

Edvo-Kit #

345

Edvo-Kit #345

Exploring the Genetics of Taste: SNP Analysis of the PTC Gene Using PCR

Experiment Objective:

In this experiment, students will isolate their own DNA and use PCR to amplify a segment of the TAS2R38 gene, which is responsible for detecting the bitter taste of PTC. Digestion of the PCR products and analysis by agarose gel electrophoresis are used to differentiate tasters and non-tasters. Finally, students' genotype is linked to phenotype by tasting the PTC paper.

See page 3 for storage instructions.

Version 345.240214

PROTOCOL HAS BEEN UPDATED!
Please review before beginning experiment!

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Experiment Components

Perishable Components

	Storage	Check (✓)
• PCR EdvoBeads™ PLUS*	NEW Update: -20°C	☐
A Universal DNA Buffer	-20°C Freezer	☐
B TE Buffer	-20°C Freezer	☐
C LyphoPrimer™ Mix	-20°C Freezer	☐
D LyphoControl™ (Complete PCR control)	-20°C Freezer	☐
E Dryzymes® Restriction Enzyme <i>Hae</i> III	-20°C Freezer	☐
F Restriction Enzyme Dilution Buffer	-20°C Freezer	☐
G 100 bp ladder	-20°C Freezer	☐
• Proteinase K	-20°C Freezer	☐
• SYBR® Safe Stain	-20°C Freezer	☐
• PTC Paper	Room temperature	☐
• Control Taste Paper	Room temperature	☐

NOTE: Components C and D are supplied in our LyphoPrimer™ and LyphoControl™ format. They will require reconstitution prior to use. Be sure to review page 21 in the Instructor's Guide for more details.

*Each PCR EdvoBead™ PLUS contains: dNTP Mixture, Taq DNA Polymerase Buffer, Taq DNA Polymerase, MgCl₂ and Reaction Buffer).

This kit contains enough reagents for 25 DNA reactions plus 5 controls.

Sample volumes are very small. It is important to quickly spin the tube contents in a microcentrifuge to obtain sufficient volume for pipetting. Spin samples for 10-20 seconds at maximum speed.



NEW Update:

PCR EdvoBeads™ are now conveniently stored at -20°C with the other perishable components.

Reagents & Supplies

Store all components below at room temperature.

• UltraSpec-Agarose™	☐
• TBE Electrophoresis Buffer Powder	☐
• Disposable plastic cups	☐
• 15 mL Conical Tube	☐
• Snap-top microcentrifuge tubes	☐
• Microcentrifuge Tubes (1.5 mL screw-cap tube – use for boiling)	☐
• 0.2 mL PCR tubes	☐
• Salt packets	☐



NOTE: Check that your PCR program matches the protocol found in the student and instructor's guides before starting the experiment!

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Requirements *(NOT included in this experiment)*

- Thermal cycler (EDVOTEK® Cat. #540 or #541-542 highly recommended) or three water baths*
- Two Water bath for 55°C and 99°C incubations (EDVOTEK® Cat. #539 highly recommended)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- UV Transilluminator or Blue light visualization (EDVOTEK® Cat #557 recommended)
- UV safety goggles
- Automatic micropipettes (5-50 µL) with tips
- Microwave or hot plate
- Pipet pump
- 250 mL flasks or beakers
- Hot gloves
- Disposable laboratory gloves
- Ice buckets and ice
- Distilled or deionized water - 3.7 L (*NOTE: for ease of preparation, we recommend purchasing one gallon [3.78 L] of distilled water.*)
- Drinking water
- Bleach solution

**If you do not have a thermal cycler, this experiment can be conducted using three water baths with proper care. However, a thermal cycler assures a significantly higher rate of success. See Appendix C for detailed instructions.*



Background Information

Four nucleotide letters specify the genetic code: A (Adenine), C (Cytosine), T (Thymine), and G (Guanine). A point mutation occurs when one nucleotide is replaced by another nucleotide. For example when an A is replaced by a C, T or G (Figure 1). When such a mutation is present in at least 1% of the population it is known as a Single Nucleotide Polymorphism or SNP (pronounced "snip".) A SNP can also occur when a single base pair has been deleted or added to a sequence.

SNPs are the most common type of genetic variation among people. They occur frequently in the non-coding regions of genes and in regions between genes. Although these SNPs do not automatically translate into amino acids they may still affect protein production through gene splicing, transcription factor binding, or non-coding RNA sequencing. An SNP can also occur in the coding sequence of a gene, where it can affect the protein product of that gene. For example sickle cell anemia occurs because a single nucleotide polymorphism causes the hydrophilic amino acid glutamic acid to be replaced with the hydrophobic amino acid valine in the β -globin chain of hemoglobin. However, in other cases, the alteration may not necessarily change the amino acid sequence of the protein due to codon degeneracy. In this experiment we will examine a SNP that occurs at the nucleotide position 145 of the Phenylthiocarbamide (PTC) Sensitivity gene *TAS2R38*.

Individuals vary greatly in their sensitivity to the bitter compound PTC (Figure 2). This fact was discovered in 1931 in a series of events that involved impressive scientific curiosity and questionable laboratory safety. A chemist named Arthur Fox was mixing a powdered chemical when he accidentally let a bit of the powder blow into the air. A nearby colleague exclaimed how bitter the powder tasted, but Fox (who was closer to the chemical) tasted nothing. Interested, both men took turns tasting the chemical. Fox continued to find the chemical tasteless while his colleague found it bitter. Next, Fox tested a large number of people. Again he found a mix of "tasters" and "non-tasters" and published his findings. This caught the interest of geneticist L.H. Snyder who tested the compound on families and hypothesized that the taster/non-taster state was genetically determined.

Ability to taste PTC compound is now linked to the presence of the protein Taste receptor 2 member 38 that is encoded by the *TAS2R38* gene. *TAS2R38* has two alleles: the dominant allele (T), which confers the ability to taste PTC, and the recessive non-taster allele (t). A person inherits one copy of the gene from each of his/her parents. The combination of these different alleles within an individual is referred to as a genotype, which in turn dictates phenotype: in this case whether an individual is a "taster" or "non-taster". PTC-tasters have one of two possible genotypes; either they are homozygous dominant and have two copies of the taster allele (TT), or they are heterozygous and have one taster allele and one non-taster allele (Tt). "Non-tasters" are homozygous recessive and have two copies of the non-taster allele (tt). Within the general population, about 70% of the people tested can taste PTC, whereas the other 30% cannot.

Sequence analysis along the coding region of *TAS2R38* revealed that PTC taster and non-taster alleles differ in 3 amino acids due to SNPs at 3 distinct locations (Table 1). There are five ver-

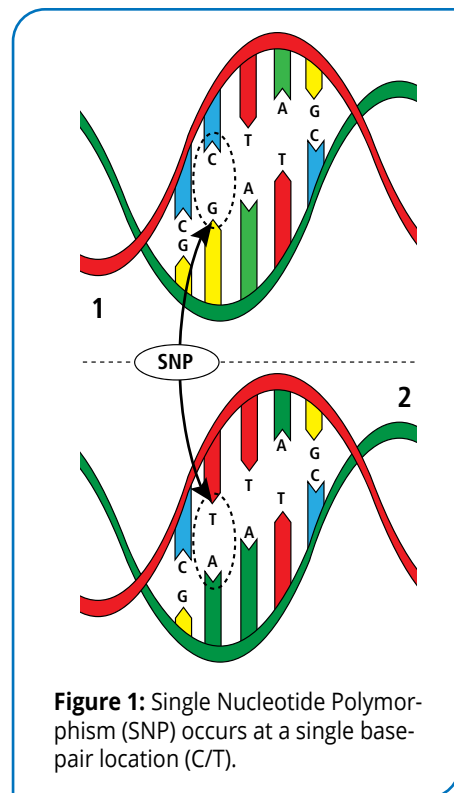
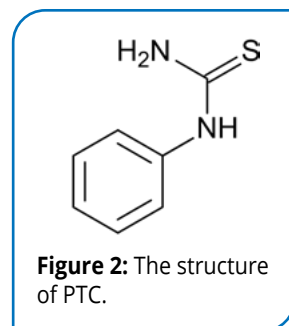


Figure 1: Single Nucleotide Polymorphism (SNP) occurs at a single base-pair location (C/T).



sions of the gene found worldwide: AVI, AAV, AAI, PAV, and PVI, named for the combination of amino acids present in the gene. The two most common haplotypes are AVI and PAV, representing non-tasters and tasters, respectively. Changes in the amino acid sequence alter the shape of the receptor protein which determines how strongly it can bind to PTC. Since all people have two copies of every gene, combinations of the bitter taste gene variants determine whether someone finds PTC intensely bitter, somewhat bitter, or without taste at all. This can be roughly quantified by a taste test or more accurately characterized by determining the nucleotides at positions 145, 785, and 886.

