Using a Single-Nucleotide Polymorphism to Predict Bitter-Tasting Ability

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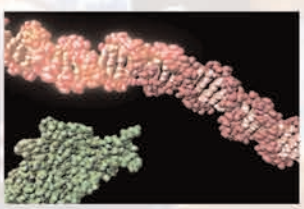
CAROLINA



21-1376 21-1377 21-1378 21-1379 21-1380 21-1381

Using a Single-Nucleotide Polymorphism to Predict Bitter-Tasting Ability

IMPORTANT INFORMATION

Storage: Upon receipt of the kit, store *Hae*III restriction enzyme, PTC primer/loading dye mix, and DNA marker pBR322/*Bst*NI in a freezer (approximately –20°C). All other materials may be stored at room temperature (approximately 25°C).

Use and Lab Safety: The materials supplied are for use with the method described in this kit only. Use of this kit presumes and requires prior knowledge of basic methods of gel electrophoresis and staining of DNA. Individuals should use this kit only in accordance with prudent laboratory safety precautions and under the supervision of a person familiar with such precautions. Use of this kit by unsupervised or improperly supervised individuals could result in injury.

Limited License: Polymerase chain reaction (PCR) is protected by patents owned by Hoffman-La Roche, Inc. The purchase price of this product includes a limited, non-transferable license under U.S. Patents 4,683,202; 4,683,195; and 4,965,188 or their foreign counterparts, owned by Hoffmann-La Roche Inc. and F. Hoffmann-La Roche Ltd. (Roche), to use only this amount of the product to practice the Polymerase Chain Reaction (PCR) and related processes described in said patents solely for the research, educational, and training activities of the purchaser when this product is used either manually or in conjunction with an authorized thermal cycler. No right to perform or offer commercial services of any kind using PCR, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted by implication or estoppel. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at The Perkin-Elmer Corporation, 850 Lincoln Center Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

Printed material: The student instructions, pages 5–20, as well as the *Carolina*BLU™ staining protocol on page 27 may be photocopied as needed for use by your students.

REAGENTS, SUPPLIES, AND EQUIPMENT CHECKLIST

Included in the kit:

DNA extraction and amplification (all kits):

- ☐ 1.5 g Chelex® resin
- ☐ 700 µL PTC primer/loading dye mix
- ☐ 25 *Ready-to-Go™ PCR Beads
- ☐ 5 mL mineral oil
- ☐ 1 200-µL tube pBR322/*Bst*NI markers (0.075 µg/µL)
- ☐ 40 µL *Hae*III restriction enzyme
- ☐ Pack of 100 PTC taste strips
- ☐ Pack of 100 control taste strips
- ☐ Instructor's manual with reproducible Student Lab Instructions
- ☐ PTC Tasting CD-ROM

***Electrophoresis with ethidium bromide staining (Kits 21-1378 and 21-1379):*

- ☐ 10 g agarose
- ☐ 200 mL 20× TBE
- ☐ 250 mL ethidium bromide, 1 µg/mL
- ☐ 12 latex gloves
- ☐ 9 staining trays

***Electrophoresis with Carolina*BLU™ *staining (Kits 21-1380 and 21-1381)*:

- ☐ 10 g agarose
- ☐ 200 mL 20× TBE
- ☐ 7 mL *Carolina*BLU™ Gel and Buffer Stain
- ☐ 375 mL *Carolina*BLU™ Final Stain
- ☐ 12 latex gloves
- ☐ 9 staining trays

Needed but not supplied:

- ☐ 0.9% saline solution (NaCl), 10 mL per student in 15-mL tube
- ☐ Micropipets and tips (1 µL to 1000 µL)
- ☐ 1.5-mL microcentrifuge tubes, polypropylene, 3 per student
- ☐ Microcentrifuge tube racks
- ☐ Microcentrifuge for 1.5-mL tubes
- ☐ 0.2-mL or 0.5-mL PCR tubes, 2 per student (1.5-mL microcentrifuge tubes may also be used.)
- ☐ 0.2-mL or 0.5-mL tube adapters for microcentrifuge (can be made from 0.5-mL and/or 1.5-mL tubes)
- ☐ Thermal cycler, programmable
- ☐ Electrophoresis chambers
- ☐ Electrophoresis power supplies
- ☐ Gel-staining trays
- ☐ UV transilluminator (ethidium bromide staining)
- ☐ White light box (*Carolina*BLU™ staining, optional)
- ☐ Camera or photo-documentary system (optional)
- ☐ Paper cup, 1 per student
- ☐ Permanent markers
- ☐ Container with cracked or crushed ice
- ☐ Boiling water bath (optional, see instructions)

*Ready-to-Go™ PCR Beads incorporate *Taq* polymerase, dNTPs, and $MgCl_2$. Each bead is supplied in an individual 0.5–mL tube or a 0.2–mL tube.

**Electrophoresis reagents must be purchased separately for Kits 21-1376 and 21-1377.

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CONTENTS

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.

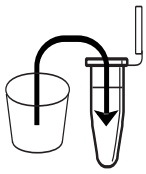
Scott, K. (2004). The Sweet and the Bitter of Mammalian Taste. *Current Opin. Neurobiol.* **14**:423-427.

