# Exploring the Genetics of Taste:

## SNP Analysis of the PTC Gene Using PCR

Thomas Cynkar
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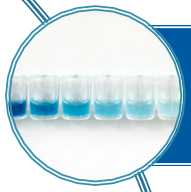
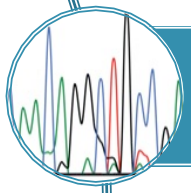
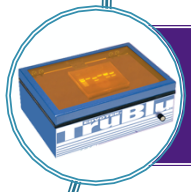


Experiments



Reagents



Equipment

Resources

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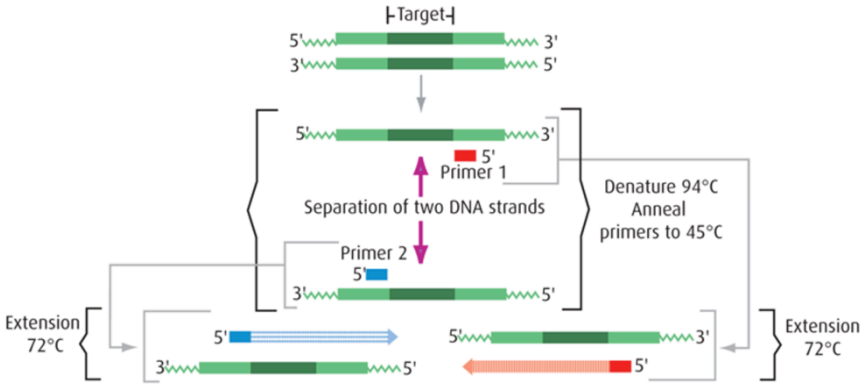
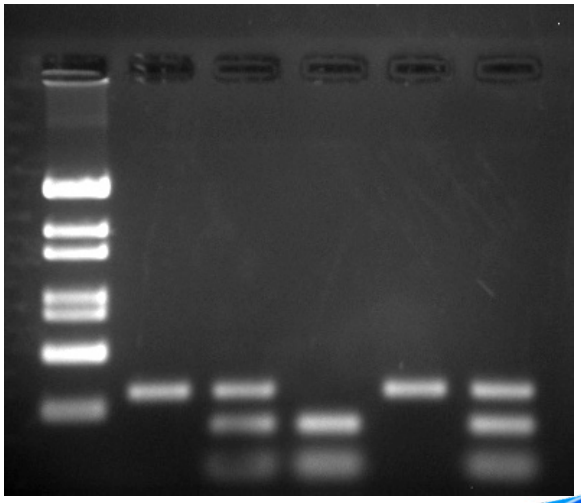
# Today's Strategy

Discuss tips and tricks for the PTC taste PCR experiment

1. **Load and Run Agarose Gels**
   - Simulation of PTC experiment
2. **Discuss DNA Extraction**
   - Tips and Tricks
3. **Discuss PCR**
   - Overview of Edvotek PCRs
4. **Discuss Restriction Digest**
   - Even more Tips and Tricks
5. **Compare PTC Taste Perception**



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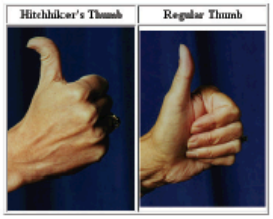
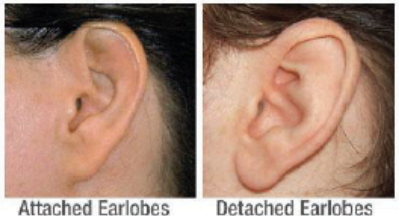
# Our Genotype Determines our Phenotype

Some traits are thought to be determined by a single gene:

| | | |
|---|---|---|
| Earlobes | Detached | Attached |
| Hairline | Widow's Peak | Straight |
| Chin | Cleft Chin | Smooth Chin |
| Thumb | Straight Thumb | Hitchhiker's Thumb |