TABLE 1:
Relationship of Variations at Specific locations in *TAS2R38* gene and Ability to Taste PTC

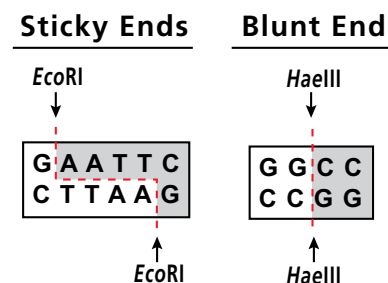
Nucleotide Position	Change in Nucleotide (Non-taster > Taster)	Change in Codon (Non-taster > Taster)	Change in Amino Acid (Non-taster > Taster)
145	G > C	GCA > CCA	Alanine > Proline
785	T > C	GTT > GCT	Valine > Alanine
886	A > G	ATC > GTC	Isoleucine > Valine

One way to detect a SNP is to use a restriction enzyme. Restriction enzymes are endonucleases that catalyze the cleavage of the phosphate bonds within both strands of DNA. The distinguishing feature of restriction enzymes is that they only cut at very specific sequences of bases. These

recognition sites are usually 4 to 8 base pairs in length and cleavage occurs within or near the site. Recognition sites are frequently symmetrical, i.e., both DNA strands in the site have the same base sequence when read 5' to 3'. Such sequences are called palindromes. A single base change in the recognition palindrome results in the inability of the restriction enzyme to cut the DNA at that location. This will alter the length and number of DNA fragments generated after digestion. These fragments can be separated according to their lengths by gel electrophoresis. The process of enzyme digestion followed by electrophoresis is often referred to as Restriction Fragment Length Polymorphism (RFLP) analysis (Figure 3). In this experiment, you will use RFLP analysis to examine the nucleotide at position 145 in your *TAS2R38* gene.

Consider the recognition site and cleavage pattern of *EcoRI* and *HaeIII*, at right. Arrows indicate the cleavage positions. Digestion with *EcoRI* produces asymmetric "sticky ends" whereas *HaeIII* restriction enzyme cleaves produces blunt ends.

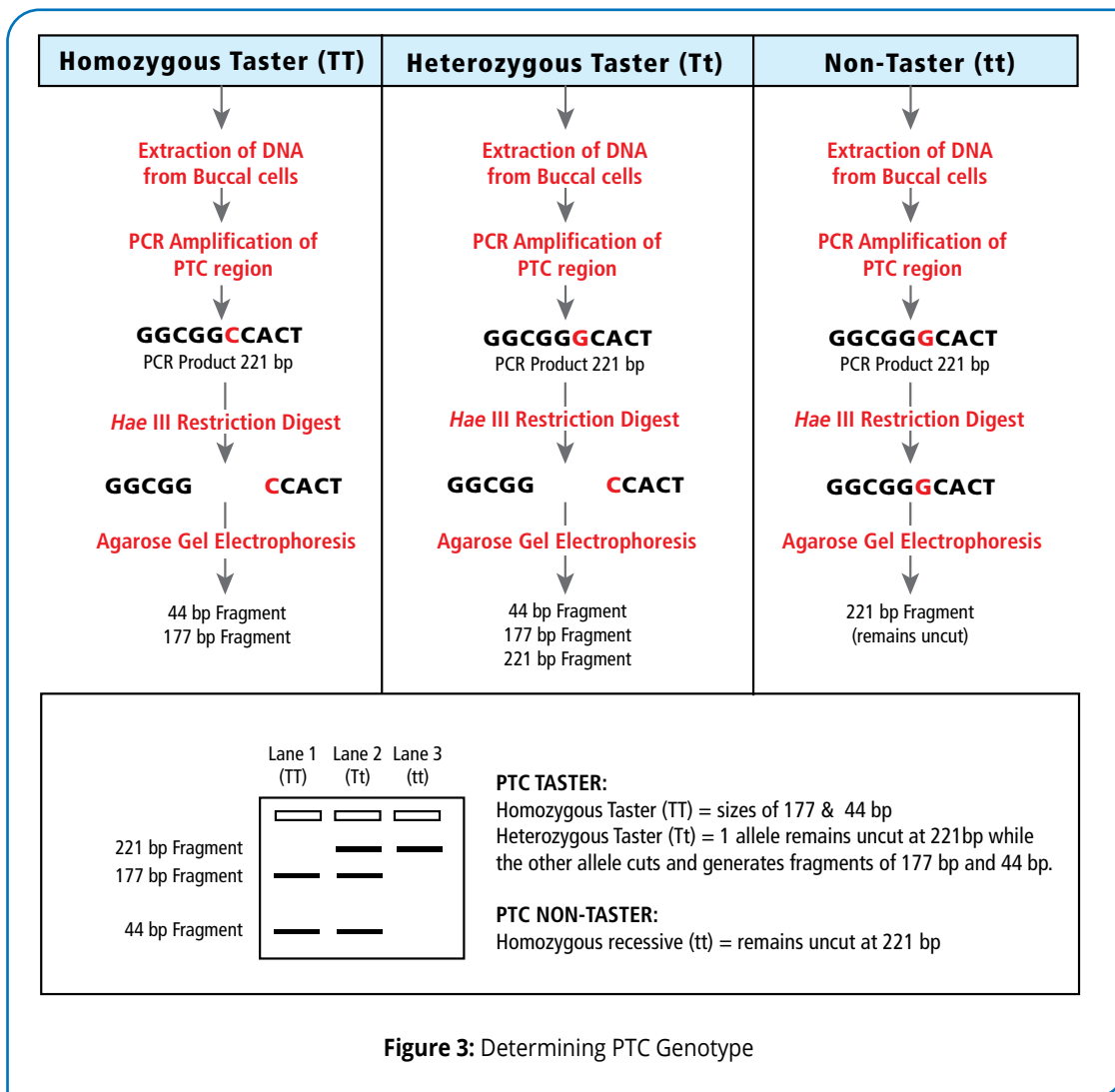
In the example of the PTC gene, *HaeIII* only cuts the taster allele (5'-GGCGGCCACT-3'). The polymorphism present in the non-taster allele (5'-GGCGGGCACT-3') changes a single base change in the restriction enzyme recognition site, so *HaeIII* can not digest non-taster DNA.



THE POLYMERASE CHAIN REACTION (PCR)

In order to visually see the effect of *HaeIII* on the first 221 bp of *TAS2R38* this DNA region must be selected and amplified. This is accomplished using the polymerase chain reaction (PCR). PCR is a technique that generates thousands to millions of copies of a particular DNA sequence. The procedure was invented by Dr. Kary Mullis while at the Cetus Corporation in 1984. Because PCR has revolutionized almost all aspects of biological research Dr. Mullis was awarded a Nobel Prize for his work in 1994. The enormous utility of PCR is based on its procedural simplicity and its specificity.

In preparation for PCR amplification, a set of two DNA "primers" is designed to target a specific region of the genomic sequence. The primers are synthetic oligonucleotides typically 15-30 base pairs in length that correspond to the start and end of the targeted DNA region. Primers bind to the template DNA -- in this case the extracted DNA from individuals who show differences in their ability to taste PTC. In addition to the two primers, four deoxynucleotides (dATP, dCTP, dGTP, and dTTP) and a thermally stable DNA polymerase are required. The most commonly used DNA polymerase is the enzyme *Taq* polymerase, which is purified from the thermophilic bacterium *Thermus aquaticus* that inhabits hot springs. This enzyme is stable at near-boiling temperatures.



The PCR process requires sequentially heating and cooling the mixture at three different temperatures (Figure 4). It is efficiently performed in a thermal cycler, an instrument that is programmed to rapidly heat, cool, and maintain samples at designated temperatures for varying amounts of time. In the first step of the PCR reaction, the mixture is heated to near boiling (94°C) in order to disrupt the hydrogen bonds between DNA strands. This step, which results in the complete separation of the two DNA strands, is known as “denaturing”. In the second PCR step, the sample is cooled to a temperature in the range of 45° - 65°C. In this step, known as “annealing,” the two primers bind to their target complements. In the third step, known as “extension” (also called DNA synthesis), the temperature is raised to an intermediate value (usually 72°C). At this temperature, the *Taq* polymerase proteins bind to each separated DNA strand + primer combo. *Taq* then adds the four free deoxynucleotides (dATP, dCTP, dGTP, and dTTP) to a growing complementary strand. The order of deoxynucleotides along this strand is determined by Watson-Crick base pairing with the original strand.

These three steps -- denaturation, annealing, and extension -- constitute one PCR “cycle.” Each cycle doubles the amount of the target DNA. Calculated mathematically, if the cycle is repeated n times the number of copies will be an exponential enlargement of 2^n . For example, ten cycles will produce 2^{10} or 1024 copies. The PCR process is typically repeated for 20-40 cycles, theoretically amplifying the target sequence to millions of copies. In practice, the amount of product reaches a maximum after about 35 cycles due to the depletion of reaction components and the loss of DNA polymerase activity.

In this experiment, the students will use the PCR-RFLP method to examine the presence of an amino acid coding SNP. Students will use the PCR to amplify a polymorphic region of the *TAS2R38* gene. The amplified DNA will be digested with the restriction enzyme *HaeIII* to determine their genotype at position 145, which correlates with the ability to taste PTC. Agarose gel electrophoresis of the restriction-digestion PCR products will reveal the 2 alleles of the *TAS2R38* gene, indicating whether a student is homozygous or heterozygous for the taster phenotype, or a homozygous non-taster. In the final module, students will test their ability to taste the bitter PTC and correlate their genotype with their phenotype.

