

EDVOTEK® • The Biotechnology Education Company®

Student's
Version

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 345.220421

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Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

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Experiment Components

Perishable Components

	Storage	Check (√)
• PCR EdvoBeads™ Plus*	Room Temperature	<input type="checkbox"/>
A Universal DNA Buffer	-20°C Freezer	<input type="checkbox"/>
B TE Buffer	-20°C Freezer	<input type="checkbox"/>
C LyphoPrimer™ Mix	-20°C Freezer	<input type="checkbox"/>
D LyphoControl™ (Complete PCR control)	-20°C Freezer	<input type="checkbox"/>
E Dryzymes® Restriction Enzyme <i>Hae</i> III	-20°C Freezer	<input type="checkbox"/>
F Restriction Enzyme Dilution Buffer	-20°C Freezer	<input type="checkbox"/>
G 100 bp ladder	-20°C Freezer	<input type="checkbox"/>
• Proteinase K	-20°C Freezer	<input type="checkbox"/>
• PTC Paper	Room temperature	<input type="checkbox"/>
• Control Taste Paper	Room temperature	<input type="checkbox"/>

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.

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, Reaction Buffer

Reagents & Supplies

Store all components below at room temperature.

• UltraSpec-Agarose™	<input type="checkbox"/>
• TBE Electrophoresis Buffer Powder	<input type="checkbox"/>
• SYBR® Safe Stain	<input type="checkbox"/>
• Disposable plastic cups	<input type="checkbox"/>
• 15 mL Conical Tube	<input type="checkbox"/>
• Snap-top microcentrifuge tubes	<input type="checkbox"/>
• Microcentrifuge Tubes (1.5 mL screw-cap tube – use for boiling)	<input type="checkbox"/>
• 0.2 mL PCR tubes	<input type="checkbox"/>
• Salt packets	<input type="checkbox"/>

LyphoPrimer™

LyphoControl™

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 System (EDVOTEK® [Cat. #557](#) or [#558](#) highly 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, PCR experiments can be conducted, with proper care, using three water baths. However, a thermal cycler assures a significantly higher rate of success.



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-

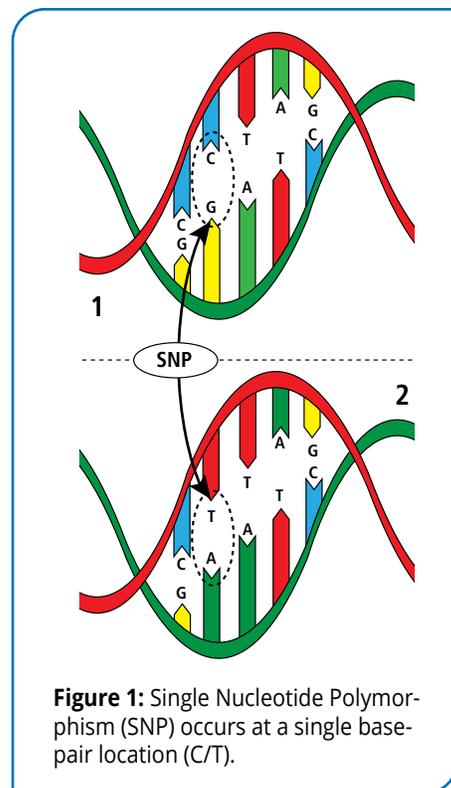


Figure 1: Single Nucleotide Polymorphism (SNP) occurs at a single base-pair location (C/T).

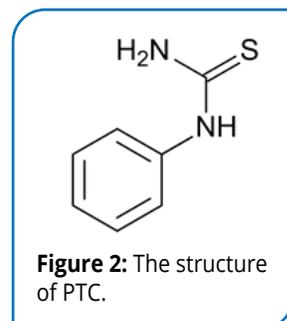


Figure 2: The structure of PTC.