Attached Earlobes Detached Earlobes

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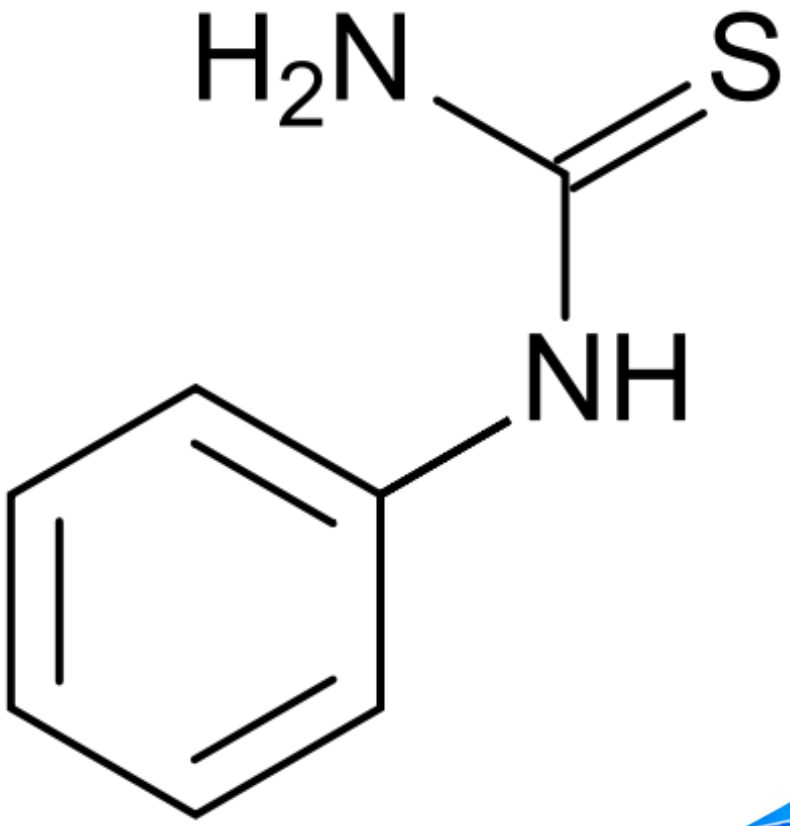
# Individuals Vary in their Sensitivity to PTC

- In 1931, Arthur Fox was mixing the chemical phenylthiocarbamide (or PTC for short) when some blew into the air.
- A nearby colleague tasted bitterness, but Fox did not taste anything.
- After testing a large number of people, it was hypothesized that the ability to taste PTC was genetically determined.

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# Characterization of the PTC Gene Using PCR-RFLP

- Step 1: DNA is extracted from cheek cells
- Step 2: Extracted DNA is amplified using PCR
- Step 3: PCR products are digested using restriction enzymes
- Step 4: Restriction digests are analyzed using electrophoresis
- Step 5: Taste PTC paper to link genotype to phenotype

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# Planning and timing requirements

- Required equipment/reagents
  - Thermal cycler
  - Agarose gel electrophoresis apparatus
  - D.C. power supply
  - Balance
  - Microcentrifuge
  - UV or Bluelight Transilluminator
  - Micropipets & tips (5-50 µL)
  - Microwave or hot plate
  - Glassware
  - Gloves
  - Ice & ice bucket
  - Distilled or deionized water
  - Drinking water

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# Planning and timing requirements

- Teacher Prep = Estimate approximately 30 mins per day
  - Prepare buffers, aliquot reagents, check equipment
- Gel Electrophoresis Prep = 45 mins
  - (Note: This can be done up to one week ahead of time)

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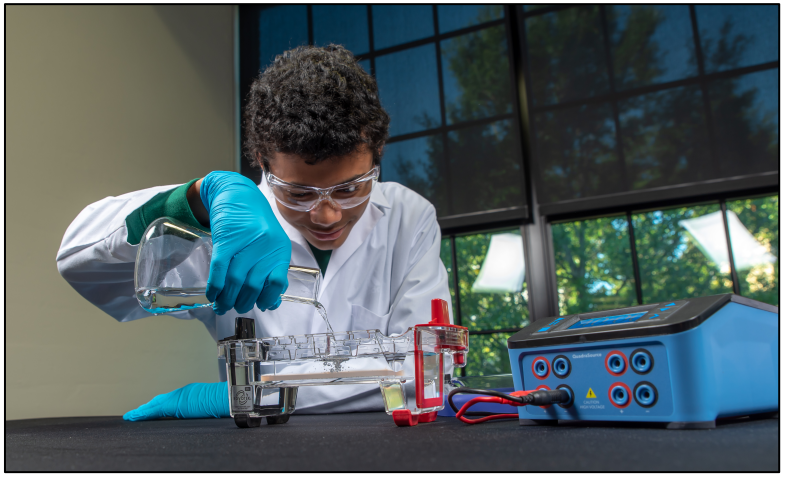
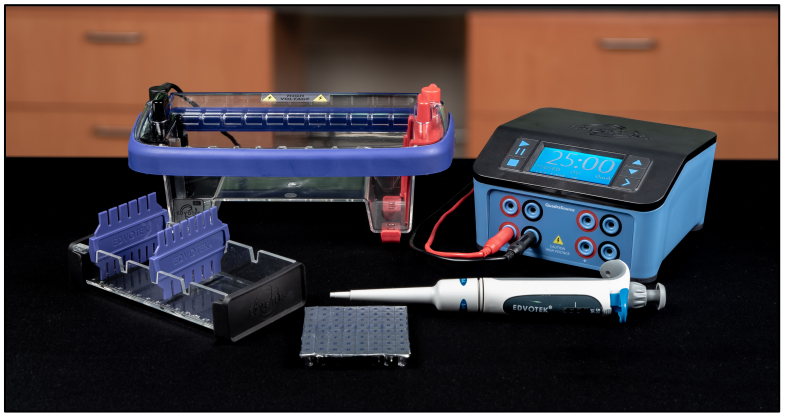
- Experiment = at least 2-3 lab days
  - Plan ahead for stopping points if necessary!