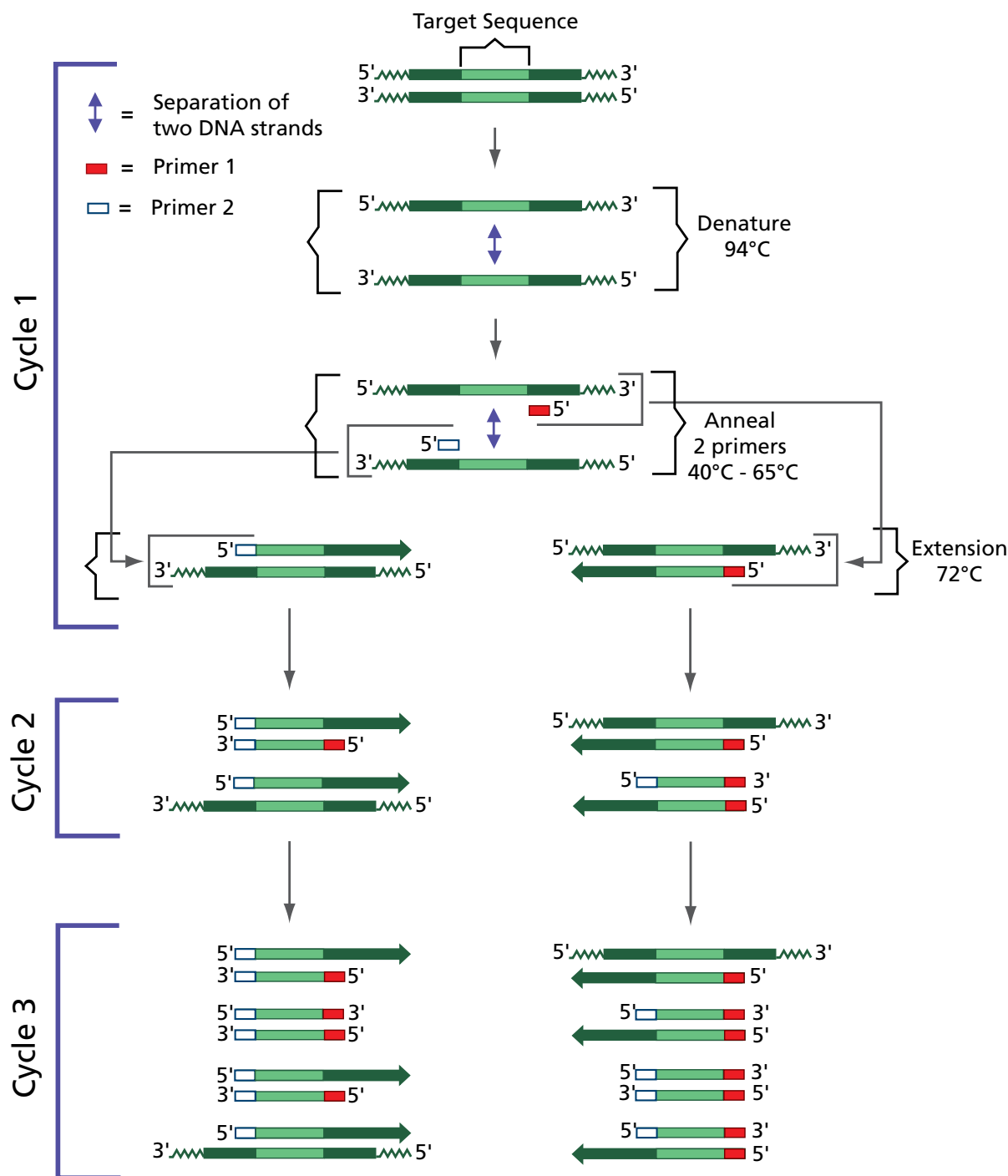


Figure 4: DNA Amplification by the Polymerase Chain Reaction

Experiment Overview

EXPERIMENT OBJECTIVE

The objective of this experiment is for students to isolate human DNA and use PCR to amplify a segment of the *TAS2R38* gene, which is responsible for detecting the bitter taste of PTC.

In **MODULE I**, Human DNA is collected from student cheek cells after a brief incubation in lysis buffer, followed by boiling the cell suspension. In **MODULE II**, the human DNA is mixed with PCR primers and a PCR EdvoBead™ PLUS, then PCR is performed in a thermal cycler. Once the *TAS2R38* gene has been amplified, the PCR product is mixed with the *HaeIII* restriction enzyme during **MODULE III**.

Finally, in **MODULES IV and V**, students will compare their genotype with their phenotype - in this case, the ability to taste PTC. Digested and undigested DNA is analyzed by agarose gel electrophoresis allowing students to detect the presence of a SNP in their *TAS2R38* gene. This genotype result is linked to phenotype by tasting the PTC paper.

IMPORTANT

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Exercise caution when working in the laboratory.
- Wear gloves and goggles at all times.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- Do not mouth pipet reagents - use pipet pumps.
- Always wash hands thoroughly with soap and water after working in the laboratory. Contaminated laboratory waste (saliva solution, cup, pipet, etc.) must be disinfected with 15% bleach solution prior to disposal.

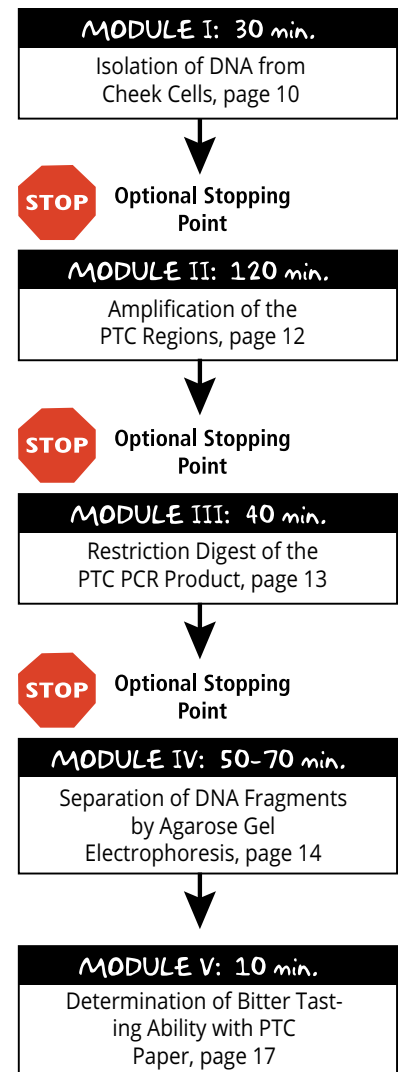
LAB PREPARATIONS

Prelab preparations begin on page 19. Please review the entire Instructor's Guide before beginning the experiment.

Before running the experiment, confirm that the program on your thermal cycler matches the PCR settings below:



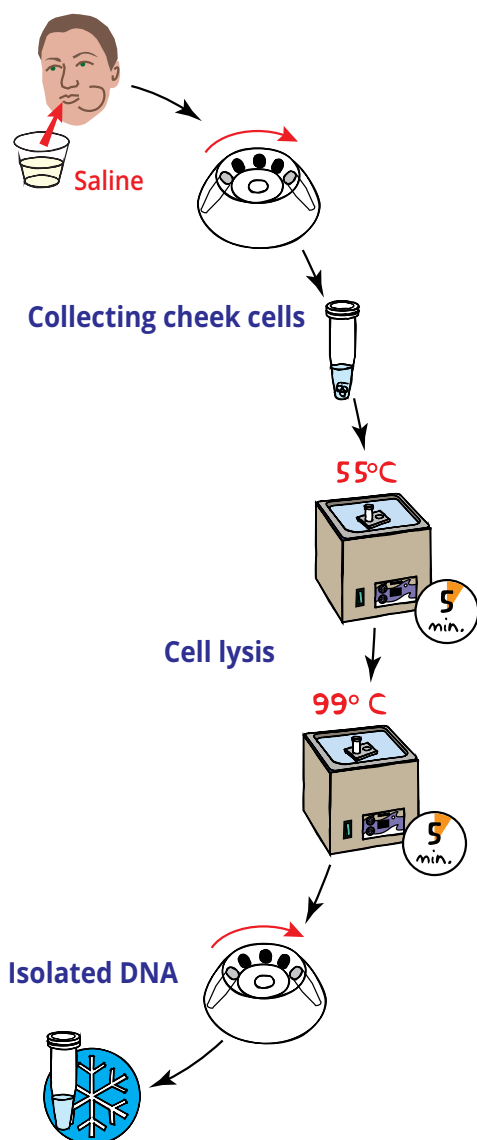
- Initial denaturation 94°C for 4 minutes.
 - 94°C for 30 seconds
 - 64°C for 30 seconds
 - 72°C for 30 seconds
 - Final extension at 72°C for 5 minutes.
- } 35 cycles