LAB FLOW

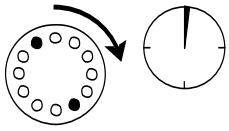
I. ISOLATE DNA BY SALINE MOUTHWASH

RINSE mouth with saline

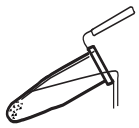
TRANSFER saline



CENTRIFUGE



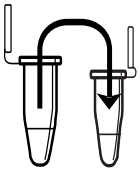
POUR OFF supernatant



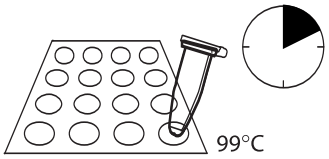
RESUSPEND

ADD Chelex

TRANSFER cell suspension

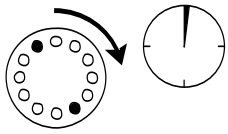


BOIL in thermal cycler

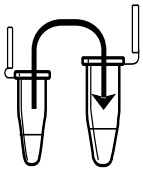


SHAKE vigorously

CENTRIFUGE



TRANSFER supernatant



STORE on ice

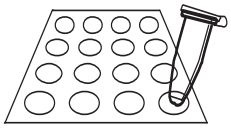
II. AMPLIFY DNA BY PCR

ADD primer/ loading dye mix

ADD DNA

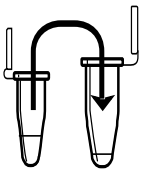
ADD mineral oil (if necessary)

AMPLIFY in thermal cycler



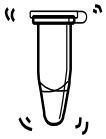
III. DIGEST PCR PRODUCTS WITH *Hae*III

TRANSFER PCR product

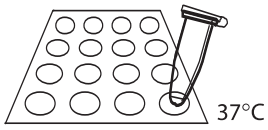


ADD *Hae*III

MIX

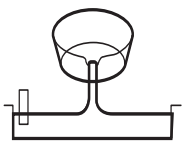


INCUBATE in thermal cycler



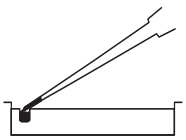
IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

POUR gel

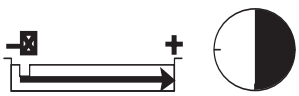


SET

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.)
7. Set a micropipet to 30 µL. Resuspend cells in the remaining saline by pipetting in and out. Work carefully to minimize bubbles.

Food particles will not resuspend.

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.

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.

Boiling step: 99°C 10 minutes

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.

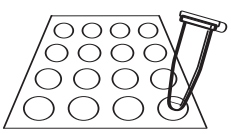
II. AMPLIFY DNA BY PCR

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 to the following profile for 30 cycles if you use ethidium bromide or 35 cycles if you are using *Carolina*BLU™. The profile may be linked to a 4°C hold program after cycling is completed.

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.

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.

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 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)
**Shared Reagents*	Microcentrifuge tube rack
*pBR322/*Bst*NI marker	Gel electrophoresis chamber
2% agarose in 1× TBE, 50 mL	Power supply
1× TBE, 300 mL	Staining trays
Ethidium bromide (1 µg/mL), 250 mL	Latex gloves
or	UV transilluminator (for use with ethidium bromide)
*Carolina*BLU™ Gel and Buffer Stain, 7 mL	White light transilluminator (for use with *Carolina*BLU™)
*Carolina*BLU™ Final Stain, 375 mL	Digital or instant camera (optional)
	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.

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.

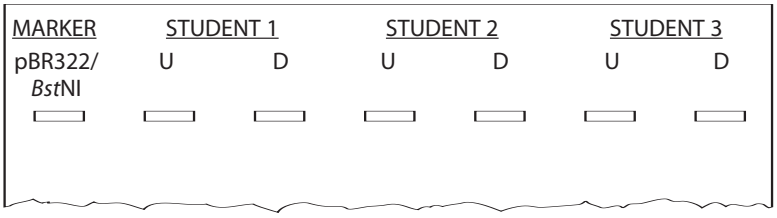
100-bp ladder may also be used as a marker.

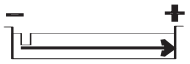
6. Use a micropipet with a fresh tip to load 20 µL of pBR322/*Bst*NI size markers into the far left lane of the gel.

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.





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.

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 *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.

BIOINFORMATICS

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′-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 *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.
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?