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

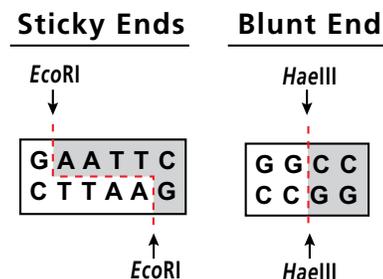
Nucleotide Position	Change in Nucleotide (Non-taster > Taster)	Change in Codon (Non-taster > Taster)	Change in Amino Acid (Non-taster > Taster)
145	G > C	GCA > CCA	Alanine > Proline
785	T > C	GTT > GCT	Valine > Alanine
886	A > G	ATC > GTC	Isoleucine > Valine

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.

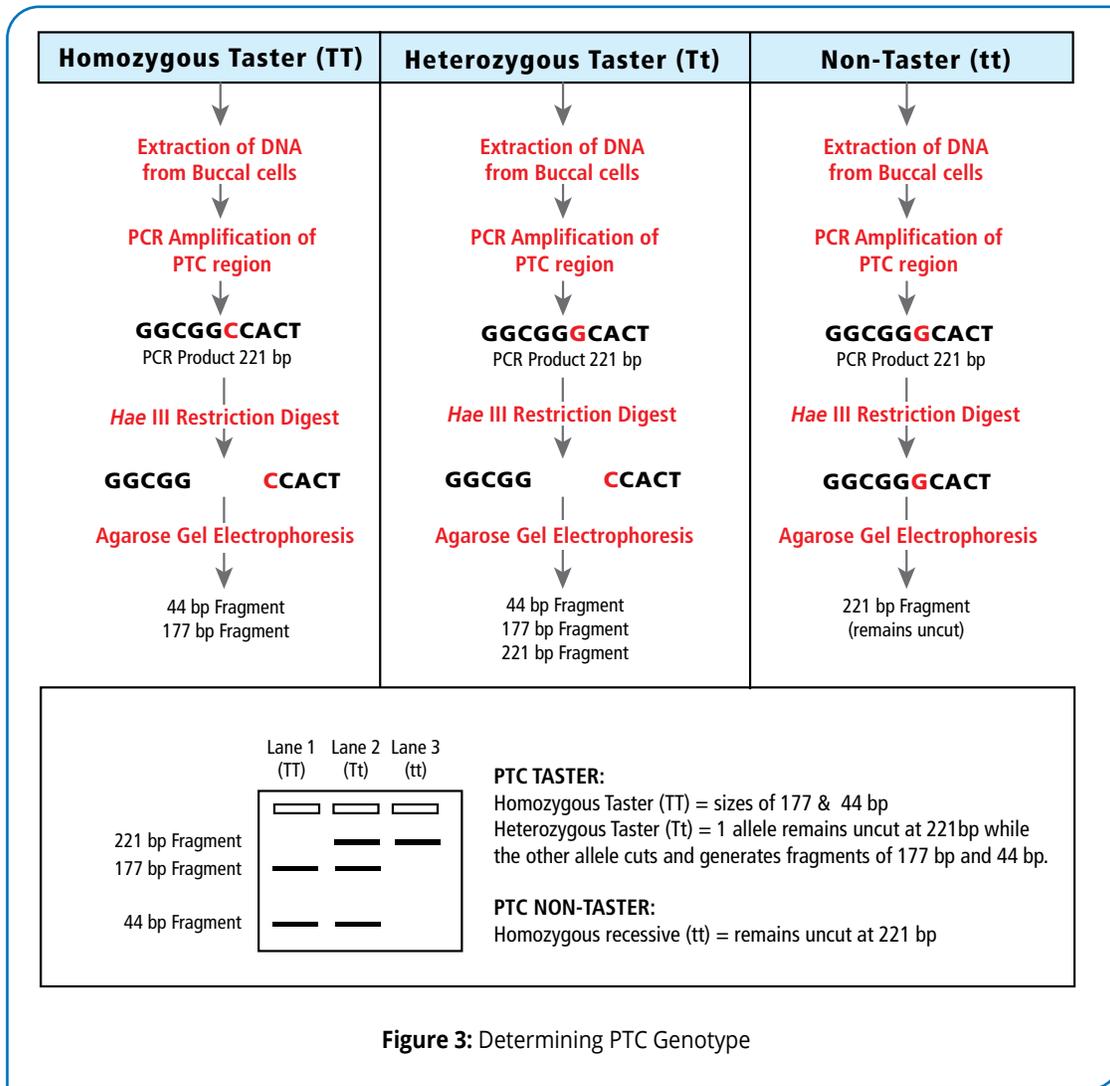
In the example of the PTC gene, *HaeIII* only cuts the taster allele (5'-GGCGGCCACT-3'). The polymorphism present in the non-taster allele (5'-GGCGGGCACT-3') changes a single base change in the restriction enzyme recognition site, so *HaeIII* can not digest non-taster DNA.