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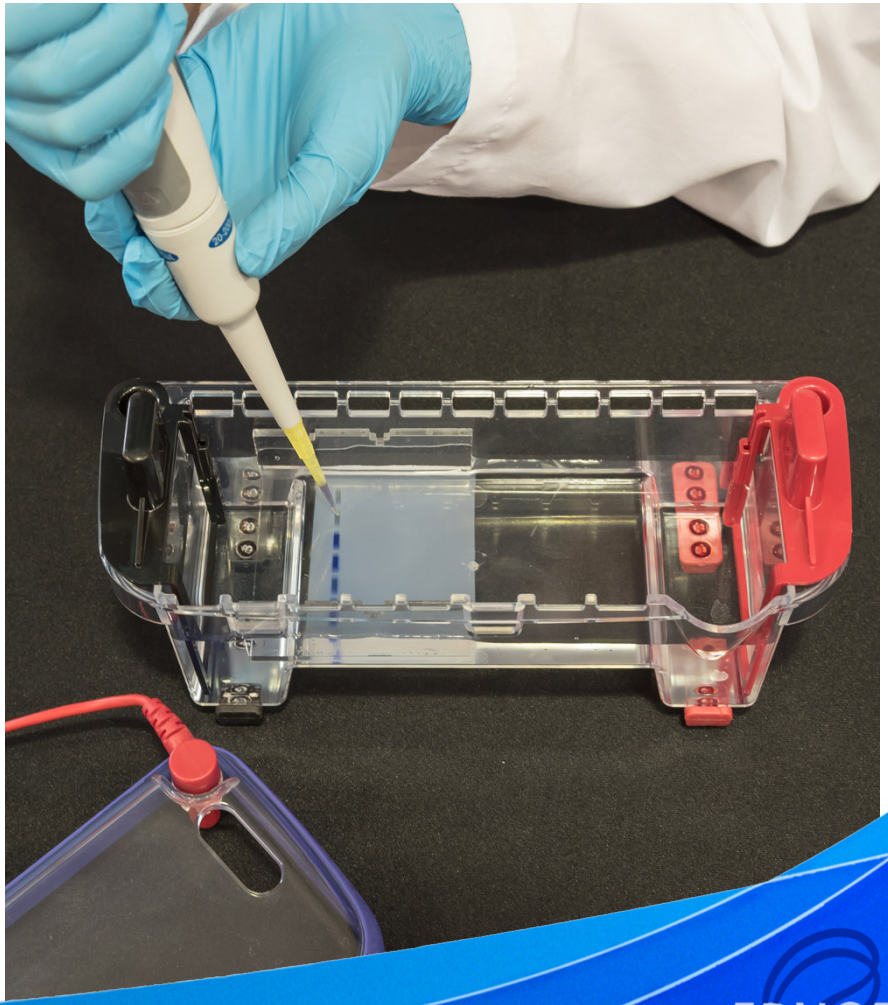

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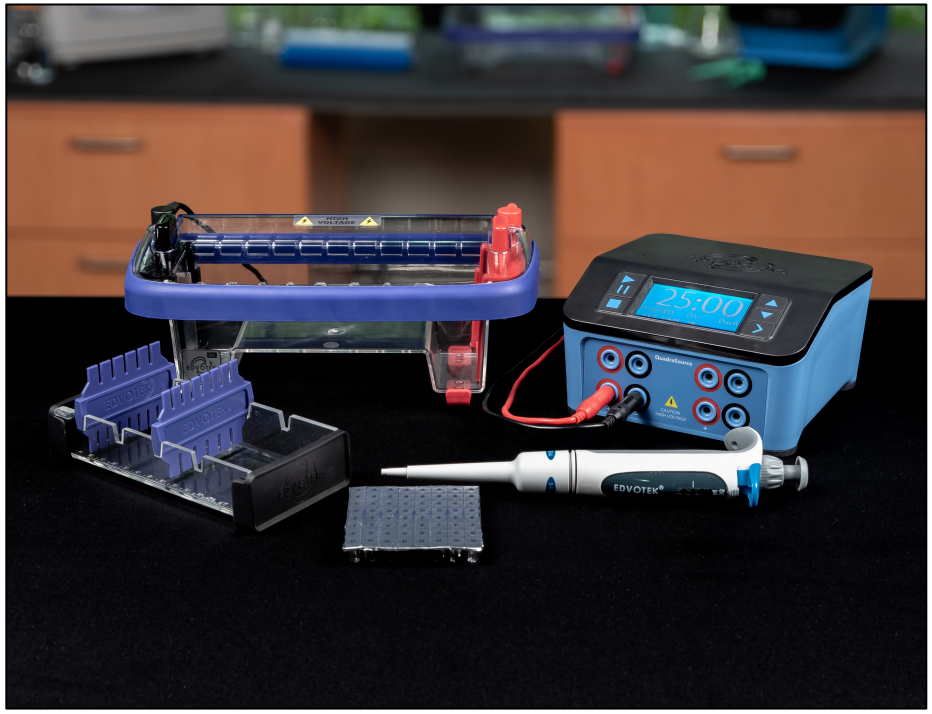
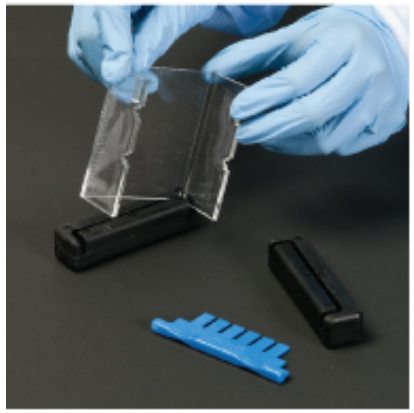
# What Do I Need to Perform Electrophoresis Experiments?

