select "Enter sequences in FASTA format." Copy and paste the sequences from the sequence dump file into the box.

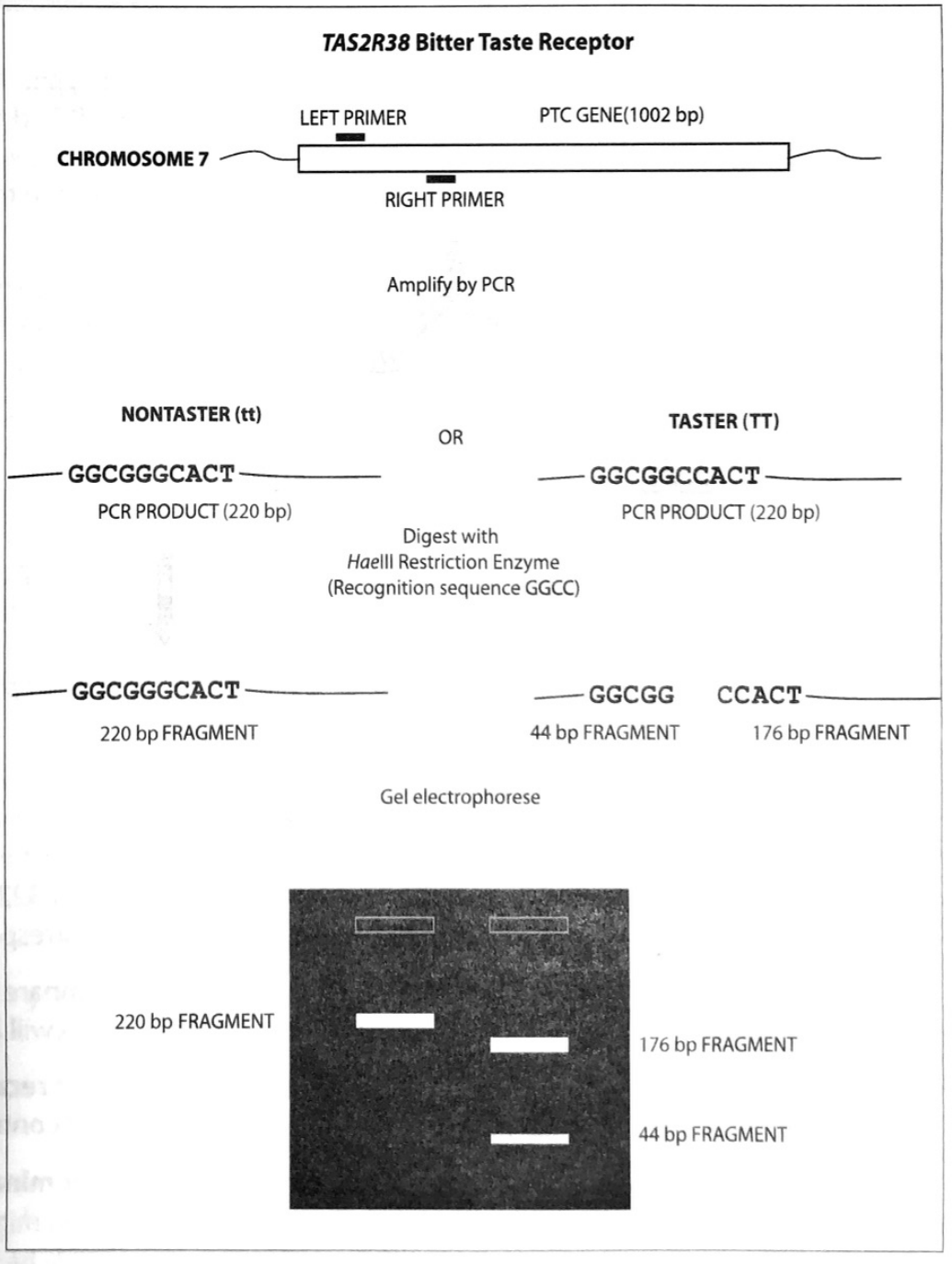
b. After running a new alignment, try to discover the ancestral (original) state of this gene at the sites with human variability.