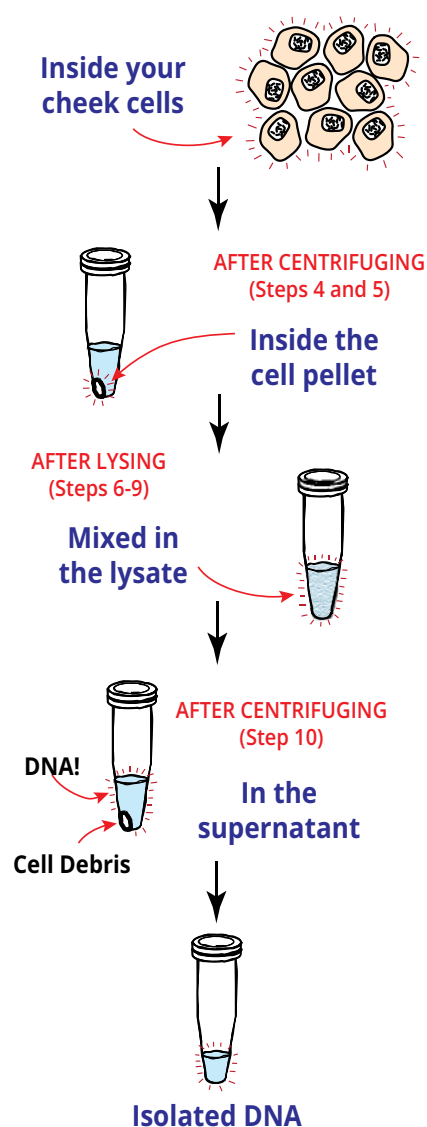
Module I Overview

In **Module I**, you will isolate DNA from your cheek cells. First, you will vigorously rinse your mouth with saline (salt water), which will dislodge cells into the solution. The cells are gathered using a centrifuge to pellet them at the bottom of a micro-centrifuge tube, allowing the saline to be removed. Next, a lysis buffer is added and the solution is incubated at 55°C and 99°C to burst (lyse) the cells and release the DNA. Finally, the cell lysate is centrifuged - this will collect the cell debris in a pellet while leaving the DNA in the supernatant. This DNA-containing supernatant will be used in **Module II**.

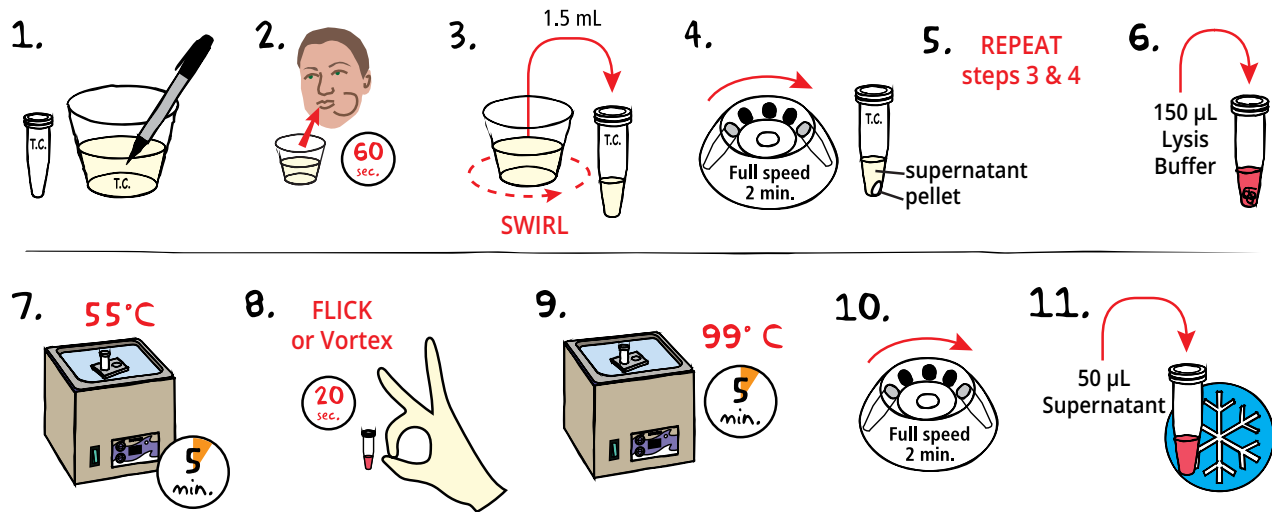
Experiment Overview



Where's my DNA



Module I: Isolation of DNA from Human Cheek Cells



1. **LABEL** an empty 1.5 mL screw top microcentrifuge tube and a cup of saline with your lab group and/or initials.
2. **RINSE** your mouth vigorously for 60 seconds using 10 mL saline solution. **EXPEL** the solution back into the same cup.
3. **SWIRL** the cup gently to resuspend the cells. **TRANSFER** 1.5 mL of the cell solution into the tube with your initials.
4. **CENTRIFUGE** the cell suspension for 2 minutes at full speed to pellet the cells. **POUR** off the supernatant (the liquid above the cell pellet) but **DO NOT DISTURB THE CELL PELLETT**!
5. **REPEAT** steps 3 and 4 once more.
6. **RESUSPEND** the cheek cell pellet in 150 µL lysis buffer by pipetting up and down or by vortexing vigorously. **NOTE:** *Ensure that the cell pellet is fully resuspended and that no clumps of cells remain.*
7. **CAP** the tube and **PLACE** it in a water bath float. **INCUBATE** the sample in a 55°C water bath for 5 minutes.
8. **MIX** the sample by vortexing or by flicking the tube vigorously for 20 seconds.
9. **INCUBATE** the sample in a 99°C water bath for 5 minutes. **NOTE:** *Students MUST use screw-cap tubes when boiling samples.*
10. **CENTRIFUGE** the cellular lysate for 2 minutes at full speed.
11. **TRANSFER** 50 µL of the supernatant to a clean, labeled microcentrifuge tube. **PLACE** the tube in ice.

The extracted DNA is now ready for Module II: Amplification of the PTC Region. If you are ready to proceed, turn to page 12. Alternatively, the extracted DNA may be stored in the **FREEZER** (-20°C) until needed.

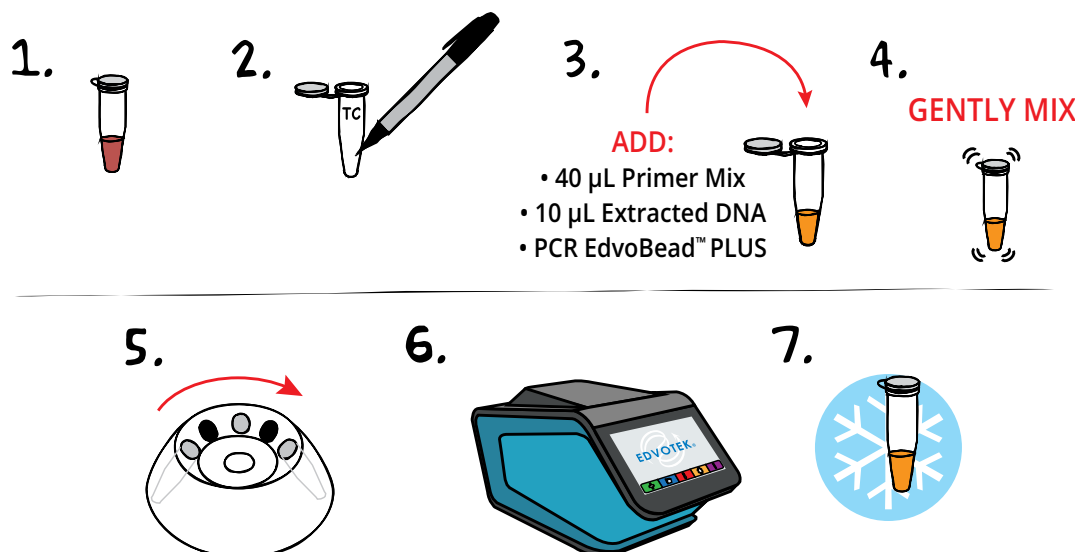


OPTIONAL STOPPING POINT:

The extracted DNA may be stored in the freezer (-20°C) until needed.

Module II: Amplification of the PTC Region

Now that you have isolated your DNA, during Module I, the next step is to amplify a specific region of the *TAS2R38* gene. First, you will combine your DNA (red) with a mixture of PTC primers (yellow) and a PCR EdvoBead™ PLUS, creating a PCR sample. Once this sample has been prepared it will be placed into a thermal cycler and the DNA will be amplified by PCR.



1. **OBTAIN** the red extracted DNA from Module I.
2. **LABEL** a fresh 0.2 mL PCR tube with your initials.
3. **ADD** 40 µL PTC primer mix (yellow), 10 µL extracted DNA (red), and a PCR EdvoBead™ PLUS.
4. **MIX** the PCR sample. Make sure the PCR EdvoBead™ PLUS is completely dissolved. If mixed correctly, the final solution will be light orange.
5. **CENTRIFUGE** the tube for a few seconds to collect the sample at the bottom of the tube.
6. **AMPLIFY** DNA using PCR guidelines listed below.



- Initial denaturation 94°C for 4 minutes.
- 94°C for 30 seconds
- 64°C for 30 seconds } 35 cycles
- 72°C for 30 seconds
- Final extension at 72°C for 5 minutes.

NOTE: If you do not have a thermal cycler, this experiment can be conducted using three water baths with proper care. *However, a thermal cycler assures a significantly higher rate of success.*

See Appendix C for detailed instructions.

The amplified DNA is now ready for Module III: Restriction Digest of the PTC PCR Product. If you are ready to proceed, turn to page 13. Alternatively, the amplified DNA may be stored in the **FREEZER** (-20°C) until needed.



OPTIONAL STOPPING POINT:

The PCR samples may be stored at -20°C for restriction digest at a later time.