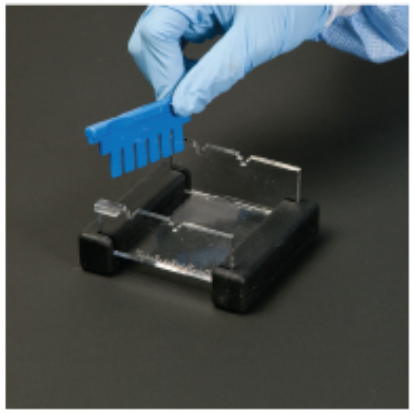



- Horizontal electrophoresis apparatus
- D.C. power source
- Micropipet or transfer pipet
- Agarose
- Electrophoresis Buffer
- Samples – dye, DNA, RNA
  - PCR products
- A way to visualize samples

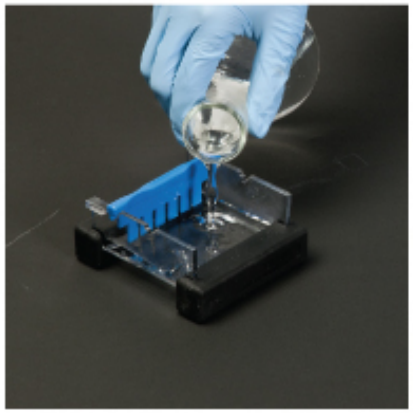
# Casting the Agarose Gels



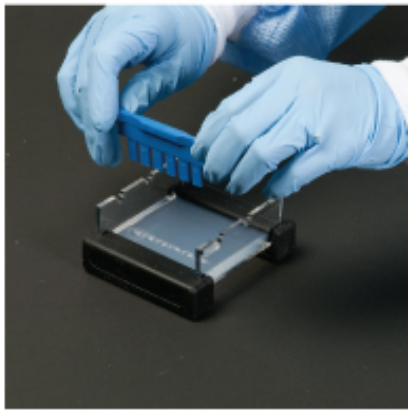
1. Prepare the tray for gel casting by sealing the ends with rubber end caps.



2. Place a comb in the appropriate notches.



3. Prepare the agarose gel solution. Cool to 60°C and then pour the gel.



4. After approx. 20 min. the gel will solidify. Remove the comb from the gel tray.

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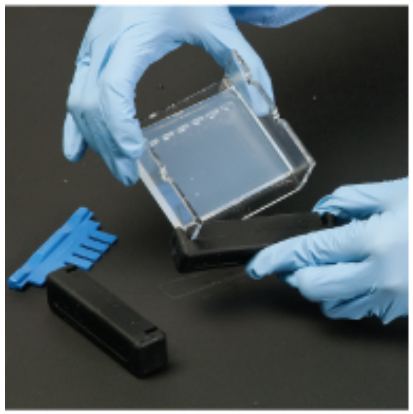
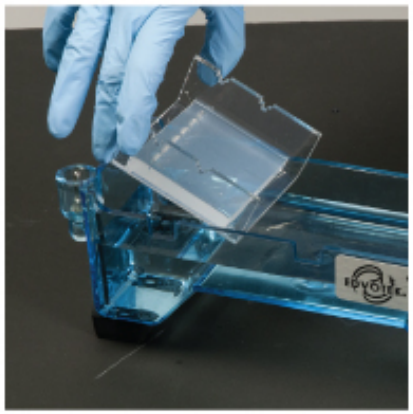
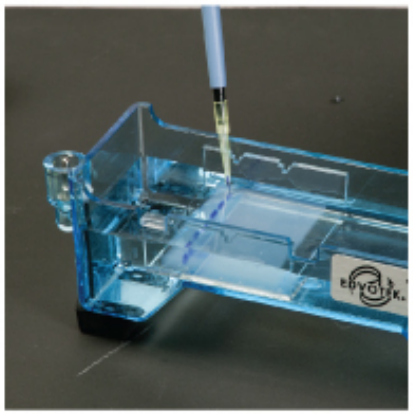
# Casting the Agarose Gels



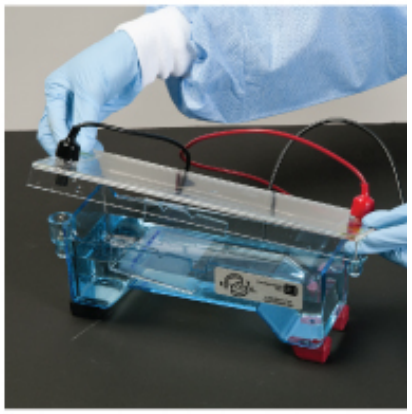
5. Slowly remove the rubber end caps. Be very careful not to damage or tear the gel!