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.

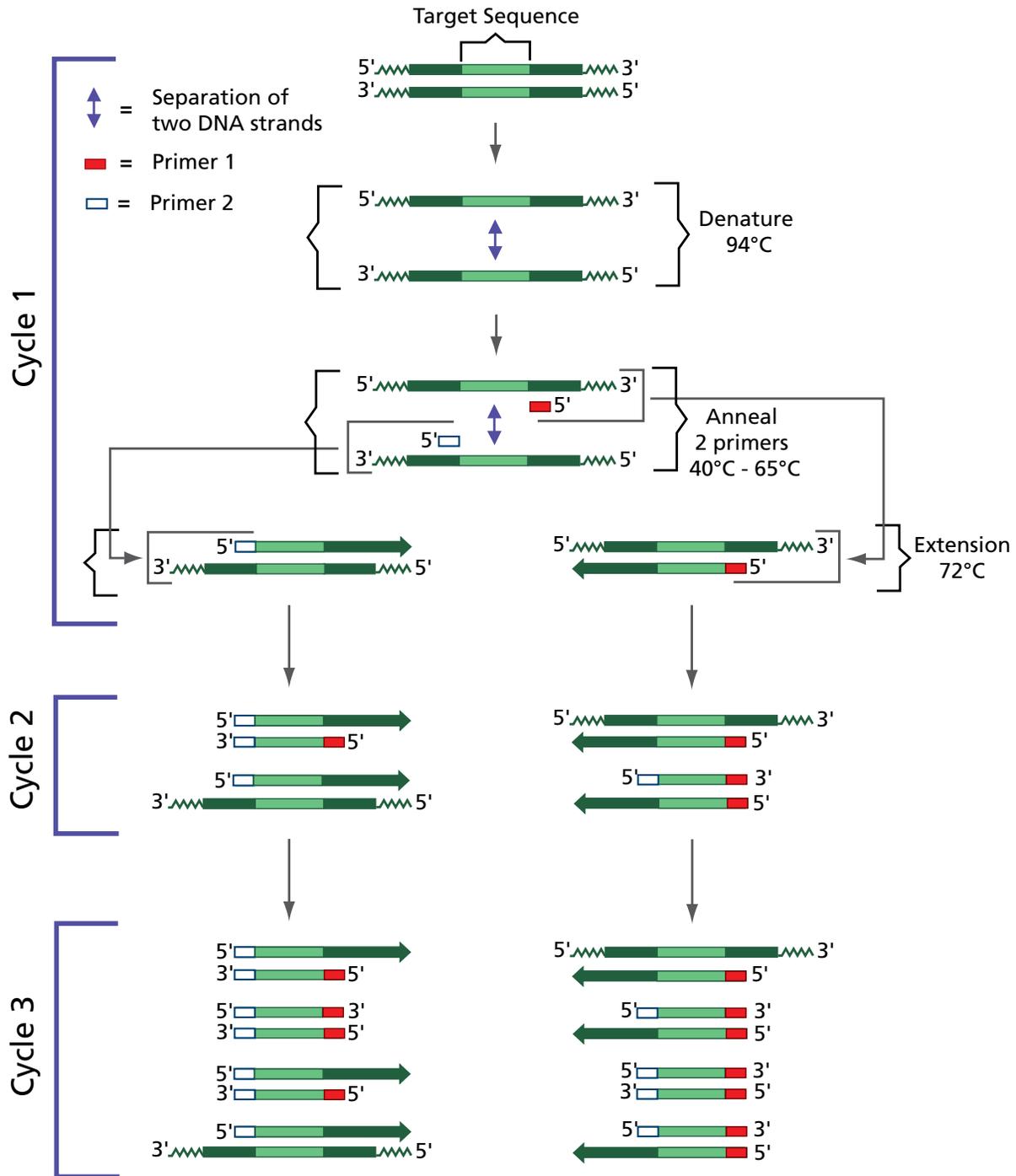


Figure 4: DNA Amplification by the Polymerase Chain Reaction

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!

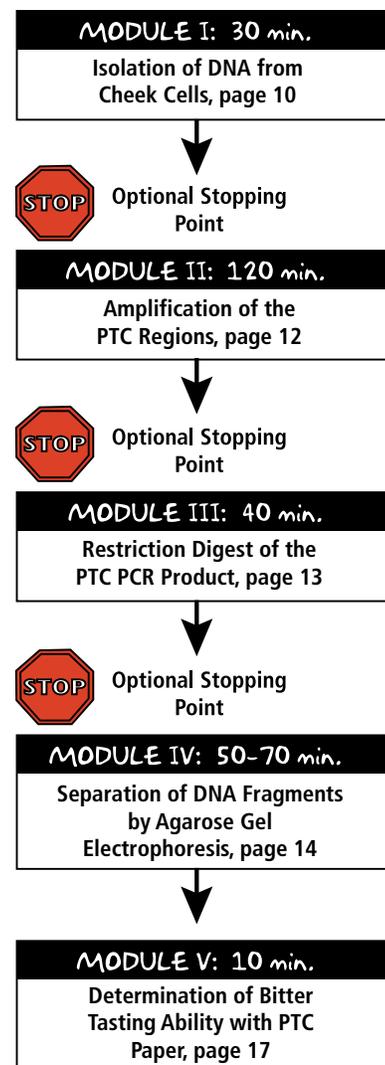
- Exercise caution when working in the laboratory.
- Wear gloves and goggles at all times.