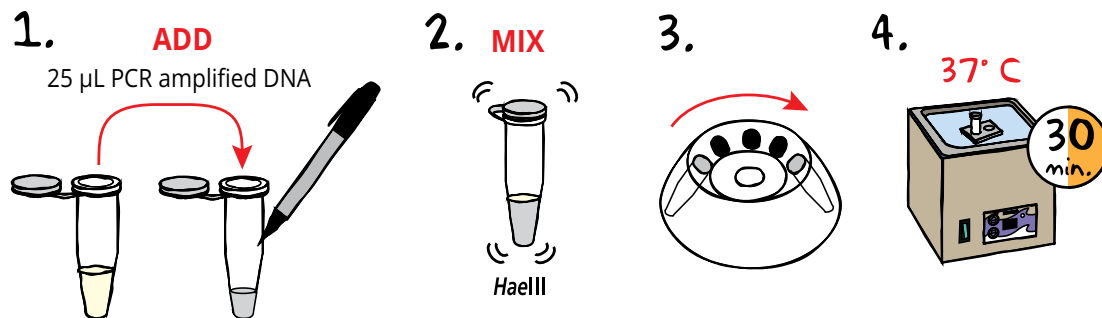


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Module III: Restriction Digest of the PTC PCR Product

After completing Module II, your PCR sample should contain millions, or billions, of copies of the *TAS2R38* gene target. In this module you will perform a restriction digest on half of the PCR product to determine if a single nucleotide polymorphism (SNP) is present at nucleotide 145. You will first remove half of the PCR mixture and add it to a tube containing the *HaeIII* restriction enzyme. This solution will be incubated at 37°C to allow the restriction enzyme to identify and cut any "GGCC" sequences present in the DNA. *For more information on the restriction digest, refer to pages 6 and 7 in the background section.*



- 1. ADD** 25 µL PCR amplified DNA to the tube containing 5 µL *HaeIII* Restriction Enzyme. **LABEL** this tube with your initials and "*HaeIII*". **SAVE** the remaining 25 µL uncut PCR product to set up as a control later.
- Gently **MIX** the restriction digest ("*HaeIII*" tube) by gently tapping the tube.
- Quickly **CENTRIFUGE** to collect sample at the bottom of the tube.
- INCUBATE** the digest for 30 minutes at 37°C.

The DNA samples are now ready for analysis in Module IV: Separation of the DNA Fragments by Electrophoresis. If you are ready to proceed, turn to page 14. Alternatively, both of your DNA samples may be stored in the **FREEZER** (-20°C) until needed.



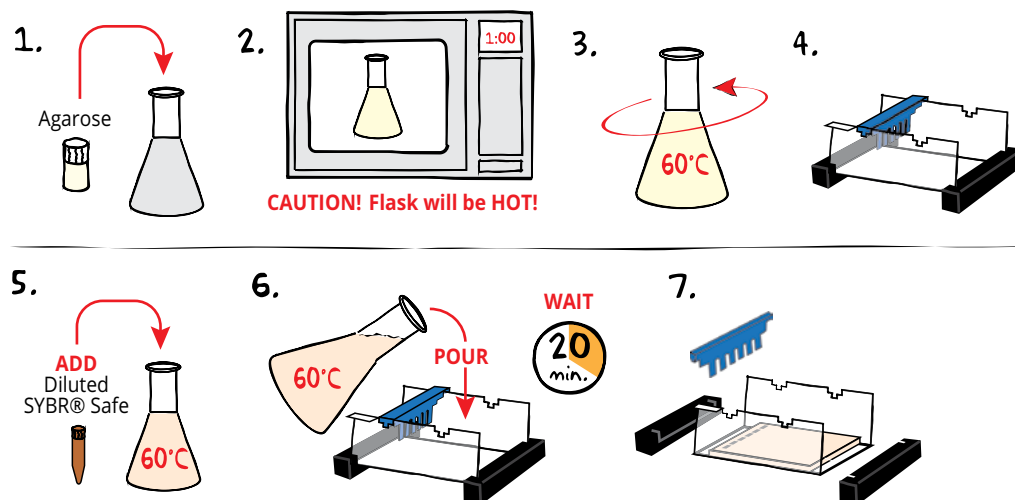
OPTIONAL STOPPING POINT:

The restriction digests may be stored at -20°C for electrophoresis at a later time.

NOTE: At this point the samples are ready for gel electrophoresis, no additional loading dye is necessary.

Module IV: Separation of DNA Fragments by Electrophoresis

In Module IV, you will perform agarose gel electrophoresis on both of your DNA samples – the digested and undigested PCR products from Module III. We recommend loading your undigested and digested samples in neighboring wells, but your instructor will provide the final guidelines on how to load the gels. Once the electrophoresis has completed you will visualize the DNA bands to determine your *TAS2R38* genotype.



IMPORTANT:

7 x 7 cm gels are recommended. Each gel can be shared by 2-3 students. Place well-former template (comb) in the first set of notches.

PREPARING THE AGAROSE GEL WITH SYBR® SAFE STAIN

- MIX** the agarose powder with 1X TBE buffer in a 250 mL flask (see Table A).
- DISSOLVE** the agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
- COOL** the agarose to 60°C by carefully swirling the flask to promote even dissipation of heat.
- While the agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the comb in the appropriate notch.
- Before casting the gel, **ADD** diluted SYBR® Safe to the cooled molten agarose and swirl the flask to mix (see Table A).
- POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
- REMOVE** the end caps and comb. Take particular care when removing the comb to prevent damage to the wells.



Table A	Individual 2.0% UltraSpec-Agarose™ with Diluted SYBR® Stain				
	Size of Gel Casting tray	1X TBE Buffer	+ Amt of Agarose	= TOTAL Volume	Diluted SYBR® (Step 5)
	7 x 7 cm	25 mL	0.5 g	25 mL	25 µL
	10 x 7 cm*	45 mL	0.9 g	45 mL	45 µL
	14 x 7 cm	50 mL	1.0 g	50 mL	50 µL

* Recommended gel volume for the EDGE™ Integrated Electrophoresis System.

STOP

OPTIONAL STOPPING POINT:

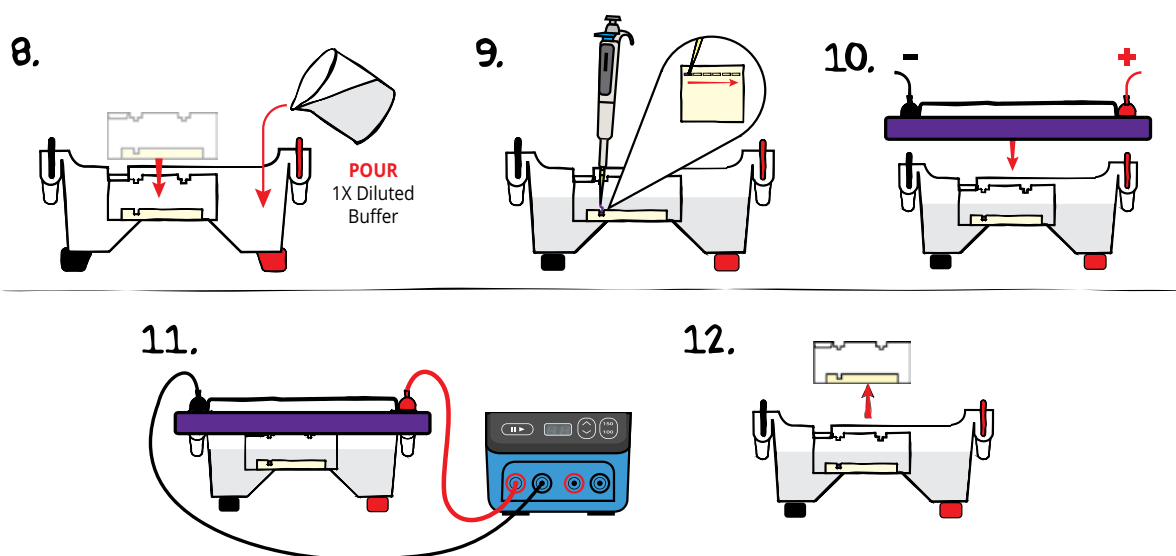
Gels can be stored for up to one week by placing them in a plastic storage bag containing 1 mL of TBE electrophoresis buffer and storing in the refrigerator. DO NOT FREEZE the gels as this will destroy them.



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Module IV: Separation of DNA Fragments by Electrophoresis, continued



RUNNING THE GEL

8. **PLACE** the gel (on the tray) into an electrophoresis chamber. **COVER** the gel with 1X TBE electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. Using Table 1 as a guide, **LOAD** the entire sample (25 μ L) into the wells in consecutive order.
10. **CHECK** that the gel is properly oriented, then **PLACE** the safety cover onto the chamber. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** the leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

Reminder:

Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



OPTIONAL STOPPING POINT:

Gels can be stored for several days. Protect from light, refrigerate, and keep hydrated by storing each gel in a watertight plastic bag with a 1 mL of TBE electrophoresis buffer.