6. Place the gel (on its tray) into the electrophoresis chamber. The gel should be completely submerged under electrophoresis buffer.

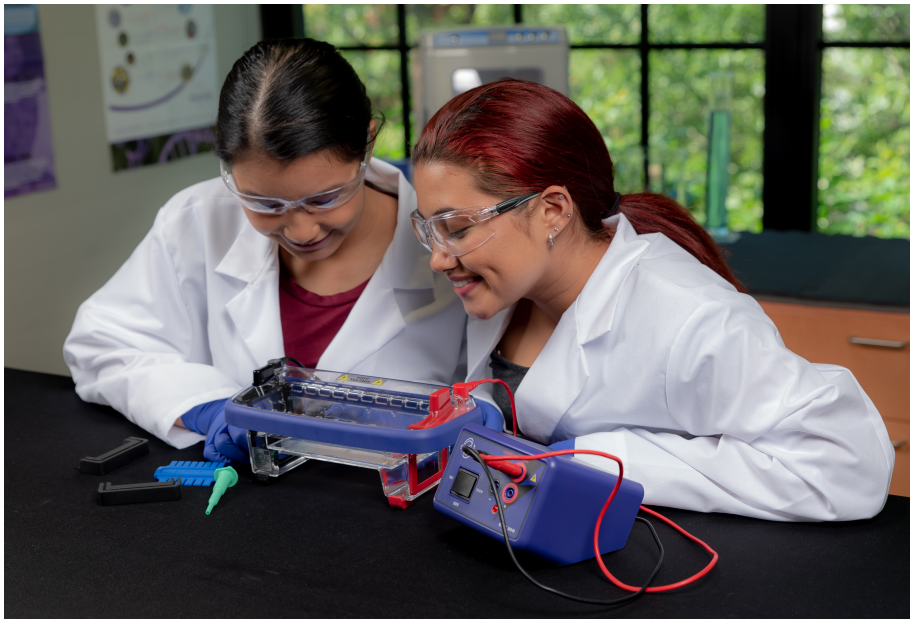


7. Load samples into wells in consecutive order, starting with the first well on the left.



8. After samples are loaded, attach the safety cover, connect the leads to the D.C power source and set the power source at the required voltage.

# Let’s run our gels!



Load 25 µL of sample per well.

| Lane | Sample |
|---|---|
| 1 | EdvoQuick™ DNA Ladder |
| 2 | Undigested DNA |
| 3 | Student 1 DNA Sample |
| 4 | Student 2 DNA Sample |
| 5 | Student 3 DNA Sample |
| 6 | Student 4 DNA Sample |

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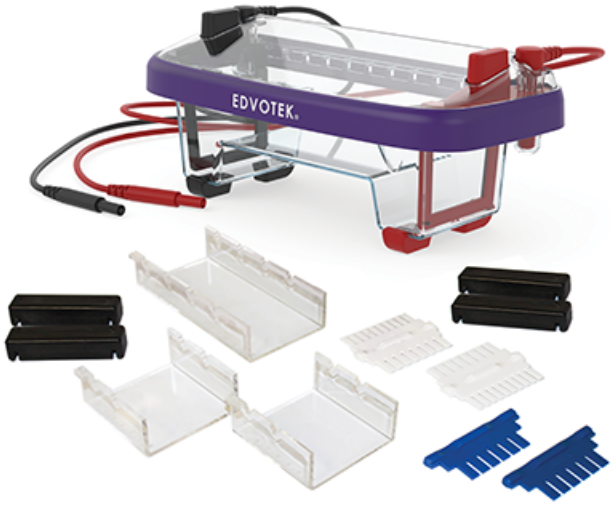
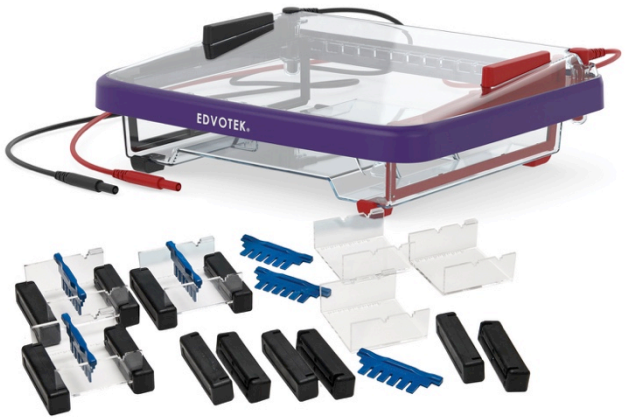
# Electrophoresis Chambers for Classrooms of all Sizes




Cat. # 504
Model M12
Two gels




Cat. # 515
Model M36
Six gels

# Power Supplies Provide Current for Electrophoresis

Cat. #509
DuoSource™ 150
(75/150 V)