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- Do not mouth pipet reagents - use pipet pumps.
- Always wash hands thoroughly with soap and water after working in 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:

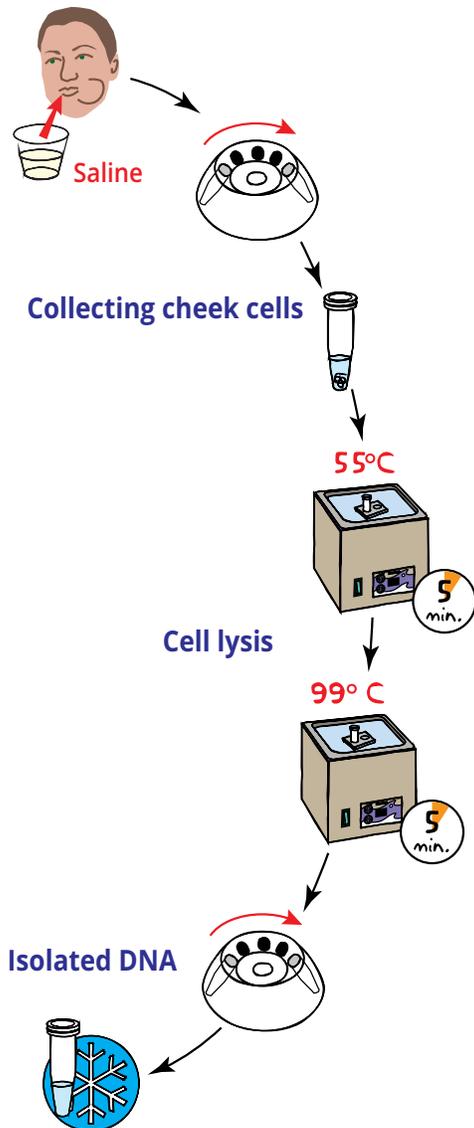
Initial denaturation: 94° C for 4 minutes
 94° C for 30 seconds
 64° C for 30 seconds } 35 cycles
 72° C for 30 seconds }
 Final Extension: 72° C for 5 minutes



