

## INFORMATION FOR INSTRUCTOR

### CONCEPTS AND METHODS

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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

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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

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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 likely 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.	<b>Pre-lab:</b> Prepare and aliquot saline solution. Prepare and aliquot 10% Chelex®. Make centrifuge adapters. Set up student stations.
		30 min.	<b>Lab:</b> Isolate student DNA.
II. Amplify DNA by PCR	1	15 min.	<b>Pre-lab:</b> Aliquot PTC primer/loading dye mix.
		15 min.	<b>Lab:</b> Set up PCR reactions.
		60–90 min.	<b>Post-lab:</b> Amplify DNA in thermal cycler.
III. Digest PCR Products with <i>HaeIII</i>	2	30 min.	<b>Pre-lab:</b> Aliquot <i>HaeIII</i> restriction enzyme. Set up student stations.
		15 min.	<b>Lab:</b> Set up <i>HaeIII</i> restriction digests.
		30 min.	Incubate restriction digests at 37°C.
IV. Analyze PCR Products by Gel Electrophoresis	2	15 min.	<b>Pre-lab:</b> Dilute TBE electrophoresis buffer.
		30 min.	<b>Lab:</b> Prepare agarose gel solution and cast gels.
	3	15 min.	Load DNA samples into gel.
		20+ min.	Electrophorese samples.
		20+ min.	<b>Post-lab:</b> Stain gels.
		30–45 min. to overnight	De-stain gels (for <i>CarolinaBLU</i> ™).
20 min.	Photograph gels.		
Results and Discussion	4	30–60 min.	Correlate <i>TAS2R38</i> 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<sup>®</sup>, 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  $\mu$ l of 10% Chelex<sup>®</sup> 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<sup>®</sup> by adding 15 mL distilled or deionized water to 1.5 g of Chelex<sup>®</sup>. For each student, aliquot 100  $\mu$ L of 10% Chelex<sup>®</sup> 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<sup>®</sup> resin quickly settles, so be sure to shake the stock tube to re-suspend the Chelex<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup>, 100  $\mu$ L (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  $\mu$ L)
- 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/ $\mu\text{L}$  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  $\mu\text{L}$ , the reaction contains 2.5 units of *Taq* DNA polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM  $\text{MgCl}_2$ , and 200  $\mu\text{M}$  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  $\mu\text{L}$  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  $\mu\text{L}$  (from Part I)
- \*PTC primer/loading dye mix, 22.5  $\mu\text{L}$
- Ready-To-Go™ PCR beads (in 0.2-mL or 0.5-mL PCR tube)
- Permanent marker
- Micropipet and tips (1–100  $\mu\text{L}$ )
- 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 ( $-20^\circ\text{C}$ ), until ready to continue.



### **Pre-lab Preparation**

Divide the *HaeIII* enzyme into 4 10- $\mu$ L 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  $\mu$ L)
- Container with cracked or crushed ice

#### **Shared Item**

- 4 10- $\mu$ L aliquots *HaeIII* 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  $\mu$ L of a 0.075- $\mu$ g/ $\mu$ L 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 $\times$  concentration of TBE by adding 75 mL of 20 $\times$  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 $\times$  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  $\mu$ L per row of gel (thaw and store on ice)

2% agarose in 1 $\times$ TBE (hold at 60°C), 50 mL per gel

1 $\times$ TBE buffer, 50 mL per gel

Ethidium bromide (1  $\mu$ g/mL), 250 mL

**or**

*Carolina*BLU™ Gel & Buffer Stain, 7 mL

*Carolina*BLU™ Final Stain, 375 mL

Micropipet and tips (1–20  $\mu$ L)

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)

## CarolinaBLU™ STAINING

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### POST-STAINING

1. Cover the electrophoresed gel with the *CarolinaBLU™* 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

*CarolinaBLU™* 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 *CarolinaBLU™* 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 *CarolinaBLU™* 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 *CarolinaBLU™* 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 *CarolinaBLU™* 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

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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

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- 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  $6 \times 10^{-12}$ , 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 *CLECF55* (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 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