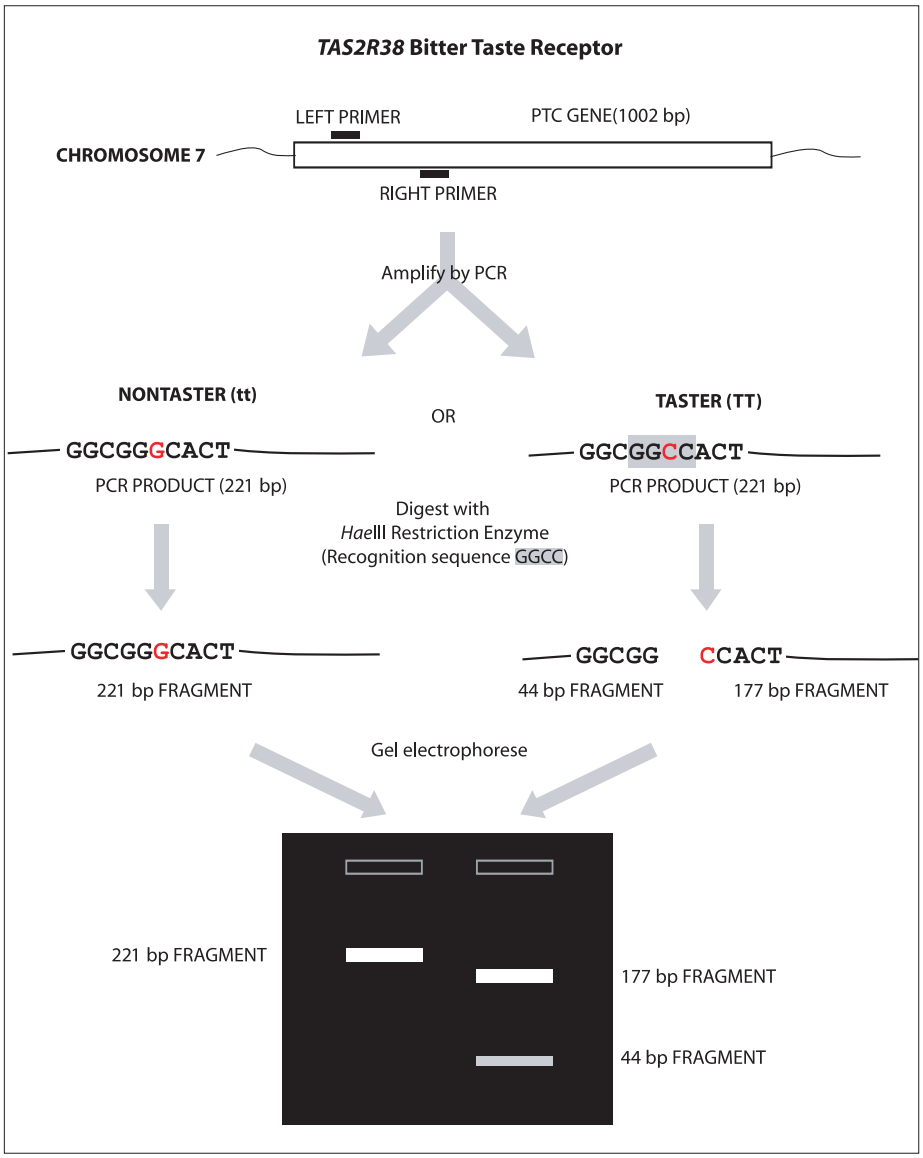
h. *Human PTC taster* vs. *human PTC non-taster.* List the nucleotide position(s) and nucleotide differences of any additional SNP(s). Count triplets of nucleotides from the initial ATG start codon to determine codon(s) affected by SNP(s). Use a standard genetic code chart to determine if an amino acid is changed by each SNP.

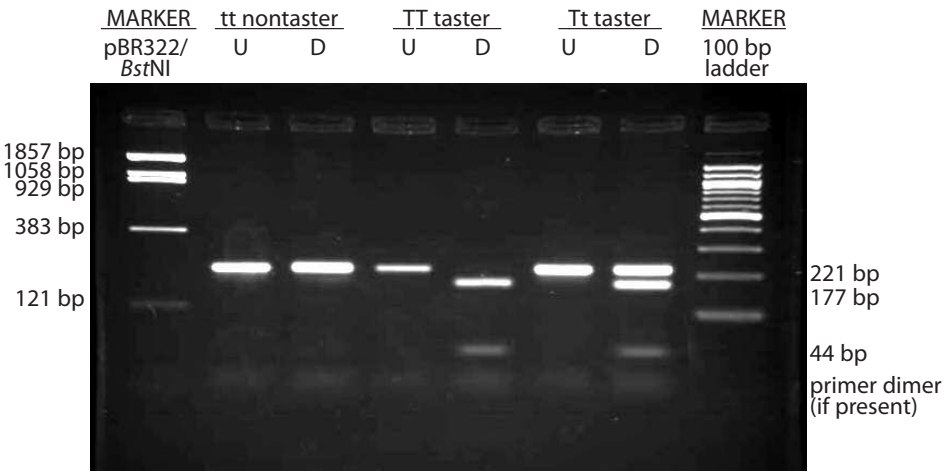
i. *Human PTC taster* vs. *human PTC non-taster* vs. *chimpanzee* vs. *bonobo* vs. *gorilla*. What is the ancestral (original) state of this gene at nucleotide positions 145, 785, and 886? Are other primates tasters or non-tasters, and what does this suggest about the function of bitter taste receptors? 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 *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 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.

	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 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?

INFORMATION FOR INSTRUCTOR

CONCEPTS AND METHODS

This laboratory can help students understand several important concepts of modern biology:

- The relationship between genotype and phenotype.
- The use of single-nucleotide polymorphisms (SNPs) in predicting drug response (pharmacogenetics).
- A number of SNPs are inherited together as a haplotype.
- The movement between in vitro experimentation and in silico computation.

The laboratory uses several methods for modern biological research:

- DNA extraction and purification.
- Polymerase chain reaction (PCR).
- DNA restriction.
- Gel electrophoresis.
- Bioinformatics.