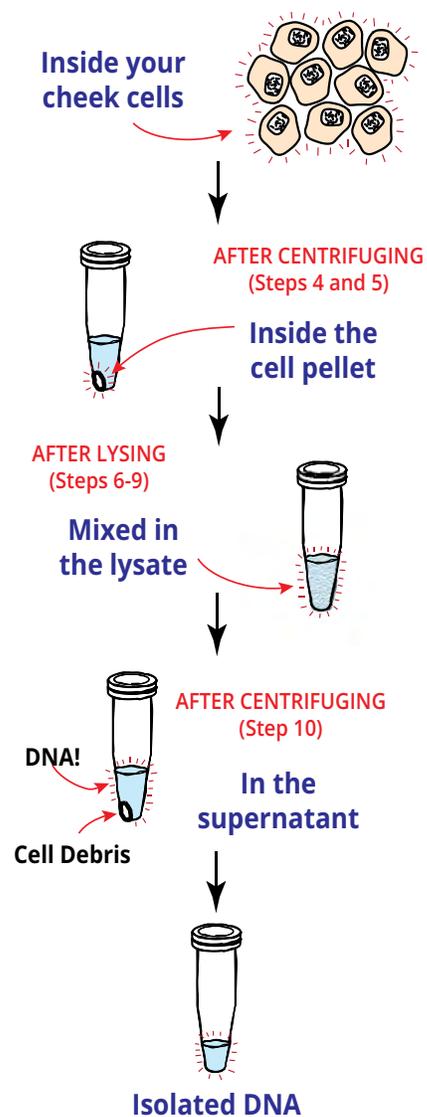
Module I Overview

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 microcentrifuge 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**.