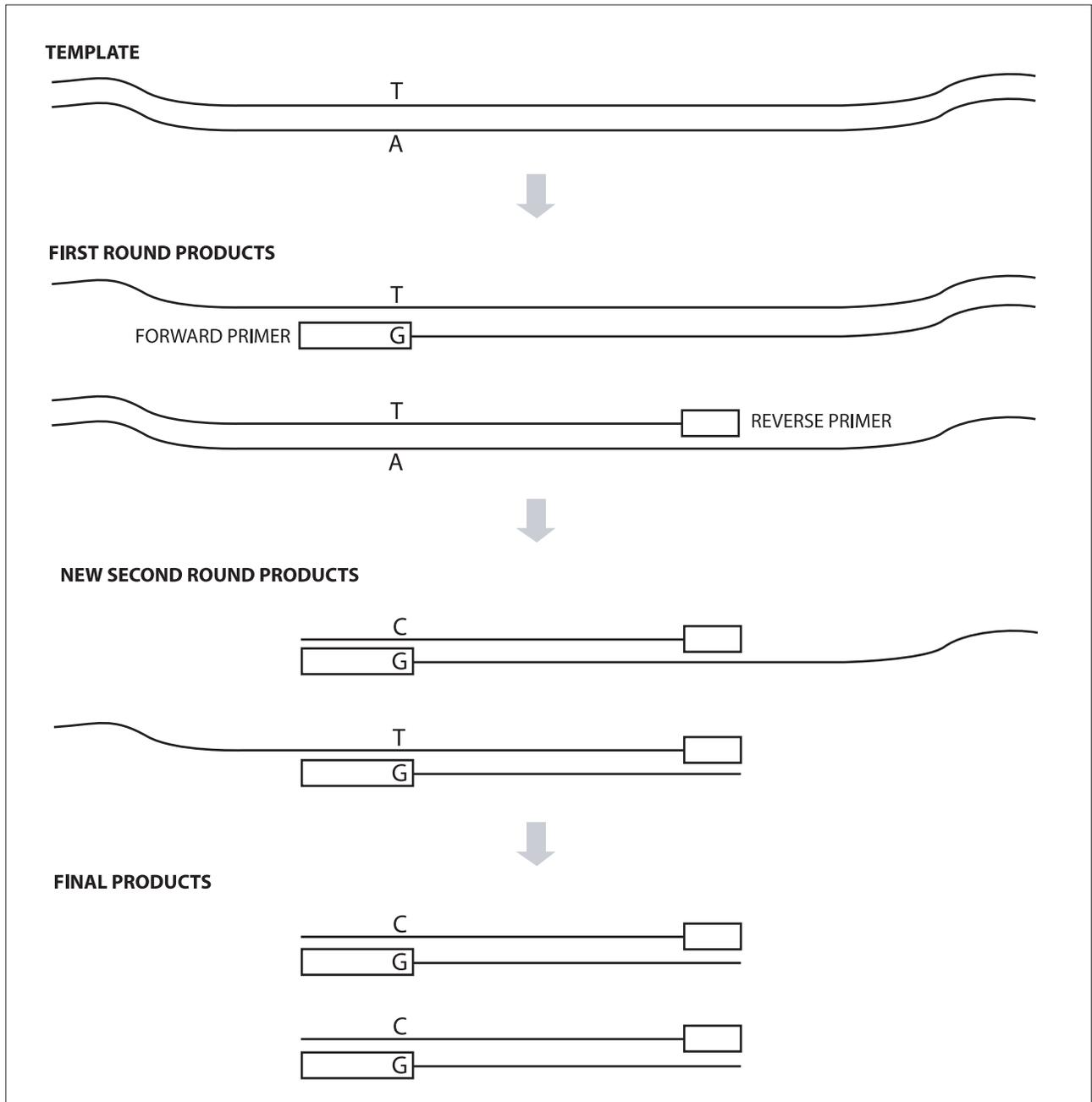
**These three SNPs are inherited as a unit, or haplotype—with C-C-G correlating most strongly with bitter tasting ability.**

- 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 *HaeIII* 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 *HaeIII* enzyme discriminate between the C-G polymorphism in the *TAS2R38* gene? ***HaeIII* 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 *HaeIII* recognition site, GGCC. How is this different from the sequence of the human *TAS2R38* gene? What characteristic of the PCR reaction allows the primer sequence to “override” the natural gene sequence? Draw a diagram to support your contention. **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 *HaeIII* 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	<u>C</u> CA	proline	<u>G</u> CA	alanine
785	G <u>C</u> T	alanine	G <u>T</u> T	valine
886	<u>G</u> TC	valine	<u>A</u> 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

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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*.