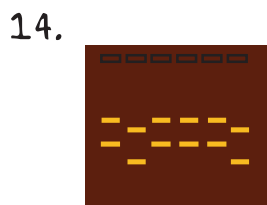
Table 1 : Gel Loading

Lane	Recommended	Sample Name
1	DNA ladder	
2	Control DNA (undigested or digested)	
3	Student 1 undigested	
4	Student 1 digested	
5	Student 2 undigested	
6	Student 2 digested	

Table B	1x TBE Electrophoresis Buffer (Chamber Buffer)	
	EDVOTEK Model #	Total Volume Required
	EDGE™	150 mL
	M12	400 mL

Table C	Time and Voltage Guidelines (2.0% Agarose Gel)		
	Electrophoresis Model		
	EDGE™ (10 x 7 cm gel)		M12 (14 x 7 cm gel)
Volts	Min/Max (minutes)		Min/Max (minutes)
150	20/30		30/40
100	35/45		45/55
75	NA		60/70

Module IV: Separation of DNA Fragments by Electrophoresis, continued



VISUALIZING THE SYBR® GEL

13. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator.
14. **TURN** the unit on. DNA should appear as bright green bands on a dark background. **PHOTOGRAPH** results.
15. **TURN** the unit off. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.



Be sure to wear UV goggles if using a UV transilluminator.

Module V: Determination of Bitter Tasting Ability with PTC Paper

In this final Module you will taste strips of control and PTC paper to determine your phenotype. First, you will taste a control strip of paper – it is important to taste the control before the PTC-coated strip since the paper will have a slight taste. Next, you will taste the PTC paper, paying attention to any differences between it and the control paper.

PROCEDURE:

1. **TASTE** the Control strip of paper first. **RECORD** your thoughts on the taste.
2. **TASTE** the PTC strip of paper. **RECORD** your thoughts on the taste.
3. **COMPARE** the taste of the Control and the PTC paper.
 - Notice what the PTC paper tastes like compared to the Control paper: intensely bitter, somewhat bitter, or tasteless.
 - If you are a taster, the PTC paper strip will be bitter. Non-tasters will not notice a difference between the strips of paper.

ANALYZE THE RESULTS:

1. Compare your genotype, from Module IV, to the phenotype observed when you taste the PTC strips. Do your results match?
2. Are you a homozygous bitter taster, a heterozygous bitter taster, or a non-taster?

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. How is PCR used to determine human genetics and identify polymorphisms in DNA?
2. What are the three steps in a PCR cycle and what does each step accomplish?
3. Based on what you have learned about the genotype of *TAS2R38* and its phenotype, fill in the table below:

Genotype	Phenotype	# of DNA bands predicted	Size of bands (bp)
TT			
Tt			
tt			

4. Based on your results, what is your genotype? Why? What is your phenotype? Why? How about your lab partners?
5. Do the control and PTC paper tasting results correlate with the DNA digest findings in your ability to taste? How about your lab partner?
6. Enter your classroom data in the Table shown below:

Genotype	Number of Individuals With Each Phenotype		
	Strong Taster	Weak taster	Non-taster
TT (homozygous)			
Tt (heterozygous)			
tt (homozygous)			

7. Considering that not everyone who can taste PTC tastes it the same way, what does this tell you about classical dominant/recessive inheritance?

Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION:

This experiment will require 3-4 class periods to complete the student experiment. Many reagents can be prepared in advance, but others require special considerations. Please consult the table below and the detailed instructions in the Instructor's Guide for more information.

Preparation For:	What to do:	When:	Time Required:
Module I: Isolation of DNA from Cheek Cells	Prepare and aliquot saline and plastic tubes	Anytime before performing the experiment.	30 min.
	Prepare and aliquot Lysis Buffer.	Prepare on the day the students will be performing the experiment OR freeze for up to one week.	15 min.
	Equilibrate water baths at 55° C and boiling.	Anytime before performing the experiment.	5 min.
Module II: Amplification of the PTC Region	Prepare and aliquot various reagents (Primer, PCR Edvo-Beads™, etc.).	Up to one week before performing the experiment. Diluted primers should be frozen until needed.	30 min.
	Program Thermal Cycler.	Anytime before performing the experiment.	15 min.
Module III: Restriction Digest of the PTC PCR Product	Prepare and aliquot <i>HaeIII</i> Restriction enzyme.	Prepare on the day the students will be performing the experiment OR freeze for up to one week.	15 min.
	Equilibrate water bath at 37° C.	Anytime before performing the experiment.	15 min.
Module IV: Separation of DNA Fragments by Electrophoresis	Prepare TBE buffer.	Up to one week before performing the experiment.	45 min.
	Prepare molten agarose and pour gels (OPTIONAL) or aliquot agarose, TBE, and SYBR® Safe.		
	Aliquot 100 bp ladder.		
Module V: Determination of Bitter Tasting Ability	Distribute PTC taste strips and Control taste strips.	During the class period you perform the experiment.	5 min.



Red = Prepare immediately before module.



Yellow = Prepare shortly before module.



Green = Flexible / prepare up to a week before the module.

Pre-Lab Preparations: Module I

In Module I, the students will isolate DNA from their cheek cells. The easiest, and recommended, method to collect cheek cells involves a brief rinse with a saline solution. The saline rinse will be centrifuged briefly to isolate the cheek cells, which are then mixed with a lysis buffer and incubated at 55 °C and 99 °C. Finally, the students will centrifuge the cell lysate and collect the DNA-containing supernatant. The isolated DNA can be safely stored at -20 °C (freezer) until needed for PCR in Module II.

Before beginning Module I the instructor must prepare the Saline Solution and Lysis Buffer. See below for preparation instructions for both solutions.

Preparation of Saline Solution

NOTE: Saline solution MUST be used for cheek cell wash. Sports drinks will inhibit amplification of DNA by Polymerase Chain Reaction in Module II. If you have used sports drinks for the cheek cell wash, please DISCARD the samples and REPEAT the DNA extraction with saline solution

1. To prepare the saline solution, dissolve all eight salt packets (~4 g) in 500 mL of drinking water. Cap and invert bottle to mix.
2. Aliquot 10 mL of saline solution per cup. Distribute one cup per student.

Preparation of Lysis Buffer

NOTE: The Lysis Buffer must be mixed with Proteinase K before performing the experiment. Once prepared, the Lysis should be used the same day or frozen.

1. Add 100 µL of Universal DNA buffer (A) to the tube of Proteinase K and allow the sample to hydrate for several minutes. After the sample is hydrated, pipet up and down several times to thoroughly mix the material.
2. Transfer the entire amount of the rehydrated Proteinase K solution to a 15 mL conical tube containing an additional 4 mL of Universal DNA buffer (A).
3. Invert the tube several times to mix. Label this tube "Lysis Buffer".

NOTE: The Lysis Buffer should be red and free of any undissolved clumps.

4. Aliquot 300 µL of Lysis Buffer into 13 labeled microcentrifuge tubes to be shared by pairs of students.

NOTE: At this point, the Lysis Buffer should be stored on ice for use within the same day (up to 6 hours) or frozen.

5. Distribute one tube of "Lysis Buffer" to each student pair. If frozen, the Lysis Buffer can be quickly thawed in a 37°C water bath or by students warming the tube in their hands.

DISINFECTING LABORATORY MATERIALS: Contaminated laboratory waste (saliva solution, cup, pipet, etc.) must be disinfected with 15% bleach solution prior to disposal. Be sure to properly dispose any biological samples according to your institutional guidelines.

NOTE:

Accurate pipetting is critical for maximizing successful experiment results. This experiment is designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students are unfamiliar with using micropipets, we recommended performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this advanced level experiment.

FOR MODULE I

Each student should receive:

- One cup containing 10 mL of saline solution
- One screw-cap tube
- One microcentrifuge tube

Reagents to be shared by two students:

- 300 µL Lysis buffer
- 15% bleach solution

Warning !!

Remind students to only use screw-cap tubes when boiling their DNA samples. The snap-top tubes can potentially pop open and cause injury.

Pre-Lab Preparations: Module II

In Module II, the students will perform PCR on the DNA that they isolated in Module I. The PCR mixture includes the student DNA, a primer mixture specific to a 221 bp region of the *TAS2R38* gene, and a PCR EdvoBead™ PLUS.

The PCR primers are provided as a lyophilized mixture that must be rehydrated by the instructor before performing the experiment. The PCR EdvoBeads™ PLUS can be distributed prior to setting up the PCR – students or instructors can gently transfer the PCR EdvoBeads™ PLUS using gloved hands. Alternatively, beads can be gently "poured" from the vial into individual PCR tubes. After distributing the beads, it is important to quickly close the PCR tubes securely to prevent the beads from absorbing moisture and becoming hard to resuspend prior to the experiment.

IMPORTANT:

The PCR EdvoBeads™ absorb moisture if left open, even for a short period of time. Open the vial only when you are ready to use the beads. If you are not using all of the beads in one session, quickly remove the required number and promptly secure the gray stopper back into the vial. Store the vial of unused beads at -20°C until needed.

This experiment contains LyphoControl™ Complete PCR control samples that should be prepared and run alongside the student PCR samples. LyphoControl™ samples are premixed with all PCR components and are ready to run immediately after rehydration. We highly recommend running the controls alongside student samples if possible, although they can also be run before or after the student PCR.

Preparation of the PTC Primer Mix

LyphoPrimer™

1. Thaw the TE Buffer (B) and mix well.
2. Ensure that the lyophilized solid is at the bottom of the LyphoPrimer™ tube (C). If not, centrifuge the tube at full speed for 10 seconds.
3. Add 1.2 mL of TE Buffer (B) to the tube. Cap and mix well, then place on ice. The solution should be light orange and clear of any solid pieces.
4. Label 13 microcentrifuge tubes "PTC Primer". Aliquot 90 µL of the diluted Primer Mix into the 13 microcentrifuge tubes. Place the tubes on ice until they are needed. If you would like to prepare the primers ahead of time, they can be aliquoted and then frozen until needed.
5. Distribute one tube of diluted PTC Primer to each student pair.