LAB SAFETY

The National Association of Biology Teachers recognizes the importance of laboratory activities using human body samples and has developed safety guidelines to minimize the risk of transmitting serious disease. ("The Use of Human Body Fluids and Tissue Products in Biology," *News & Views*, June 1996.) These are summarized below:

- Collect samples only from students under your direct supervision.
- Do not use samples brought from home or obtained from an unknown source.
- Do not collect samples from students who are obviously ill or are known to have a serious communicable disease.
- Have students wear proper safety apparel: latex or plastic gloves, safety glasses or goggles, and lab coat or apron.
- Supernatants and samples may be disposed of in public sewers (down lab drains).
- Have students wash their hands at the end of the lab period.
- Do not store samples in a refrigerator or freezer used for food.

The risk of spreading an infectious agent by this lab method is much less likely than from natural atomizing processes, such as coughing or sneezing. Several elements further minimize any risk of spreading an infectious agent that might be present in mouthwash samples:

- Each experimenter works only with his or her sample.
- The sample is sterilized during a 10-minute boiling step.
- There is no culturing of the samples that might allow growth of pathogens.
- Samples and plasticware are discarded after the experiment.

INFORMED CONSENT AND DISCLOSURE

Student participation in this experiment raises real-life questions about the use of personal genetic data: What is my DNA sample being used for? Does my DNA type tell me anything about my life or health? Can my data be linked personally to me?

There is consensus that a human DNA sample should be obtained only with the willing consent of a donor, who understands the purpose for which it is being collected. Thus, this experiment should be explained ahead of time and students given the option to refrain from participating. (Some teachers may wish to have parents sign a consent form, such as those filled out for a field trip.) There is also consensus that a DNA sample be used only for the express purpose for which it is collected. Thus, student DNA samples should be thrown away after completing the experiment.

The *TAS2R38* polymorphism was specifically selected to demonstrate the relationship between genotype and PTC-tasting phenotype, because it has no known relationship to disease states or sex determination.

TAS2R38 alleles are inherited in a Mendelian fashion and can give indications about family relationships. To avoid the possibility of suggesting inconsistent inheritance, it is best not to generate genotypes from parent-child pairs. In any event, this two-allele system would be less likley to turn up an inconsistency than the ABO blood groups. Furthermore, the chance that student samples can be mixed up when isolating DNA, setting up PCR reactions, and loading electrophoresis gels provides no certainty to any of the genotypes obtained in the experiment. (A forensic laboratory would use approved methods for maintaining "chain of custody" of samples and for tracking samples.)

INSTRUCTOR PLANNING, PREPARATION, AND LAB FINE POINTS

The following table will help you to plan and integrate the four parts of the experiment.

Part		Day	Time	Activity	
I.	Isolate DNA	1	60 min.	**Pre-lab:**	Prepare and aliquot saline solution. Prepare and aliquot 10% Chelex®. Make centrifuge adapters. Set up student stations.
			30 min.	**Lab:**	Isolate student DNA.
II.	Amplify DNA by PCR	1	15 min. 15 min. 60–90 min.	**Pre-lab:** **Lab:** **Post-lab:**	Aliquot PTC primer/loading dye mix. Set up PCR reactions. Amplify DNA in thermal cycler.
III.	Digest PCR Products with *Hae*III	2	30 min. 15 min. 30 min.	**Pre-lab:** **Lab:**	Aliquot *Hae*III restriction enzyme. Set up student stations. Set up *Hae*III restriction digests. Incubate restriction digests at 37°C.
IV.	Analyze PCR Products by Gel Electrophoresis	2 3	15 min. 30 min. 15 min 20+ min. 20+ min. 30–45 min. to overnight 20 min.	**Pre-lab:** **Lab:** **Post-lab:**	Dilute TBE electrophoresis buffer. Prepare agarose gel solution and cast gels. Load DNA samples into gel. Electrophorese samples. Stain gels. De-stain gels (for *Carolina*BLU™). Photograph gels.
	Results and Discussion	4	30–60 min.	Correlate *TAS2R38* genotypes with PTC tasting.	

I. ISOLATE DNA BY SALINE MOUTHWASH

Saline mouthwash is the most effective method of cell collection for PCR. Cells are gently loosened from the inside of the cheek, yielding small groups of several cells each. This maximizes the surface area of cells, allowing for virtually complete lysis during boiling. Experience has shown that the mouthwash procedure produces interpretable PCR results in 85%–95% of samples. Food particles rinsed out with the mouthwash have little effect on PCR amplification but may obstruct passage of fluid through pipet tips and make pipetting difficult. For that reason, it is advisable not to eat immediately before the experiment—especially fruits.

It is worth a diversion to allow students to view their own squamous epithelial cells under a compound microscope. Touch a toothpick to the cell pellet formed in Methods, Step I.6. Smear the cell debris on a microscope slide, add a drop of 1% methylene blue (or other stain), and add a coverslip.

DNA is liberated from cheek cells by boiling in 10% Chelex®, which binds contaminating metal ions that are the major inhibitors of PCR. The boiling step is most easily accomplished using the same thermal cycler used for PCR. To do this, provide each student with 100 µl of 10% Chelex® suspension in a PCR tube that is compatible with the thermal cycler you will be using: either 0.2 ml or 0.5 ml. It is not necessary to use a "thin-walled" tube. Alternatively, use 1.5 ml tubes in a heat block or a boiling water bath. *Watch out for lids opening as the tubes heat*. (Make a simple water bath by maintaining a beaker of water at a low boil on a hot plate. Place 1.5 ml tubes in a floating rack or in holes punched in a double layer of aluminum foil over the top. If using aluminum foil, insure that tubes are immersed, and add hot water as necessary to maintain water level.)

