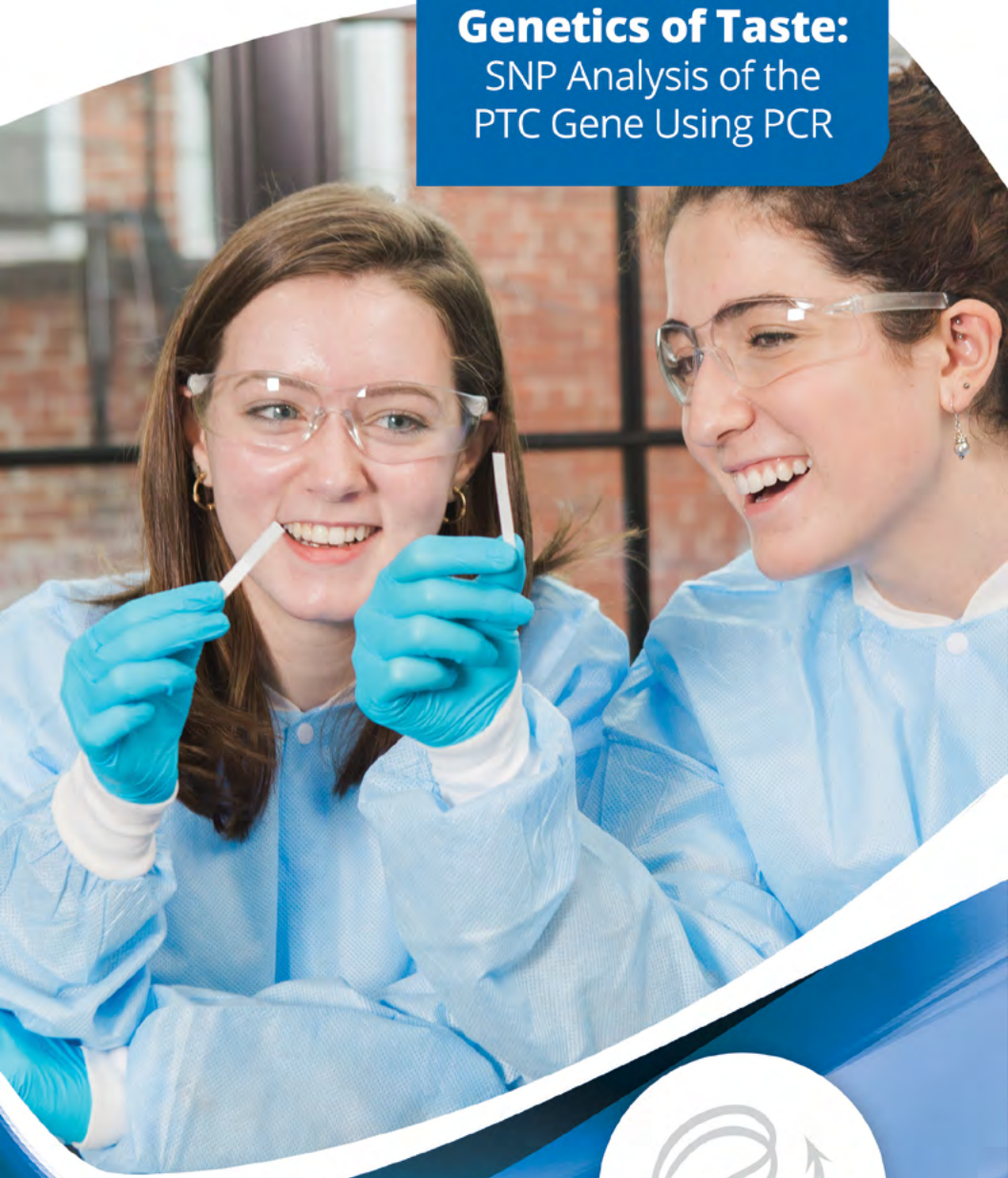
WORKSHOP

Exploring the Genetics of Taste:

SNP Analysis of the PTC Gene Using PCR



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Introduction

Explore the relationship between genotype and phenotype using Phenylthiocarbamide (PTC). Some think PTC tastes bitter, while others find it tasteless. The ability to taste PTC has been linked to variations in a taste receptor gene. In this workshop, you will learn to use PCR to distinguish between PTC alleles.