Preparation of the LyphoControl™

LyphoControl™

NOTE: This kit includes enough reagents to set up 6 control reactions. At least one control reaction should be performed per class to confirm that PCR was successful.

1. Ensure that the lyophilized solid is at the bottom of the LyphoControl™ tube (D). If not, centrifuge the tube at full speed for 10 seconds.
2. Add 320 µL of TE Buffer (B) to the tube. Cap and mix well then place on ice. The solution should be light red and clear of any solid pieces.
3. Dispense 50 µL of the diluted control mixture for each control reaction.

NOTES:

- The LyphoControl™ already contains all necessary PCR components and does not need a PCR EdvoBead™ PLUS. Once diluted, the LyphoControl™ is ready to be amplified by the PCR alongside student PCR samples, if there is room, or run prior to the student experiment. After PCR, the control reactions can be stored at -20°C until needed in Module III.
- Distilled water can be used to rehydrate the lyophilized components. Just be sure that the proper volumes are used.

Pre-Lab Preparations: Module II, continued

IMPORTANT:

The PCR EdvoBeads™ absorb moisture if left open, even for a short period of time. Only open the vial when you are ready to use the beads. If you are not using all of the beads in one session, quickly remove the required number and promptly secure the gray stopper back into the vial. Store the vial of unused beads at -20°C (desiccated) until needed.

PCR EdvoBeads™

NOTE: The beads are vacuum-sealed and may move around once the stopper is removed. Only open the vial when you are ready to use the beads and quickly recap the vial.

Dispense the PCR EdvoBeads™ just prior to the students performing their PCR reactions. Alternatively, student pairs can dispense PCR EdvoBeads™ during Module II. Each student will require 1 bead.

1. Open the vial of PCR EdvoBeads™ (carefully remove metal crimp) and gently/slowly remove the gray rubber stopper.
2. With gloved hand, forceps or sterile loop, remove one single bead and transfer to a PCR tube. Do this for the number of reactions you wish to perform. Quickly cap each of the tubes and distribute to the student groups.

PCR Amplification:

The Thermal cycler should be programmed as outlined in Module II in the Student's Experimental Procedure.

- Accurate temperatures and cycle times are critical. A pre-run for one cycle (takes approximately 3 to 5 minutes) is recommended to check that the thermal cycler is properly programmed.
- For thermal cyclers that do not have a heated lid, it is necessary to place a layer of oil or wax above the PCR reactions in the microcentrifuge tubes to prevent evaporation.

FOR MODULE II

Each student should receive:

- One PCR tube and PCR EdvoBead™ PLUS

Reagents to be shared by two students:

- 90 µL PTC Primer mix

IMPORTANT NOTE: Before running the experiment, confirm that your PCR program matches the settings below.



- Initial denaturation 94°C for 4 minutes.
 - 94°C for 30 seconds
 - 65°C for 30 seconds
 - 72°C for 30 seconds
- } 35 cycles
- Final extension at 72°C for 5 minutes.

Pre-Lab Preparations: Module III

RESTRICTION DIGEST OF THE PTC PRODUCT

During Module III students will digest some of their PCR product, saved from Module II, using the *HaeIII* restriction enzyme. For best results, the *HaeIII* Dryzyme® restriction enzyme must be prepared shortly before use and kept on ice, or stored at -20°C immediately after aliquoting. Each student will receive a single tube of the rehydrated enzyme for use during the experiment.

Remind students to save the remaining, undigested sample for use in Module IV. Once the digest has completed the digested and undigested samples can be stored together at -20°C (freezer) until needed.

Dilution of *HaeIII* Restriction Enzyme

1. Make sure that the solid material is at the bottom of the Dryzyme® Restriction Enzyme *HaeIII* tube (E). If not, centrifuge the tube at full speed for 20 seconds.
2. Add 200 µL of Restriction Enzyme Dilution Buffer (F) to the tube containing the *HaeIII* Dryzyme®.
3. Allow the sample to hydrate for 1 minute.
4. Mix the tube for 30 seconds (gently vortex or tap bottom of the tube) and set on ice for 1 minute.
5. Centrifuge the rehydrated enzyme for 20 seconds at max. speed.
6. Dispense 5 µL of the *HaeIII* Restriction Enzyme into 25 tubes. Label these tubes "*HaeIII*"

FOR MODULE III
Reagents to be shared by
two students:

- Two tubes of *HaeIII* Restriction Enzyme

NOTE: The rehydrated restriction enzyme can be stored on ice or in the refrigerator for up to 6 hours, or frozen for up to one week. We do not recommend overnight storage in the refrigerator.

Pre-Lab Preparations: Module IV

SEPARATION OF DIGESTION PRODUCTS BY ELECTROPHORESIS

Preparation of Electrophoresis Buffer:

For this experiment, we recommend preparing the TBE electrophoresis buffer in bulk for sharing by the class. Unused diluted buffer can be used at a later time.

1. Measure 3.7 L of distilled or deionized water and place in a large vessel. (*NOTE: If using purchased water in a gallon jug, remove and discard 80 mL water.*)
2. Add the entire amount of TBE electrophoresis buffer powder to the vessel and mix well.
3. Label the vessel as "1X Electrophoresis Buffer (TBE)".
4. Use within 60 days of preparation.

SYBR® Safe Stain Preparation:

Prepare diluted SYBR® Safe by adding 400 µL of 1X TBE buffer to the tube of SYBR® Safe and tapping the tube several times to mix. Diluted SYBR® Safe will be used during agarose gel preparation.

FOR MODULE IV

Each group receives:

- 1X TBE buffer
- UltraSpec-Agarose™ Powder
- Tube of diluted SYBR® Safe (25 µL)
- 100 bp ladder (30 µL)

Preparation of Agarose Gels:

This experiment requires one 2.0% agarose gel for every group of two students (13 gels total). 7 x 7 cm gels are recommended. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow 30-40 minutes for this procedure.

Individual Gel Preparation:

Each student group can be responsible for casting its own individual gel prior to conducting the experiment (see Module IV in the Student's Experimental Procedure). Each 7 x 7 cm gel will require 25 mL of 1X TBE buffer, 0.5 g of agarose powder, and 25 µL of diluted SYBR® Safe Stain.

Batch Gel Preparation:

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. See Appendix B for instructions.

Preparing Gels in Advance:

Gels may be prepared ahead and stored for later use. Solidified gels can be stored for up to 1 week in the refrigerator in water-tight bags with a small amount of buffer to prevent drying. We recommend adding 2 mL of buffer to the bag; excess buffer can lead to diffusion of SYBR® Safe out of the gels.

Do not store gels at -20° C as freezing will destroy them.

Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

Pre-Lab Preparations: Module IV, continued

Additional Materials:

- Aliquot 30 μ L of the 100 bp ladder (G) into labeled microcentrifuge tubes and distribute one tube of 100 bp ladder per gel.

Each 2.0% gel should be loaded with the 100 bp ladder and samples from two students. The control PCR reaction can also be loaded in one of the wells. If desired, you can designate one student or pair of students from each gel to load the controls.

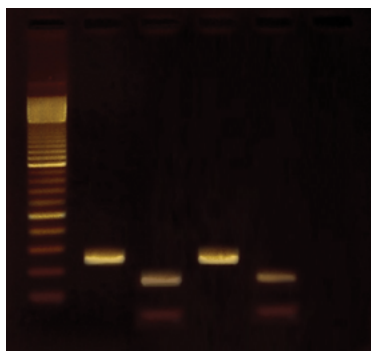
Visualizing SYBR® Safe-Stained Gels:

SYBR® Safe is a DNA stain that fluoresces when bound to double-stranded DNA, allowing us to visualize our samples. This DNA stain is compatible with both UV and blue-light transilluminators. For best results, we recommend the TruBlu™ 2 Blue Light Transilluminator ([Cat #557](#)).

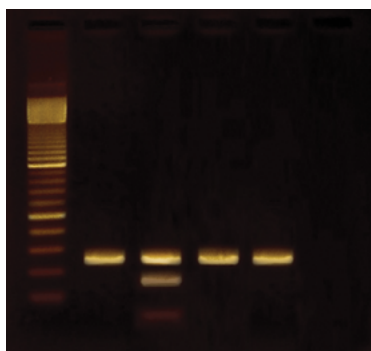
Photodocumentation of DNA (Optional):

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

Experiment Results and Analysis



Lane	Sample	Size
1	100 bp ladder	Bands range in size from 100 bp – 4000 bp in 100 bp increments. High intensity reference band at 500 bp.
2	Control DNA, uncut	221 bp
3	Control DNA, cut	177, 44 bp
4	Student DNA, uncut	221 bp
5	Student DNA, cut	177, 44 bp



Lane	Sample	Size
1	100 bp ladder	Bands range in size from 100 bp – 4000 bp in 100 bp increments. High intensity reference band at 500 bp.
2	Student 1 DNA, uncut	221 bp
3	Student 1 DNA, cut	221, 177, 44 bp
4	Student 2 DNA, uncut	221 bp
5	Student 2 DNA, cut	221 bp

NOTE: In some samples, a diffuse, low molecular weight band known as a "primer dimer" may be present. This is a PCR artifact and can be ignored.