Cat. #5010
QuadraSource™
(10-300V)

# Characterization of the PTC Gene Using PCR-RFLP

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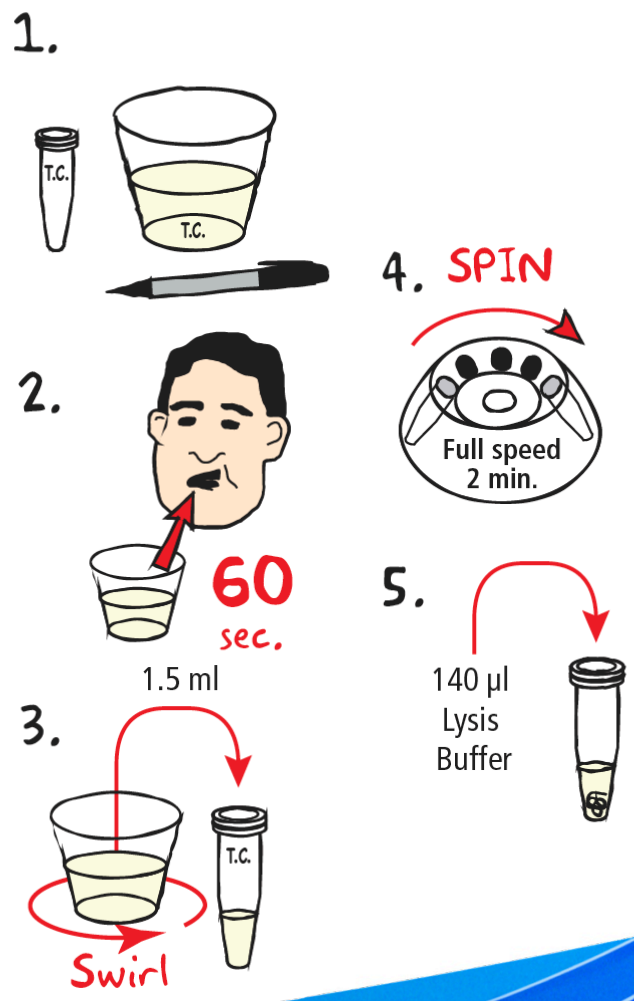


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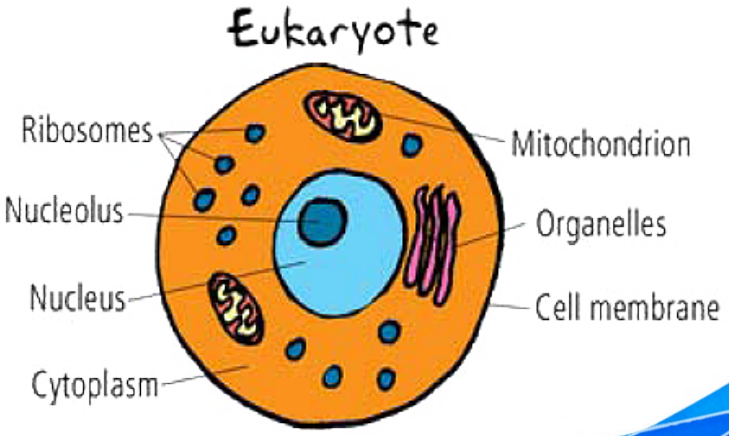
# Isolation of DNA from Cheek Cells

- Cheek cells are homogenized in a solution that releases the DNA from the cell by breaking down cellular and nuclear membranes in a process called **lysis**.
  - a buffer stabilizes the sample's pH (Tris-HCl)
  - a chelating agent removes ions (inactivates DNAses)
  - a detergent dissolves lipids (SDS)
  - an enzyme breaks down proteins (Proteinase K).

- After a short incubation, the solution is boiled to lyse the cells.

- The lysate is centrifuged to remove insoluble components.

- The lysate contains nuclear DNA for PCR-RFLP analysis.

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# Isolation of DNA from Cheek Cells

## Teacher preparation:

| Preparation For: | What to do: | When: | Time Required: |
|---|---|---|---|
| **Module I: Isolation of DNA from Cheek Cells** | Prepare and aliquot various reagents | Up to one day before performing the experiment. | 30 min. |
| | Prepare and aliquot Lysis Buffer | **IMPORTANT: Prepare the Lysis buffer no more than one hour before performing the experiment.** | 15 min. |
| | Equilibrate waterbaths at 55° C and boiling. | One hour before performing the experiment. | 5 min. |

- Student experiment should take 30-40 minutes.
- Extracted DNA can be frozen until needed.