Exploring the Genetics of Taste *Excerpts from EDVO-Kit 345*

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS)

Single Nucleotide Polymorphisms (SNPs) are DNA sequence variations that occur in the genome sequence when a single nucleotide is altered. The genetic code is specified by the **four nucleotide letters:** A (Adenine), C (Cytosine), T (Thymine), and G (Guanine). SNP occurs when a single nucleotide, such as a T, replaces one of the other three nucleotides A, G, or C (Figure 1). Each person has many SNPs that together create a unique DNA fingerprint profile for that individual.

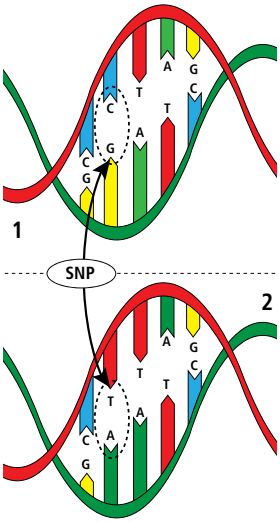


Figure 1: Single Nucleotide Polymorphism (SNP) occurs at a single base-pair location (C/T).

SNPs may fall within any region of the genome – the coding sequences of genes, the non-coding sequences of genes, or regions in-between genes (the intergenic regions). When an SNP falls within a coding sequence, that alteration may not necessarily change the amino acid sequence of the subsequent protein produced due to degeneracy of the genetic code. On the other hand, SNPs that do not fall into a coding sequence may still affect that region of DNA in such a way by inducing gene splicing, transcription factor binding, or by altering the sequence of non-coding RNA.

Variations in our DNA sequences can affect many aspects of human physiology - how we develop diseases, respond to pathogens, process chemicals, drugs , and many more. For example, sickle cell anaemia is caused by a single nucleotide mutation in the ß-globin chain of hemoglobin. This, in turn, causes the hydrophilic amino acid glutamic acid to be replaced with the hydrophobic amino acid valine, thus altering the shape of the blood cells. Instead of having the usual disc-like shape that is flexible, enabling them the pass even the smallest blood vessels, sickle cells are long "sickle-shaped" red blood cells that block the flow of blood through the blood vessels. Sickle cells can lead to various complications, including acute chest syndrome, organ damage, gallstone, and stroke.

THE BITTER TASTE AND THE BITTER COMPOUND PHENYLTHIOCARBAMIDE (PTC)

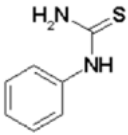


Figure 2: The structure of PTC.

Individuals vary greatly in their sensitivity to the bitter compound Phenylthiocarbamide (PTC). This is one of the best-known genetic traits in the human population and has been a popular teaching tool for genetic inheritance. In this experiment, we examine variable regions to distinguish differences in individuals by identifying two different forms (alleles) of a gene related to one's ability to taste PTC (Figure 2).

The famous European geneticist Professor Ed V. Otek, tested his rather large genetics class of students for the ability to taste PTC. He discovered that this gene 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 the 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.

THE PTC SENSITIVITY GENE – TAS2R38

Studies show that there is an inherited component that effects how people taste PTC. In 2003, more than 70 years after its original discovery, the gene responsible for PTC sensitivity, TAS2R38, was identified. Analysis of SNPs within the coding region of TAS2R38 revealed that PTC taster and non-taster alleles differ in 3 amino acids. There are two common forms (or alleles) of the PTC gene: a dominant tasting allele and a recessive non-tasting allele. The shape of the receptor protein determines how strongly it can bind to PTC. Since all people have two copies of every gene, combinations of the alleles determine whether someone finds PTC intensely bitter, somewhat bitter, or tasteless.