Pre-lab Preparation

Prepare saline by dissolving 0.9 g NaCl in 100 mL distilled or deionized water. For each student, aliquot 10 mL into a 15-mL polypropylene tube.

Prepare 10% Chelex® by adding 15 mL distilled or deionized water to 1.5 g of Chelex®. For each student, aliquot 100 µL of 10% Chelex® into either a 0.2-mL or 0.5-mL tube (whichever format is accommodated by your thermal cycler). Alternatively, use a 1.5-mL microcentrifuge tube if you are planning to use a heat block or water bath instead of a thermal cycler. *The Chelex® resin quickly settles, so be sure to shake the stock tube to re-suspend the Chelex® each time before pipetting a student aliquot.*

Remove caps from 1.5-mL tubes to use as adapters in which to centrifuge the 0.5-mL PCR tubes used for Chelex® extraction. Two adapters are needed to spin 0.2-mL PCR tubes—a capless 0.5-mL PCR tube is nested within a capless 1.5-mL tube.

Pre-lab Set Up for DNA Isolation (per student station)

Saline solution (0.9% NaCl), 10 mL (in 15-mL tube)
10% Chelex®, 100 µL (in 0.2- or 0.5-mL tube, depending on thermal cycler)
2 1.5-mL microcentrifuge tubes
Permanent marker
Micropipets and tips (10–1,000 µL)
Microcentrifuge tube rack
Container with cracked or crushed ice
Paper cup

Shared Items

Microcentrifuge
Microcentrifuge adapters for 0.2-mL or 0.5-mL PCR tubes
Thermal cycler
Vortexer (optional)

II. AMPLIFY DNA BY PCR

The primer/loading dye mix incorporates the appropriate primer pair (0.26 picomoles/µL of each primer), 13.8% sucrose, and 0.0081% cresol red. The inclusion of the loading dye components, sucrose and cresol red, allows the amplified product to be directly loaded into an agarose gel for electrophoresis. Each Ready-To-Go™ PCR Bead contains reagents so that when brought to a final volume of 25 µL, the reaction contains 2.5 units of *Taq* DNA polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, and 200 µM of each dNTP.

The lyophilized *Taq* DNA polymerase in the bead becomes active immediately upon addition of the primer/loading dye mix and template DNA. In the absence of thermal cycling, "nonspecific priming" at room temperature allows the polymerase to begin generating erroneous products, which can show up as extra bands in gel analysis. *Therefore, work quickly. Be sure the thermal cycler is set and have all experimenters set up their PCR reactions as a coordinated effort. Add primer/loading dye mix to all reaction tubes, then add each student template, and begin thermal cycling as quickly as possible. Hold reactions on ice until all are ready to load into the thermal cycler.*

PCR amplification from crude cell extracts is biochemically demanding, and requires the precision of automated thermal cycling. However, amplification of the *TAS2R38* locus is not complicated by the presence of repeated units. Therefore, the recommended amplification times and temperatures will work adequately for all types of thermal cyclers.

Pre-lab Preparation

Aliquot 25 µL of PTC primer/loading dye mix per student. The primer/loading dye mix may collect in the tube cap during shipping; pool the reagent by spinning the tube briefly in a microcentrifuge or by sharply tapping the tube bottom on the lab bench.

Pre-lab Setup for DNA Amplification (per student station)

*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
Container with cracked or crushed ice

*Store on ice

Shared Items

Mineral oil, 5 mL (depending on thermal cycler)
Thermal cycler

III. DIGEST PCR PRODUCTS WITH *Hae*III

The PCR buffer provides adequate salt and pH conditions for the *Hae*III enzyme, so no additional restriction buffer is required for the reaction.

Thirty minutes is the minimum time needed for complete digestion. If time permits, incubate reactions for 1 or more hours. After several hours, the enzyme will denature and lose activity. Stop the reactions whenever it is convenient, and store them in a freezer (–20°C), until ready to continue.

Pre-lab Preparation

Divide the *Hae*III enzyme into 4 10-µL aliquots, each of which will be shared by 7–8 students. Keep aliquots on ice at a central station.

Pre-lab Set Up for DNA Restriction (per student station)

PCR product from Part II (store on ice)
1.5 mL microcentrifuge tube
Microcentrifuge tube rack
Micropipet and tips (1-20 µL)
Container with cracked or crushed ice

Shared Item

4 10-µL aliquots *Hae*III restriction enzyme (store on ice)
Thermal cycler (or water bath or heat block)

IV. ANALYZE AMPLIFIED DNA BY GEL ELECTROPHORESIS

The cresol red and sucrose in the primer mix function as loading dye, so that amplified samples can be loaded directly into an agarose gel. This is a nice time saver. However, since it has relatively little sugar and cresol red, this loading dye is more difficult to use than typical loading dyes. So, encourage students to load carefully.