**For best results, you MUST use saline for this module!**

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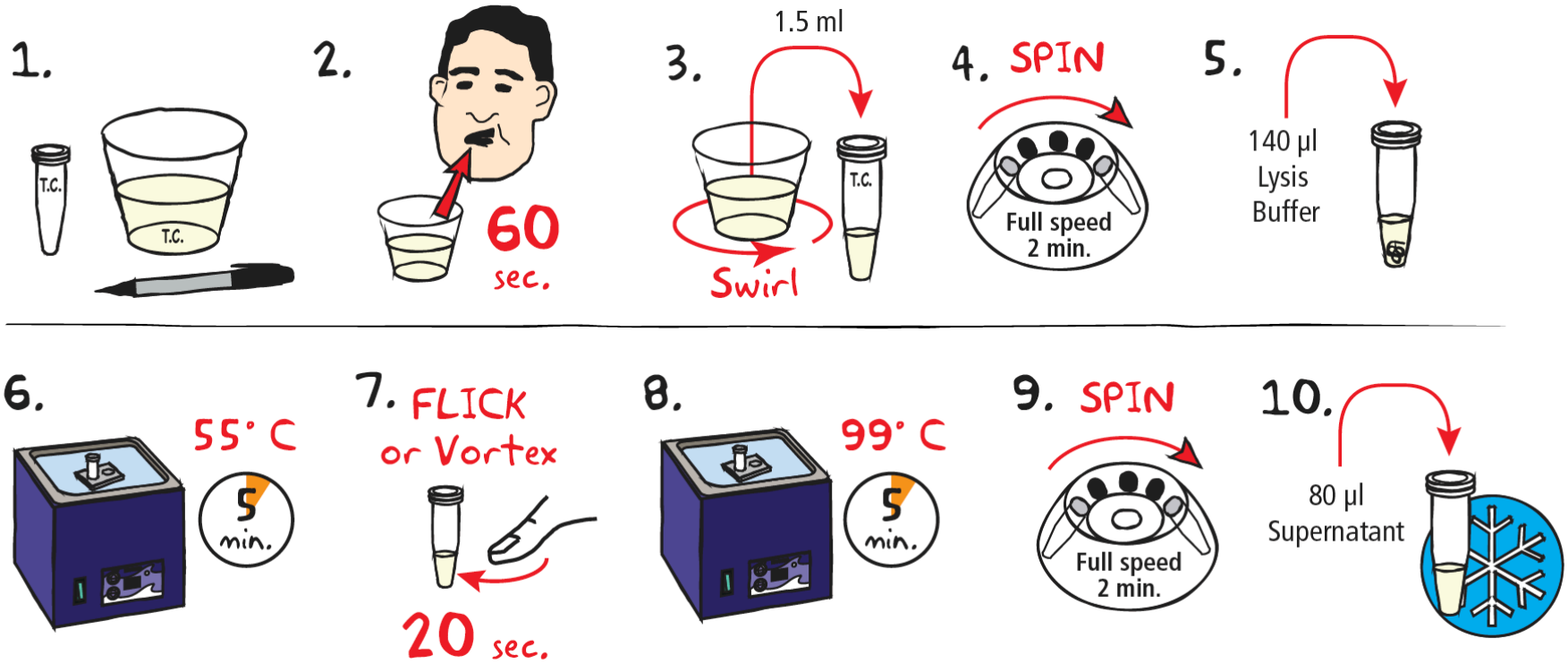
# Extraction of DNA from Cheek Cells

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# Characterization of the PTC Gene Using PCR-RFLP

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- Step 5: Taste PTC paper to link genotype to phenotype

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# Amplification of the PTC region

## Teacher preparation:

| Preparation For: | What to do: | When: | Time Required: |
|---|---|---|---|
| **Module II: Amplification of the PTC Region** | Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.) | One day to 30 min. before performing the experiment. | 30 min. |
| | Program Thermal Cycler | One hour before performing the experiment. | 15 min. |

- Student experiment should take 15-20 minutes.
- PCR reactions should be setup and run in the same class period.
- PCR products can be frozen and stored until needed.

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# DNA Amplification using PCR