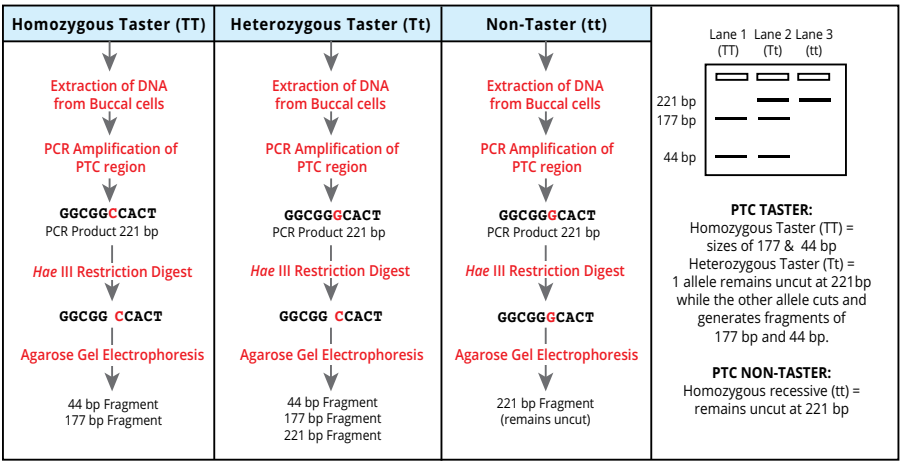
As discussed earlier, there are 3 positions within the gene that control the ability to taste PTC (as shown in table below).

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

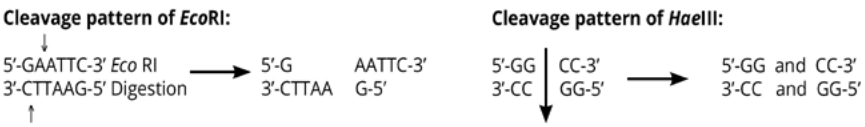
RESTRICTION ENZYME DIGESTION

Restriction enzymes are endonucleases which 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. A single base change in the recognition results in the inability of the restriction enzyme to cut the DNA at that sequence location. Differences in the sequence of DNA at that specific location can be quickly identified using restriction enzyme digestion.

A restriction enzyme requires a specific double-stranded recognition sequence of nucleotide bases to cut DNA. Recognition sites are usually 4 to 8 base pairs in length. 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.



Consider the recognition site and cleavage pattern of *Eco*RI and *Hae*III below. The cleavage positions are indicated by arrows. Digestion with *Eco*RI produces asymmetric "sticky ends", whereas *Hae*III restriction enzyme cleavage produces "blunt' ends.



In the example of the PTC gene, *Hae*III 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 *Hae*III can not digest non-taster DNA.

THE POLYMERASE CHAIN REACTION (PCR)

The PCR reaction is a DNA amplification technique that revolutionized almost all aspects of biological research. The procedure was invented by Dr. Kary Mullis while at Cetus Corporation in 1984. Dr. Mullis was awarded a Nobel Prize for his work in 1994. PCR amplification can produce millions of copies from a small quantity of DNA. The enormous utility of PCR is based on its procedural simplicity and specificity. Since the first application of PCR to diagnose sickle cell anemia, a large number of amplifications have successfully been developed. PCR has made amplification of DNA an effective alternative to cloning. It is currently routinely used in forensics, paternity/kinship testing, and the identification of human remains.

In preparation for PCR amplification, a set of two DNA "primers" are designed to target a specific region of the genomic sequence containing the gene of interest. The primers are two synthetic oligonucleotides typically 15-30 base pairs in length that are synthesized so that they correspond to the start and end of a specific region of the DNA sequence to be amplified. In this experiment, the template DNA is from individuals who show differences in their ability to taste PTC. The extracted DNA is called the "template." Freshly isolated DNA from biological sources will yield the best amplification. DNA extracted from stored specimens may be degraded and therefore less suitable for amplification.

In addition to the two primers, the four deoxynucleotides (dATP, dCTP, dGTP, and dTTP) that are the precursors building blocks of DNA 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 a sequential heating and cooling cycle of the mixture at three different temperatures. 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),to allow the two complementary DNA strands to be denatured. This step, known as "denaturation," disrupts the hydrogen bonds between the strands and causes the complete separation of the two DNA strands. In the second PCR step, the sample is cooled to a temperature in the range of 45°C - 65°C. In this step, known as "annealing," the two primers, which are present in great excess to the separated DNA template strands, 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 is maximally functional. It adds the precursor nucleotides to the primers to complete the synthesis of the new complementary strands based on the traditional Watson-Crick base pairing. 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 1,048,576 copies. The PCR process is typically repeated for 20-40 cycles, theoretically amplifying the target sequence to millions of copies. In practice, however, the amount of product often reaches a maximum after about 35 cycles. This is due to the depletion of reaction components and loss of Taq polymerase activity. The exact temperature and incubation time required for each of the three steps depends upon several factors, including the length of the DNA target and the Guanine /Cytosine (GC) content of the primer/ target.