Plasmid pBR322 digested with the restriction endonuclease *Bst*NI is an inexpensive marker and produces fragments that are useful as size markers in this experiment. The size of the DNA fragments in the marker are 1,857 bp, 1,058 bp, 929 bp, 383 bp, and 121 bp. Use 20 µL of a 0.075-µg/µL stock solution of this DNA ladder per gel. Other markers or a 100-bp ladder may be substituted.

View and photograph gels as soon as possible after appropriate staining/destaining. Over time, the small-sized PCR products will diffuse through the gel and lose sharpness. Refrigeration will slow diffusion somewhat, but for best results view and photograph gels as soon as staining/destaining is complete.

Pre-lab Preparation

Prepare a 1× concentration of TBE by adding 75 mL of 20× concentrated stock into 1,425 mL of deionized or distilled water. Mix thoroughly.

Prepare a 2% agarose solution by adding 2 g of agarose to 100 mL of 1× TBE in a 500-mL flask or beaker. Heat the flask or beaker in a boiling water bath (approximately 15 minutes) or in a microwave oven (approximately 4 minutes) until the agarose is completely dissolved. You should no longer see agarose particles floating in solution when the beaker is swirled. Allow the agarose to cool to approximately 60°C, and hold at this temperature in a hot water bath. Cover beaker or flask with aluminum foil, and skim any polymerized "skin" off the top of the solution before pouring.

Pre-lab Setup for Gel Analysis (per student station)

PCR product and restriction digest from Part III (store on ice)
Container with cracked or crushed ice

Shared Items

pBR322/*Bst*NI markers, 20 µL per row of gel (thaw and store on ice)
2% agarose in 1× TBE (hold at 60°C), 50 mL per gel
1× TBE buffer, 50 mL per gel
Ethidium bromide (1 µg/mL), 250 mL
or
*Carolina*BLU™ Gel & Buffer Stain, 7 mL
*Carolina*BLU™ Final Stain, 375 mL
Micropipet and tips (1–20 µL)
Microcentrifuge tube rack
Gel electrophoresis chambers
Power supplies
Water bath for agarose solution (60°C)
Latex gloves
Staining tray
Transilluminator with digital or instant camera (optional)

*Carolina*BLU™ STAINING

POST-STAINING

1. Cover the electrophoresed gel with the *Carolina*BLU™ Final Stain and let it sit for 20–30 minutes. Agitate gently (optional).
2. After staining, pour the stain back into the bottle for future use. (The stain can be used 6–8 times.)
3. Cover the gel with deionized or distilled water to destain. Chloride ions in tap water can cause the staining to fade.
4. Change the water 3 or 4 times over the course of 30–40 minutes. Agitate the gel occasionally.
5. Bands that are not immediately present will become more apparent with time and will reach their maximum visibility if the gel is left to destain overnight in just enough water to cover the gel. Gels left overnight in a large volume of water may destain too much.

PRE-STAINING

*Carolina*BLU™ can also be used to stain the DNA while it is being electrophoresed. Pre-staining will allow students to visualize their results prior to the end of the gel run. However, post-staining is still required for optimum viewing.

To pre-stain the gel during electrophoresis, add *Carolina*BLU™ Gel and Buffer Stain in the amounts indicated in the table below. Note that the amount of stain added is dependent upon the voltage used for electrophoresis. *Do not use more stain than recommended. This may precipitate the DNA in the wells and create artifact bands.*

Gels containing *Carolina*BLU™ may be prepared one day ahead of the lab day, if necessary. However, gels stored longer tend to fade and lose their ability to stain DNA bands during electrophoresis.

Use the table below to add the appropriate volume of *Carolina*BLU™ stain to the agarose gel:

Voltage	Agarose Volume	Stain Volume
<50 Volts	30 mL	40 µL (1 drop)
	200 mL	240 µL (6 drops)
	400 mL	520 µL (13 drops)
>50 Volts	50 mL	80 µL (2 drops)
	300 mL	480 µL (12 drops)
	400 mL	640 µL (16 drops)

Use the table below to add the appropriate volume of *Carolina*BLU™ stain to 1× TBE buffer:

Voltage	Agarose Volume	Stain Volume
<50 Volts	500 mL	480 µL (12 drops)
	3000 mL	3 mL (72 drops)
>50 Volts	500 mL	960 µL (24 drops)
	2600 mL	5 mL (125 drops)

BIOINFORMATICS

Have students do the bioinformatics exercises before starting the experiment—or analyzing results. This should improve conceptual and practical understanding.

The onscreen Bio-i Guide *can be played from the included CD-ROM or from the Internet site http://bioinformatics.dnalc.org/ptc/. The default version (640 × 480 pixels) allows one to follow along with an open browser window. The full-screen version (1024 × 768 pixels) is best for demonstrations.*

ANSWERS TO BIOINFORMATICS QUESTIONS

I.2. c. Also notice that position 43 of the forward primer is missing. What does this mean? **This nucleotide did not match the genomic sequence.**

I.3. a. What does the *E-value* of *6e-12* mean? **This denotes 6×10^{-12}, or 6 preceded by 11 decimal places! Which is to say that the query has found strong matches in the database.**