	Lane 1 (TT)	Lane 2 (Tt)	Lane 3 (tt)
221 bp Fragment	—	—	—
177 bp Fragment	—	—	
44 bp Fragment	—	—	

PTC TASTER:

Homozygous Taster (TT) = sizes of 177 & 44 bp

Heterozygous Taster (Tt) = 1 allele remains uncut at 221bp while the other allele cuts and generates fragments of 177 bp and 44 bp.

PTC NON-TASTER:

Homozygous recessive (tt) = remains uncut at 221 bp

- **Homozygous Taster:**
Both copies of the gene contained the polymorphism, allowing it to be digested by *HaeIII*.
- **Heterozygous Taster:**
One copy of the gene contained the polymorphism, allowing it to be digested by *HaeIII*. The other copy of the gene did not have the restriction site and was not digested
- **Homozygous Non-taster:**
Neither copy of the gene contained the polymorphism, so *HaeIII* could not digest this DNA.

NOTE: The 221 and 177 bp bands will appear brighter than the 44 bp band.

**Please refer to the kit
insert for the Answers to
Study Questions**

Appendices

- A EDVOTEK® Troubleshooting Guide
- B Bulk Preparation of Electrophoresis Buffer and Agarose Gels
- C Performing the PCR Experiment Using Three Water Baths

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Appendix A

EDVOTEK® Troubleshooting Guides

DNA EXTRACTION

PROBLEM:	CAUSE:	ANSWER:
There is no cell pellet after centrifuging the cheek cell suspension.	Not enough cheek cells in suspension	Mouth must be vigorously rinsed for at least 60 sec. to harvest loose cheek cells.
	Sample not centrifuged fast enough	Spin cells at maximum speed for 2 min. If your centrifuge's max RCF is less than 2000 x g, spin at highest available speed for 4 min.
Poor DNA extraction	Samples not mixed well enough during extraction	In addition to flicking the tube, vortex or pipet up and down to mix the sample.
	Proteinase K inactive because it was prepared too far in advance.	Prepare Lysis buffer the same day that it will be used or immediately freeze until needed.
	Water baths not at proper temperature	Use a thermometer to confirm water bath set point.
	Not enough DNA	Ensure students are centrifuging saline rinse TWICE during Module I.
	Sports drink was used for DNA extraction.	Repeat DNA extraction with saline solution.
The extracted DNA is very cloudy.	Cellular debris from pellet transferred to tube	Centrifuge sample again and move supernatant to a fresh tube. Take care to avoid pellet.
	Cellular debris not separated from supernatant	Centrifuge sample again. If possible, centrifuge at a higher speed. Move cleared supernatant to a fresh tube.

Appendix A

EDVOTEK® Troubleshooting Guides

RESTRICTION ENZYME DIGESTION

PROBLEM:	CAUSE:	ANSWER:
Undigested or incompletely digested DNA	Impure DNA – some contaminants (EDTA, salts) might partially or completely inhibit activity of <i>HaeIII</i> restriction enzyme	Poor DNA extraction. Extract new DNA. Cheek cell extraction usually results in higher DNA yield.
	Improper dilution of enzyme	Ensure that <i>HaeIII</i> restriction enzyme was correctly diluted.
	Improper addition of enzyme	Ensure that correct amount of <i>HaeIII</i> restriction enzyme was added to the restriction digest.
	Incorrect incubation temperature	Use a thermometer to confirm water bath temperature and adjust, if necessary.
	Abbreviated incubation time	Samples must be incubated 30 min. Slower incubations may lead to incomplete digestions.
Unexpected cleavage pattern	DNA sample is contaminated	Prepare a new DNA sample.
Smearing of digested DNA on gel	Nuclease contamination	Care should be taken to avoid cross contamination when setting up reactions.
	Agarose running conditions	Use fresh electrophoresis buffer and appropriate voltage.

Appendix A

EDVOTEK® Troubleshooting Guides

PCR AND ELECTROPHORESIS

PROBLEM:	CAUSE:	ANSWER:
There is very little liquid left in tube after PCR.	Sample has evaporated.	Make sure the heated lid reaches the appropriate temperature.
		If your thermal cycler does not have a heated lid, overlay the PCR reaction with oil or wax.
	Pipetting error.	Make sure students close the lid of the PCR tube properly.
The ladder, control DNA, and student PCR products are not visible on the gel.	The gel was not prepared properly.	Make sure students pipet 40 µL primer mix and 10 µL extracted DNA into the 0.2 mL tube.
		Ensure that the electrophoresis buffer was correctly diluted.
	The gel was not stained properly.	Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels.
After staining, the ladder and control PCR products are visible on the gel but some student samples are not present.	The proper buffer was not used for gel preparation. Make sure to use 1x Electrophoresis Buffer.	
	Malfunctioning electrophoresis unit or power source.	Ensure that SYBR® Safe was added to the gel before casting. If staining with FlashBlue™, either re-stain with FlashBlue™ solution or destain until bands are seen.
	Student DNA sample was not concentrated enough.	Contact the manufacturer of the electrophoresis unit or power source.
	Student DNA sample was degraded.	Poor DNA extraction. Repeat Module I (Isolation of DNA from Human Cheek Cells).
Some student samples have more/less amplification than others.	Wrong volumes of DNA and primer added to PCR reaction.	If DNA is not used right after extraction, store sample at -20° C.
	Sports drink was used for DNA extraction.	Practice using micropipets.
Low molecular weight band in PCR samples.	Primer dimer	Repeat DNA extraction with saline solution.
DNA bands were not resolved.	Concentration of DNA varies by sample.	There is an inherent variability in the extraction process.
	To ensure adequate separation, make sure the tracking dye migrates at least 4 cm on 7 x 7 cm gels and 6 cm on 7 x 14 cm gels.	Low concentration of extracted DNA in PCR reaction.
		Be sure to run the gel the appropriate distance before staining and visualizing the DNA.

Appendix B

Bulk Preparation of Electrophoresis Buffer and Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities that the whole class can share. Leftover diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

BULK 1X TBE ELECTROPHORESIS BUFFER

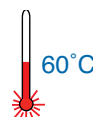
For this experiment, we recommend preparing the 1X TBE Electrophoresis Buffer in bulk for sharing by the class. Unused diluted buffer can be used at a later time.

1. Measure 3.7 L of distilled or deionized water and place in a large vessel. (*NOTE: If using purchased water in a gallon jug, remove and discard 80 mL water.*)
2. Add the entire amount of TBE Electrophoresis Buffer powder to the vessel and mix well.
3. Label the vessel as "1X TBE Electrophoresis Buffer".
4. Use within 60 days of preparation.

BATCH AGAROSE GELS (2.0%)

Bulk preparation of 2.0% agarose gel is outlined in Table D.

1. Measure 400 mL of 1X TBE Electrophoresis Buffer and pour into a 500 mL flask.
2. Pour 8.0 g of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.
6. If staining with SYBR® Safe, add the entire volume of diluted SYBR® Safe from page 24 to the cooled agarose and mix well.
7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 25 mL for each 7 x 7 cm gel, 50 mL for each 7 x 14 cm gel. ***For this experiment, 7 x 7 cm gels are recommended.***
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis. Alternatively, gels can be stored in water-tight plastic bags with 2 mL of 1X electrophoresis buffer for up to 1 week in the refrigerator.



60°C

Table
E

Batch Prep of 2.0% UltraSpec-Agarose™

Amt of Agarose	+	1x Electrophoresis Buffer
8.0 g		400 mL

NOTE:

The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

Appendix C

Performing the PCR Experiment Using Three Water Baths

This experiment can be modified to use three water baths in place of a thermal cycler. In this method, the PCR samples are cycled through three water baths, each maintained at a different temperature, for a specified period. The sequential placement of the reaction sample through the three water baths constitutes one PCR cycle. Please note that results obtained when using three water baths are often variable. **A thermal cycler assures a significantly higher rate of success.**

Set up the samples as outlined in Module II steps 1-5. Before cycling the samples, be sure to:

- Allow at least 15 minutes for the water baths to reach the temperatures specified in Module I (94°C, 65°C, 72°C).
- Cover the water baths when not in use to maintain the proper temperature and to prevent water evaporation.
- The volume of the PCR sample is small and can evaporate easily. To prevent this, transfer one wax bead to each PCR sample. (*Wax beads are not provided.*) The melted wax bead forms a barrier over the PCR sample to prevent its evaporation during heating.
- Make sure that the sample remains undisturbed at the bottom of the tube. If necessary, centrifuge or shake the tube to get the sample to the bottom of the tube.
- Place the PCR samples in a water bath float before placing them in the water bath.

Continue with step 6 (thermal cycling), following the below protocol for 35 cycles. Refer to the protocol in Module II for detailed instructions.

- 94°C for 30 seconds
- 65°C for 30 seconds
- 72°C for 30 seconds

Handle the samples carefully when shifting between water baths. Use forceps to carefully raise/lower the float into the water baths.

Remove samples at time points specified by Module II. **Take care to avoid the liquid wax layer when removing the sample.** We recommend placing the tube on ice for a few seconds to solidify the wax. Use a clean pipette tip to gently break through the wax layer, making enough room to fit a clean pipette tip. Using a fresh, clean pipette tip, remove the PCR product and transfer to the appropriate tube.

- Final extension at 72°C for 5 minutes.