One common problem that occurs during PCR is the production of unwanted amplification products. This may be due to contamination of the sample or nonspecific annealing (to the wrong segment). If this were to occur in an early cycle, the incorrect copy will also be amplified. To reduce contamination, autoclaved tubes and pipet tips, as well as sterile water should be used. Gloves should always be worn when performing PCR. Minimizing the concentration of the primers may curtail the production of unwanted PCR due to nonspecific annealing. Another common technique is a "hot start" step, in which the PCR reagents are introduced in the reaction only after the DNA is fully denatured at 94°C.

In this experiment, the students will use the PCR-RFLP method to examine the presence of the polymorphism. Students will use the PCR to amplify the 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-digested PCR products will reveal the 2 alleles of the TAS2R38 gene, indicating whether a student is homozygous or heterozygous for the taster phenotype. In the final module, students will test their ability to taste the bitter PTC and correlate their genotype with their phenotype.

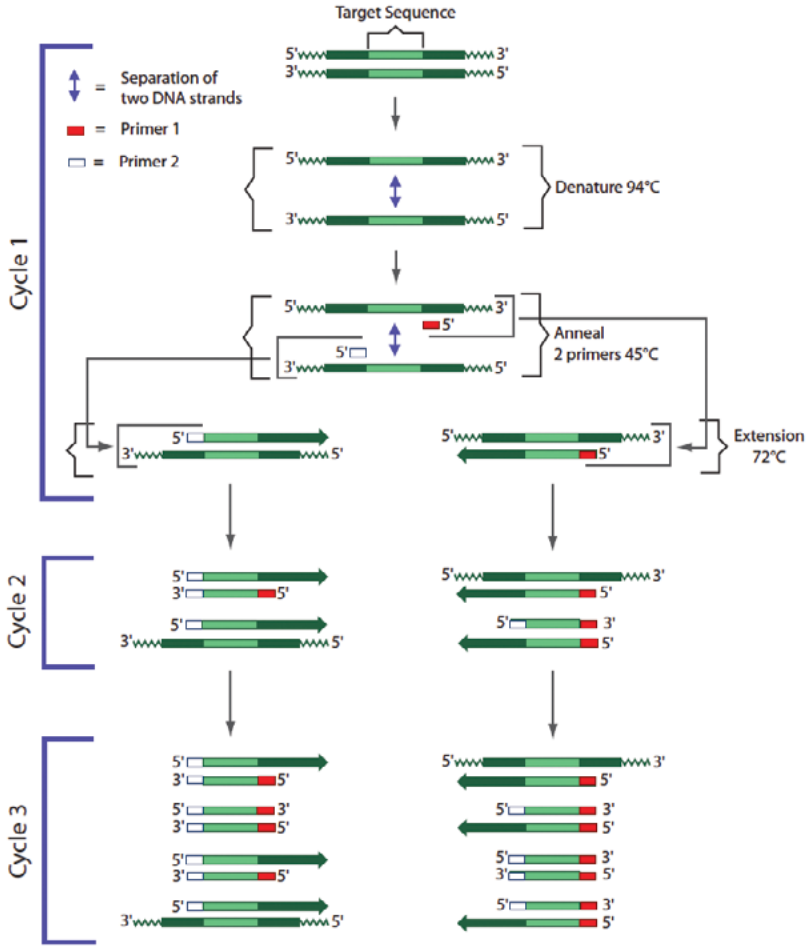
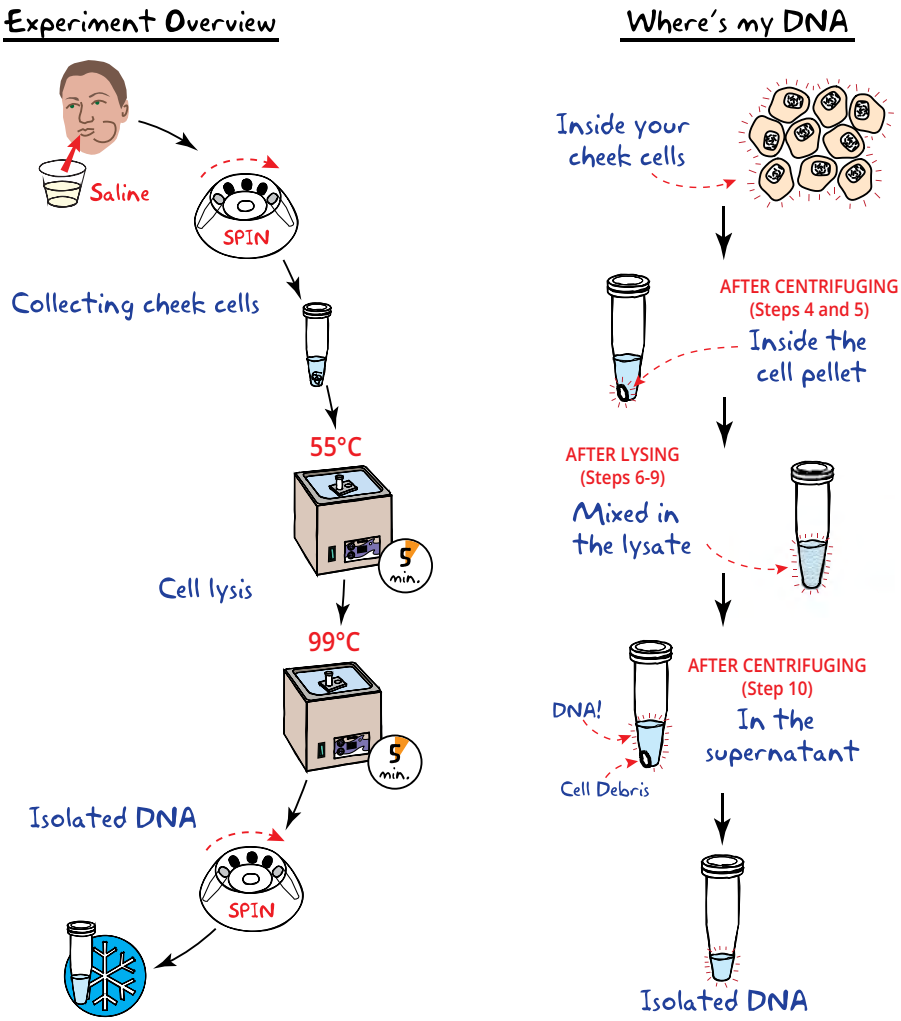


Figure 3: Polymerase Chain Reaction

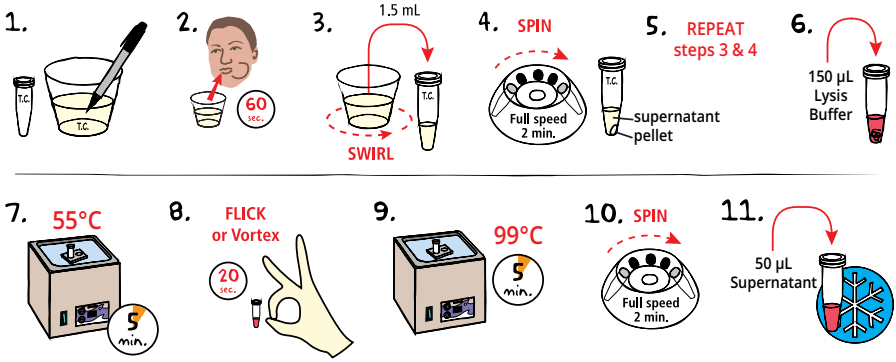


GEL SPECIFICATIONS

This experiment requires a gel with the following specifications:

Recommended Gel Size:	7 x 7 cm or 7 x 14 cm
Number of Samples Wells:	6
Placement of the Well-former Template:	First set of notches
Gel Concentration Required:	2.0%

Isolation of DNA from Human Cheek Cells

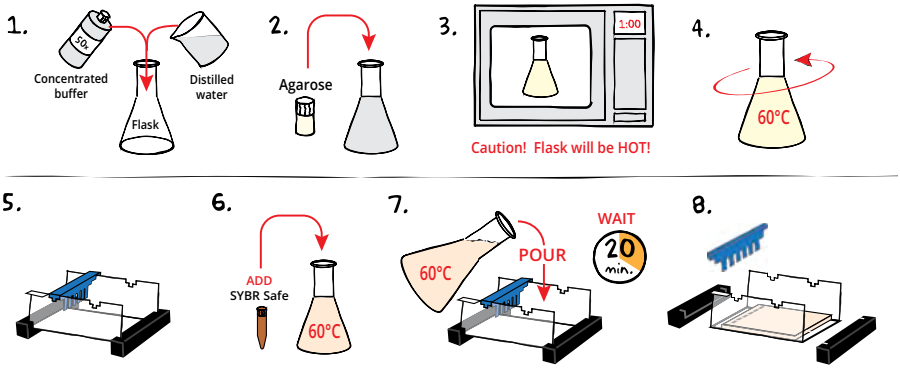


1. **LABEL** an empty 1.5 mL screw top microcentrifuge tube and a cup of saline with your lab group and/or initials.
2. **RINSE** your mouth vigorously for 60 seconds using 10 mL saline solution. **EXPEL** the solution back into the same cup.
3. **SWIRL** the cup gently to resuspend the cells. **TRANSFER** 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 PELLET**!
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 Amplification of the PTC Region.

OPTIONAL STOPPING POINT:
The extracted DNA may be stored at -20°C for amplification at a later time.

Agarose Gel Electrophoresis



CASTING THE AGAROSE GEL

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** 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).
4. **COOL** agarose to 60°C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. Before casting the gel, **ADD** diluted SYBR® Safe to the molten agarose and swirl to mix (see Table A).
7. **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.
8. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

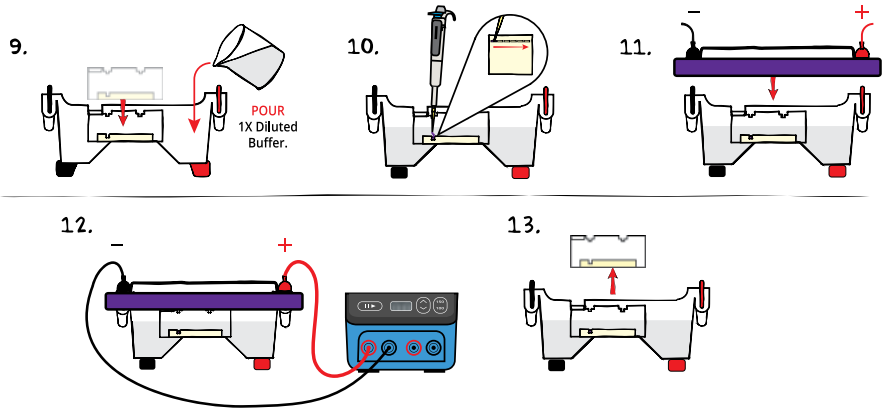
Table A

Individual 2.0% UltraSpec-Agarose™ Gel with Diluted SYBR® Safe Stain

Size of Gel Casting tray	1X TBE Buffer	+	Amt of Agarose	=	TOTAL Volume	ADD Diluted SYBR (Step 6)
7 x 7 cm	25 mL		0.5 g		25 mL	25 µL
7 x 14 cm	50 mL		1.0 g		50 mL	50 µL

Wear gloves and safety goggles

Agarose Gel Electrophoresis



RUNNING THE GEL

9. **PLACE** gel (on the tray*) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
10. **LOAD** the entire sample (25 µL) into the well in the order indicated by Table 1.
11. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
12. **CONNECT** leads to the **power sourc**e and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
13. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

Table B

Individual 2.0% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	1X Dilluted Buffer	+ Amt of Agarose
7 x 7 cm	25 mL	0.50 g
7 x 14 cm	50 mL	1.0 g

Table C

Time and Voltage Guidelines
(2.0% Agarose Gels)

Volts	Time: 7 x 7 cm gel ~4.0 cm migration	Time: 7 x 14 cm gel ~6.5 cm migration
125	30 min.	60 min.
70	60 min.	120 min.
50	90 min.	150 min.