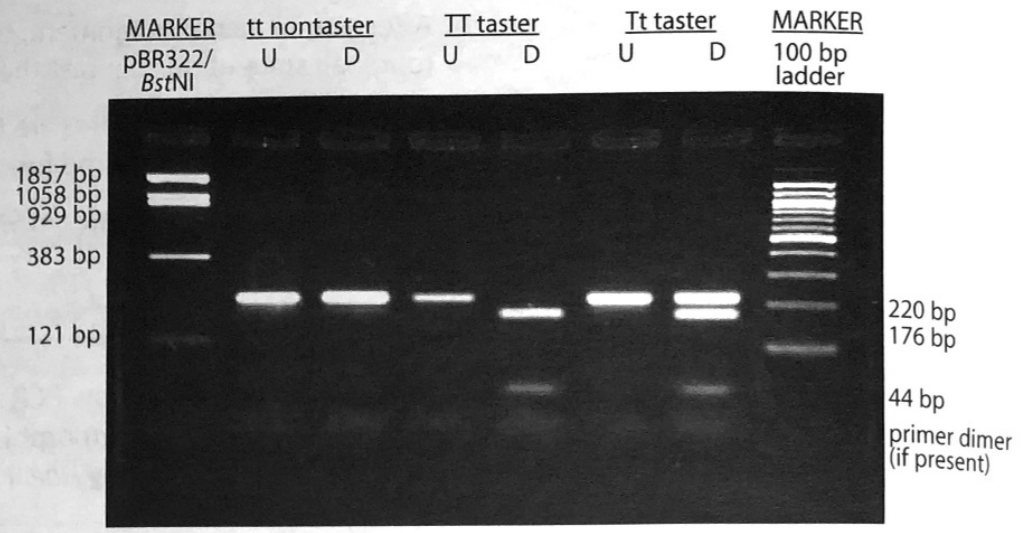
c. Do you think other primates are tasters or non-tasters, and what does that suggest about the function of bitter taste receptors?

d. What patterns do you notice in SNPs at other locations in the gene?

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 *Hae*III 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 220 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 176 bp and 44 bp. The 176-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 220-bp position, but this band should be clearly fainter than the 176-bp band.)

Tt taster (heterozygous) shows three bands that represent both alleles—220 bp, 176 bp, and 44 bp. The 220-bp band must be stronger than the 176-bp band. (If the 220-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.

	Phenotype		
Genotype	**Strong taster**	**Weak taster**	**Nontaster**
TT (homozygous)			
Tt (heterozygous)			
tt (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 *Hae*III enzyme discriminate between the C-G polymorphism in the *TAS2R38* gene?

5. The forward primer used in this experiment incorporates part of the *Hae*III 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 non-tasting 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?

9. Note the FASTA entries in the sequence dump: each sequence record begins with a line starting with a right angle bracket (>) and a descriptive label. Following this format, label the taster and non-taster amplicons from part II, above, ">Taster" and "Non-taster." You don't have to remove the spaces or numbers from the sequences, but must ensure you have a return at the end of the labels for each sequence.
10. Name your project (required) and enter a description if desired, then click "Continue" to load the project into DNA Subway.
11. Click on "Upload Data" to enter the amplicon sequences. To the right of "Enter Sequences in FASTA format," copy and paste the labeled sequences into the box and click "Add sequence(s)." Enter the amplicon sequences one at a time.
12. Compare the human PTC taster and non-taster cds to the taster and non-taster amplicons:
    a. Click "Select Data." Click on the boxes beside the taster and non-taster amplicons and cds, then click "Save Selections."
    b. Click on Muscle. This will run a multiple sequence alignment. Click on Muscle again once a green V appears to view the results. In the resulting alignment, differences are highlighted in color, while similarities as shown in tan. Dark grey indicates no sequence. Looking across the alignments, are any of the sequences shorter (missing sequence compared to the others)?
    c. Are there any differences between the taster and non-taster allele within the amplicon? What is the difference? (To figure that out, zoom in on the region by clicking on the "+" above until nucleotides are displayed. In the alignment, differences will be shown for one variant, while the other sequence will be shown in the consensus, above.) Outside the amplicon? Does the experiment test for all the variants in the gene? Do the results of the alignment agree with the results from the Genome Data Viewer?
13. Figure out where the forward primer lies compared to the variant in the amplicon.
    a. First, exit from the Alignment Viewer.
    b. Next, find the forward primer sequence (it is the longer primer).
    c. Click "Upload Data," then type ">Forward_Primer" followed by enter, then enter the forward primer sequence in to the box and hit "Add sequence(s)."
    d. Return to Select Data, add the primer sequence, then save your selections. Rerun MUSCLE and view the resulting alignment. How does the forward primer line up with the variation? Does the primer sequence exactly match the sequence of the taster and non-taster alleles?
14. Compare the different primate sequences.
    a. Select the human PTC taster, human PTC non-taster, chimpanzee, bonobo, and gorilla cds and save the selections.