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? **Yes, they are all examples of the *TAS2R38* bitter taste receptor from humans and other primates.**

e. **The primer set amplifies a 221-bp product.**

III.5. On what chromosome have you landed? **Chromosome 7.**

III.8. 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. **Its nearest neighbors are *CLECF5S* (a lectin domain gene) and *MGAM* (a gene that encodes a starch-digesting enzyme). These genes have multiple coding exons, with intervening introns—*TAS2R38* has a single coding exon.**

III.9. What do most of these have in common with *TAS2R38*, and what can you conclude? **They are primarily taste and olfactory receptors. *TAS2R38* is part of a "cluster" of sensory receptors, each having a single exon. In the first screen, there is a an olfactory receptor, designated OR, followed by three nonfunctional OR pseudogenes. In the next screen are three other members of the *TAS2R* family of taste receptors. Clustering of genes according to function is seen in many areas of the human and other genomes.**

IV.6. 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 is the SNP examined in the experiment, and what is the difference between taster and non-taster alleles? **The beginning of the gene is not amplified by the primers in this experiment. The amplicon tracks along with the taster and non-taster alleles from position 101 to 321. The SNP is at position 145: with a C in the taster allele and a G in the non-taster allele.**

IV.6. 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.

Nucleotide Position	TASTER		NONTASTER	
	Codon	Amino Acid	Codon	Amino Acid
145	CCA	proline	GCA	alanine
785	GCT	alanine	GTT	valine
886	GTC	valine	ATC	isoleucine

These three SNPs are inherited as a unit, or haplotype—with C-C-G correlating most strongly with bitter tasting ability.

IV.6. i. *Human PTC taster* vs. *human PTC non-taster* vs. *chimpanzee* vs. *bonobo* vs. *gorilla.* What is the ancestral 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? **The ancestral state of the *TAS2R38* gene is C145, C785, G886—so the nontasting alleles arose after the human lineage split from other primates. Other primates are PTC tasters, suggesting that the ability to detect bitter tastes has a selective advantage in avoiding poisonous plants, many of which are bitter. At some positions, one of the apes shares the SNP with humans. At other positions, apes share one SNP and humans share another. The bonobo differs from humans and other apes at a number of positions.**

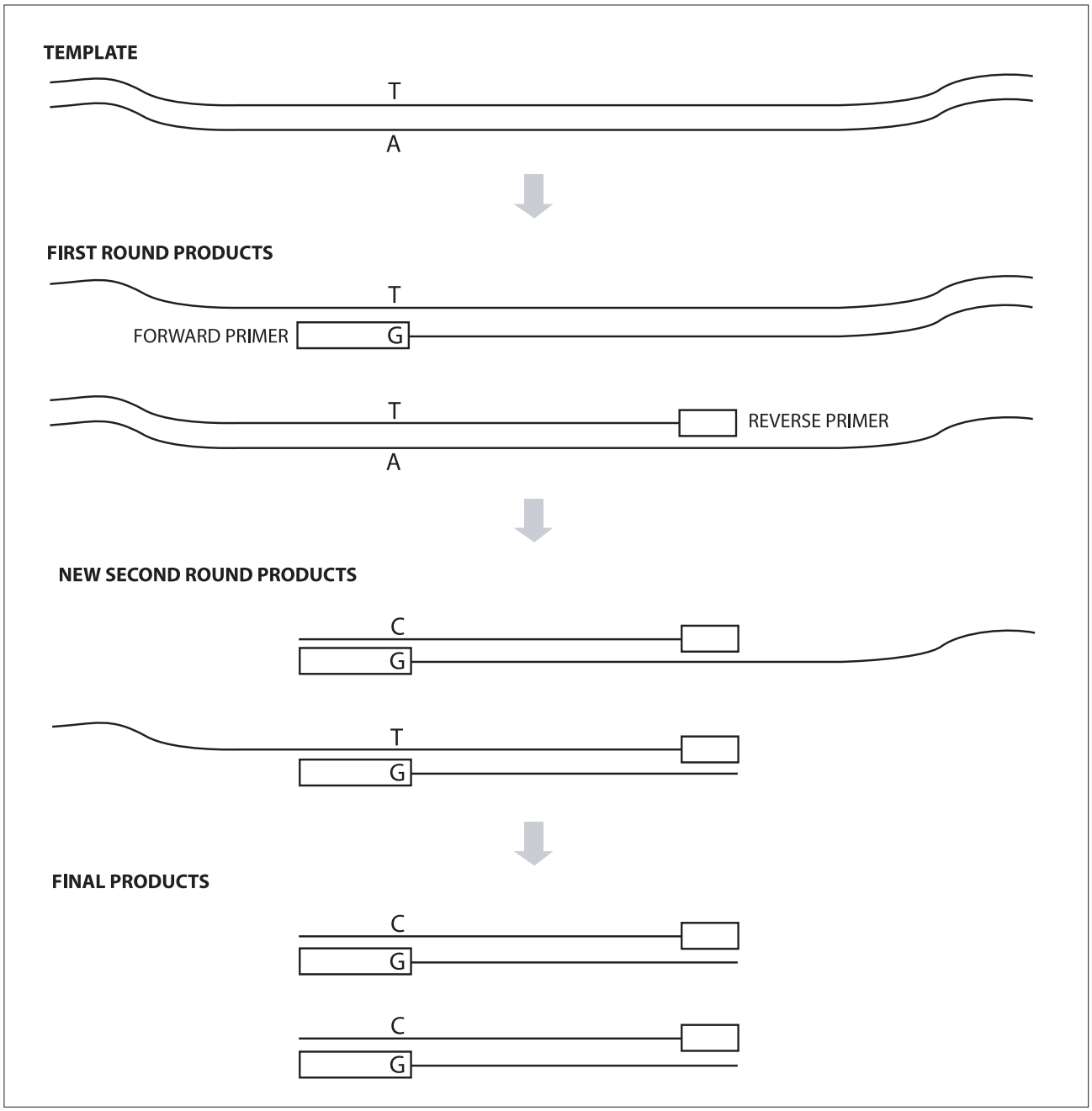
IV.6. j. *Forward primer* vs. *human PTC taster* vs. *human PTC non-taster.* Where does the forward primer bind? What discrepancy do you notice between the primer sequence and the *TAS2R38* gene sequence? Of what importance is this to the experiment? **The forward primer binds within the *TAS2R38* gene, from nucleotides 101–144. There is a single mismatch at position 143, where the primer has a G and the gene has an A. This mismatch is crucial to the PCR experiment, because the A in the PTC sequence is replaced by a G in each of the amplified products. This creates the first G of the *Hae*III recognition sequence GGCC, allowing the amplified taster allele to be cut. The amplified nontaster allele reads GGGC and is not cut.**