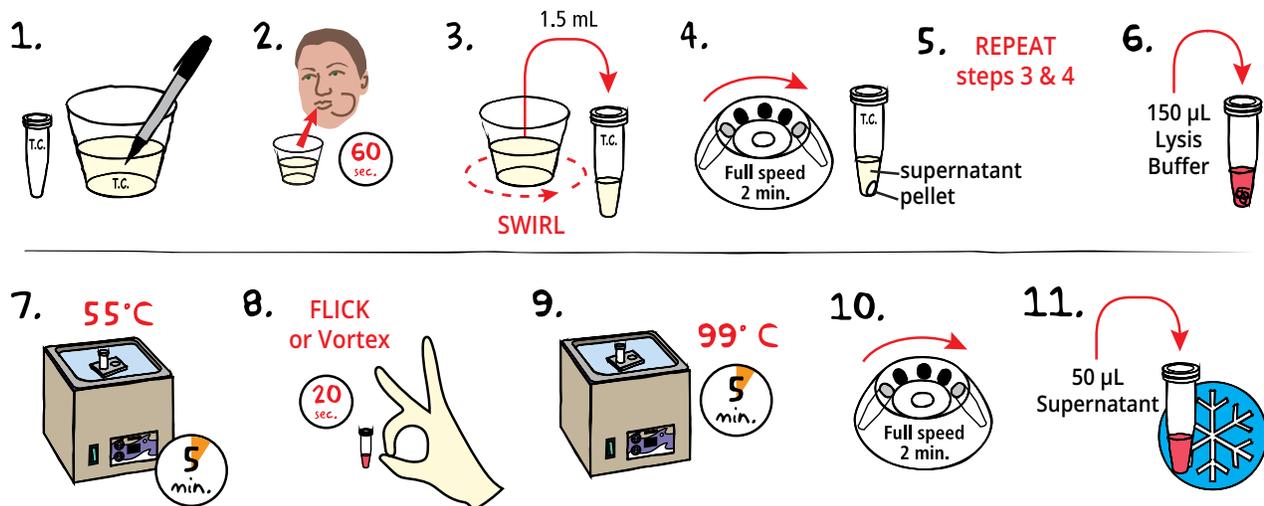
Experiment Overview



Where's my DNA



Module I: Isolation of DNA from Human Cheek Cells



- LABEL** an empty 1.5 mL screw top microcentrifuge tube and a cup of saline with your lab group and/or initials.
- RINSE** your mouth vigorously for 60 seconds using 10 mL saline solution. **EXPEL** the solution back into the same cup.
- SWIRL** the cup gently to resuspend the cells. **TRANSFER** 1.5 mL of the cell solution into the tube with your initials.
- 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!**
- REPEAT** steps 3 and 4 once more.
- 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.*
- CAP** the tube and **PLACE** it in a water bath float. **INCUBATE** the sample in a 55°C water bath for 5 minutes.
- MIX** the sample by vortexing or by flicking the tube vigorously for 20 seconds.
- INCUBATE** the sample in a 99°C water bath for 5 minutes. **NOTE:** *Students MUST use screw-cap tubes when boiling samples.*
- CENTRIFUGE** the cellular lysate for 2 minutes at full speed.
- 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.

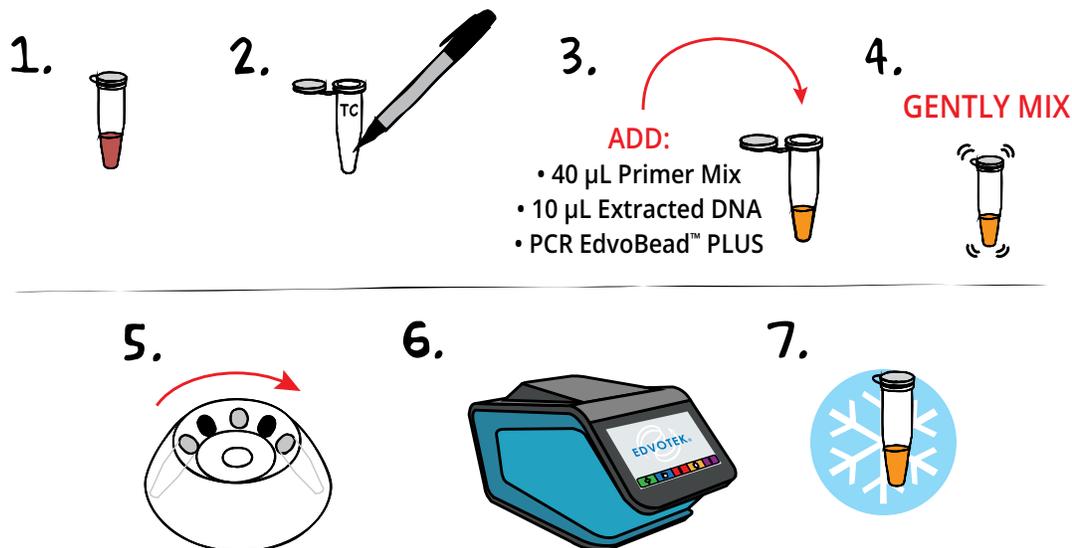


OPTIONAL STOPPING POINT:

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.