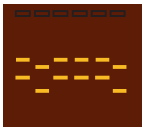
REMINDER:
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

Table 1: Gel Loading

Lane	Label	Sample Name
1	A	EdvoQuick™ DNA Ladder
2	B	Undigested DNA
3	C	Student 1 Digested DNA Sample
4	D	Student 2 Digested DNA Sample
5	E	Student 3 Digested DNA Sample
6	F	Student 4 Digested DNA Sample

Agarose Gel Electrophoresis

14. 

15. 

16. 

VISUALIZING THE SYBR® GEL

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

PTC Tasting *(Excerpts from EDVO-Kit #345)*

For this module, each student should receive the following materials:

- PTC Paper
- Control Taste Paper

PROCEDURE:

1. **TASTE** the Control strip of paper first.
2. **TASTE** the PTC strip of paper.
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 either strip of paper.

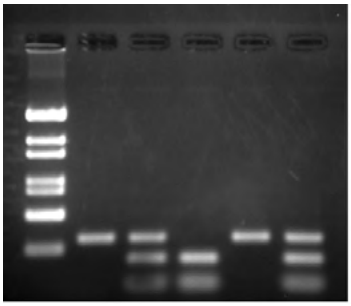
ANALYZE THE RESULTS:

1. Verify the outcome of your bitter tasting ability using the PTC paper with your genotype in the PCR-RFLP analysis in previous module.
2. Are you a homozygous bitter taster, a heterozygous bitter taster, or a non-taster?

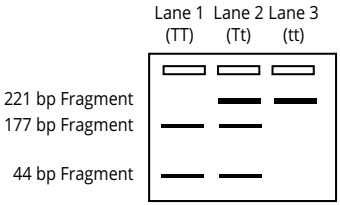
Enter your classroom data in a table as shown below:

	PHENOTYPE		
GENOTYPE	Strong Taster	Weak taster	Non-taster
TT (homozygous)			
Tt (heterozygous)			
tt (homozygous)			

Experimental Results and Analysis



Lane	Sample	Genotype	Sizes (in bp)
1	EdvoQuick™ DNA Ladder	----	----
2	Undigested DNA	----	221
3	Student 1	Taster heterozygous	221, 177, 44
4	Student 1	Taster homozygous	177, 44
5	Student 1	Non-Taster homozygous	221
6	Student 1	Taster heterozygous	221, 177, 44



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.

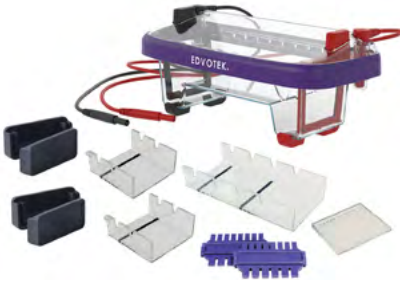
Related Products

EDGE™ Integrated Electrophoresis System
Cat# 500

EdvoCycler™ Jr. Personal PCR Machine
Holds 16 x 0.2 mL Samples
Cat# 540

EDVOTEK® Variable Micropipette
5-50 µL Micropipette
Cat. # 590

Fixed Volume MiniPipet™
35 µL MiniPipet™
Cat. # 587-2



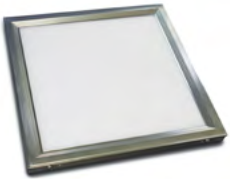
M12 Complete™ Electrophoresis Package
Cat# 502-504

TruBlu™ Jr Blue Light Transilluminator
Cat# 555

TruBlu™ 2 Blue/White Transilluminator
Cat# 557

DuoSource™ 150
75/150 V, for 1 or 2 units
Cat# 509

QuadraSource™
10-300 V, for up to 4 units
Cat# 5010



White Light LED Transilluminator
Cat# 552

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