ANSWERS TO DISCUSSION QUESTIONS

3. According to your class results, how well does *TAS2R38* genotype predict PTC-tasting phenotype? What does this tell you about classical dominant/recessive inheritance? **The presence of a T allele generally predicts tasting, although heterozygotes are more likely to be weak tasters. Even in a relatively simple genetic system, such as PTC tasting, one allele rarely has complete dominance over another. This experiment examined only one of several mutations in the *TAS2R38* gene that influence bitter tasting. Furthermore, variability in taste perception is likely affected by processing in the brain, which involves numerous other genes.**

4. How does the *Hae*III enzyme discriminate between the C-G polymorphism in the *TAS2R38* gene? ***Hae*III cuts at the sequence GGCC. This sequence is found at nucleotide positions 143–146 of the *TAS2R38* gene segment amplified. A G nucleotide at position 145 of the non-taster allele changes the sequence so that it is no longer recognized by the restriction enzyme.**

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. **The dynamics of replication demand that every PCR product incorporates each of the two primers. Thus, the G in the forward primer is carried forward into all products of PCR amplification, but the A (in the template) is not. This G "creates" a *Hae*III recognition sequence that is not naturally present in the *TAS2R38* gene.**



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? **A synonymous mutation specifies the same amino acid as the wild-type allele; this is due to the redundancy of the genetic code. A nonsynonymous mutation creates a new codon, which specifies a different amino acid. The G-to-C change at position 145 changes the codon CCA (proline) to GCA (alanine). This amino acid change alters the ability of the *TAS2R38* receptor to bind PTC in a lock-and-key fashion.**

7. Research other mutations in the *TAS2R38* gene and how they may influence bitter taste perception. **The *TAS2R38* gene contains five SNPs, three of which particularly influence bitter taste perception. These SNPs are inherited as a unit, with one combination, or haplotype—proline/alanine/valine (PAV)—correlating most strongly with bitter-tasting ability.**

Nucleotide Position	TASTER		NONTASTER	
	Codon	Amino Acid	Codon	Amino Acid
145	**C**CA	proline	**G**CA	alanine
785	G**C**T	alanine	G**T**T	valine
886	**G**TC	valine	**A**TC	isoleucine

8. The frequency of PTC nontasting is higher than would be expected if bitter-tasting ability was 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? **Scientists are uncertain, but it may be that the nontasting alleles produce receptors that bind different sorts of bitter molecules. In this case, heterozygotes would have the advantage of detecting a greater range of potentially toxic molecules.**

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. **a) The FBI Combined DNA Index System (CODIS) uses a panel of 13 STR (short tandem repeat) polymorphisms for forensic DNA typing. b) Each STR locus is labeled with one of four fluorescent dyes, and the alleles are differentiated by DNA sequencing. c) Forensic DNA laboratories use a strict "chain of custody" to insure that samples remain with their correct identifying label. Validated lab methods insure that labels are checked during each step of the procedure.**

10. What ethical issues are raised by human DNA typing experiments? **Has the DNA sample been obtained with the willing consent of the donor, who understands the purpose for which it is being collected? Is the DNA used only for the express purpose for which it is collected, or is it also used for reasons other than those described to the donor? Is the DNA sample destroyed after its intended use, or is it stored for future use? Are the experimental results stored anonymously? Who has access to the results? Does the result of the experiment provide any unintended information—for example, about disease susceptibility or paternity?**

CD-ROM CONTENTS

The valuable companion CD-ROM is for exclusive use of purchasers of this DNA Learning Center Kit. To accommodate home or computer lab use by students, all materials may also be reached at the companion Internet site http://bioinformatics.dnalc.org/ptc/.

- **Protocol:** a unique online lab notebook with the complete experiment, as well as printable PDF files.
- **Resources:** 13 animations on key techniques of molecular genetics and genomic biology, from the award-winning Internet site, *DNA Interactive*.