- OBTAIN** the red extracted DNA from Module I.
- LABEL** a fresh 0.2 mL PCR tube with your initials.
- ADD** 40 µL PTC primer mix (yellow), 10 µL extracted DNA (red), and a PCR EdvoBead™ PLUS.
- MIX** the PCR sample. Make sure the PCR EdvoBead™ PLUS is completely dissolved. If mixed correctly, the final solution will be light orange.
- CENTRIFUGE** the tube for a few seconds to collect the sample at the bottom of the tube.
- AMPLIFY** the DNA using PCR.
PCR cycling conditions:
 - Initial denaturation 94°C for 4 minutes
 - 94°C for 30 seconds
 - 65°C for 30 seconds
 - 72°C for 30 seconds
 - Final Extension 72°C for 5 minutes

NOTE:

If your thermal cycler does not have a heated lid, it is necessary to overlay the PCR reaction with oil or wax to prevent evaporation. Visit our website for more information.

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.

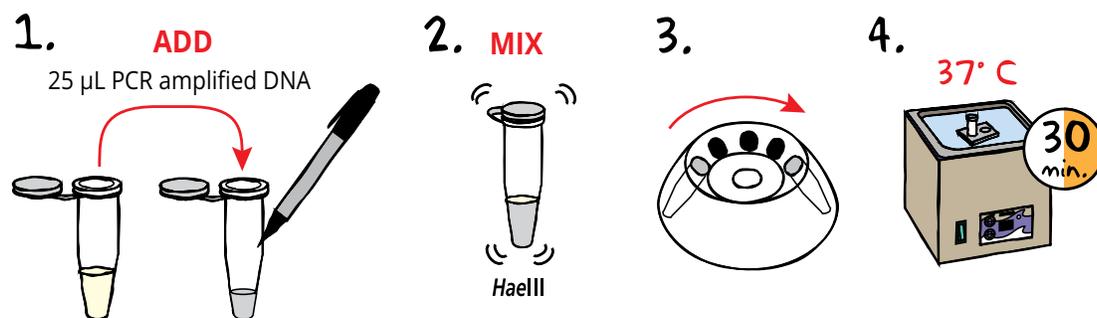


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Module III: Restriction Digest of the PTC PCR Product

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.
2. Gently **MIX** the restriction digest ("*HaeIII*" tube) by gently tapping the tube.
3. Quickly **CENTRIFUGE** to collect sample at the bottom of the tube.
4. **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.



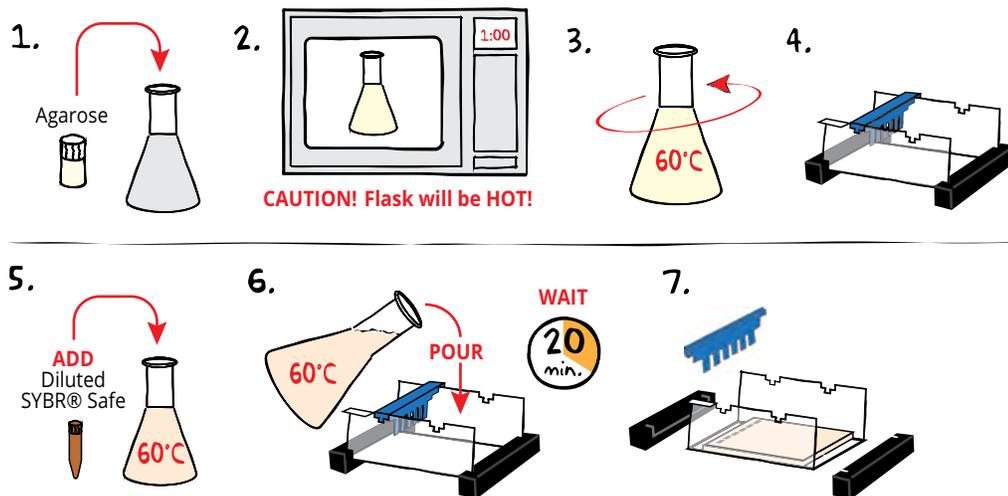
OPTIONAL STOPPING POINT:

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	29.5 mL		0.3 g		30 mL	30 µL
10 x 7 cm*	49.0 mL		0.5 g		50 mL	50 µL
14 x 7 cm	58.8 mL		0.6 g		60 mL	60 µL

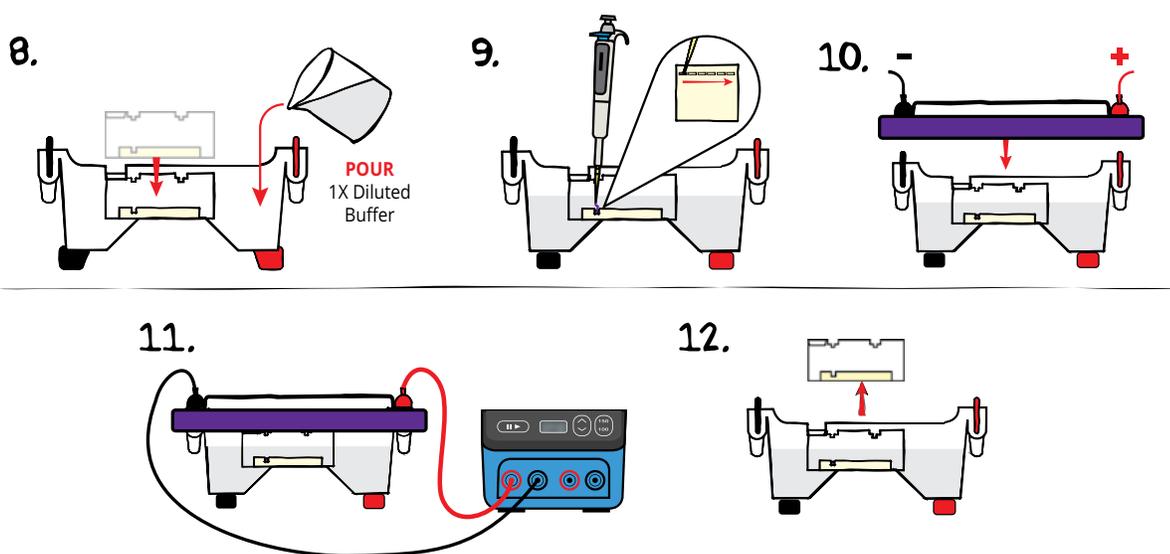
* Recommended gel volume for the EDGE™ Integrated Electrophoresis System.



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.

Module IV: Separation of DNA Fragments by Electrophoresis, continued



RUNNING THE GEL

- 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.
- Using Table 1 as a guide, **LOAD** the entire sample (25 μ L) into the wells in consecutive order.
- 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.
- CONNECT** the leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
- 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:

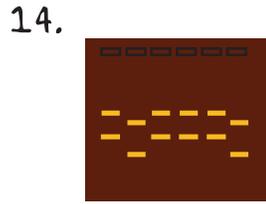
Gels can be stored for several days. Protect from light, refrigerate, and keep hydrated by storing each gel in a watertight plastic bag with a 1 mL of TBE electrophoresis buffer.

EDVOTEK Model #	Total Volume Required
EDGE™	150 mL
M12	400 mL

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	

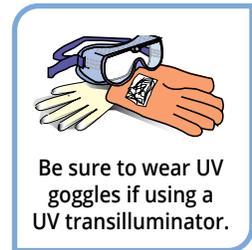
Volts	Electrophoresis Model	
	EDGE™ (10 x 7 cm gel)	M12 (14 x 7 cm gel)
150	Min/Max (minutes) 20/30	Min/Max (minutes) 30/40
100	Min/Max (minutes) 35/45	Min/Max (minutes) 45/55
75	NA	60/70

Module IV: Separation of DNA Fragments by Electrophoresis, continued



VISUALIZING THE SYBR® GEL

13. **SLIDE** the gel off the casting tray onto the viewing surface of the transilluminator and turn the unit on. **ADJUST** the brightness to the desired level to maximize band visualization. DNA should appear as bright green bands on a dark background.
14. **PHOTOGRAPH** the results.
15. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.



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?