## Process that amplifies short segments of DNA from a longer template

- **DNA template**

  The original DNA sequence to be copied

- **Primers**

  Short piece of DNA that defines the area to amplify

- **Thermostable DNA polymerase (*Taq*)**

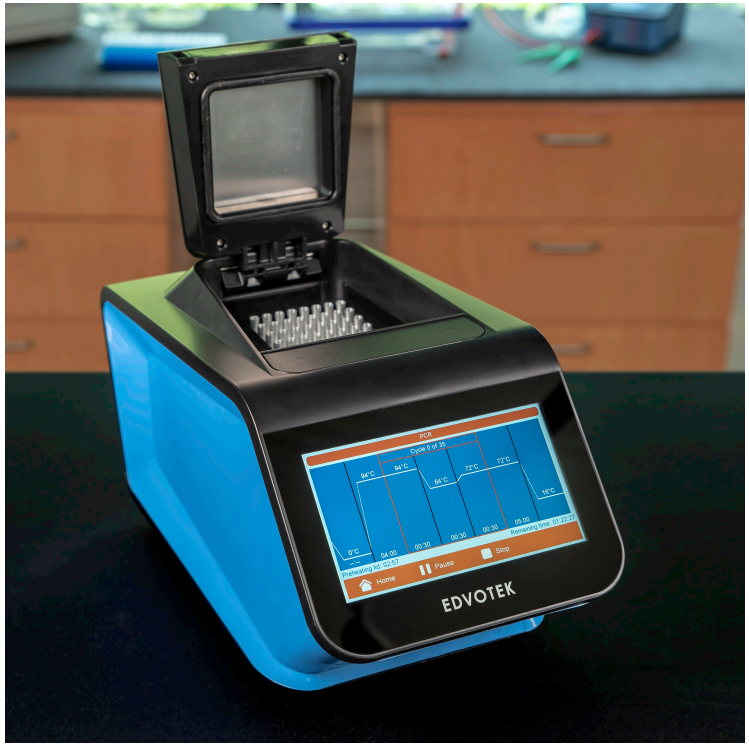
  Enzyme that replicates DNA

- **Free nucleotides (dNTPs)**

  Excess DNA building blocks

- **Buffer**

  Regulates pH, provides magnesium

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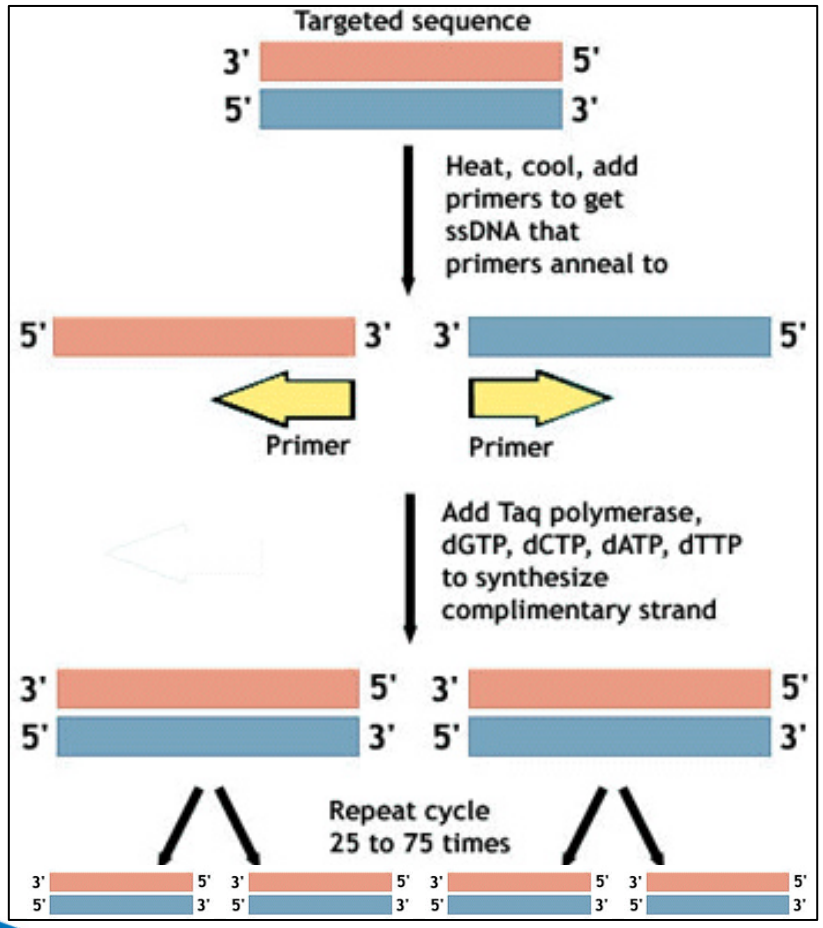
# PCR Amplifies Specific DNA Sequences




- **Denaturation (94°C)**
  Double-stranded DNA is "unzipped" into single strands.
- **Annealing (40-65°C)**
  Primers base pair with the target DNA sequence.
- **Extension (72°C)**
  *Taq* polymerase extends the primer and synthesizes a new strand of DNA.

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# Convenient PCR EdvoBeads™

# Characterization of the PTC Gene Using PCR-RFLP

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# Restriction Digest of the PTC PCR Product

## Teacher preparation:

| Preparation For: | What to do: | When: | Time Required: |
|---|---|---|---|
| **Module III: Restriction Digest of the PTC PCR Product** | Prepare and aliquot *Hae*III Restriction enzyme | **IMPORTANT: Prepare the diluted *Hae*III Restriction Enzyme no more than one hour before performing the experiment.** | 15 min. |
| | Equilibrate waterbath at 37° C | One hour before performing the experiment. | 15 min. |

- Student experiment should take 40-70 minutes.
- Digested DNA can be frozen and stored until needed.

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# The Genetic Basis of the Bitter Tasting Ability

- Ability to taste PTC compound has been linked to the *TAS2R38* gene.
- The taster phenotype (T) is dominant to the non-taster phenotype (t).
- A person inherits one copy of the gene from each of his/her parents.

| | T | t |
|---|---|---|
| T | TT | Tt |
| t | Tt | tt |

Genotype:

| | |
|---|---|
| 1/4 | TT |
| 1/2 | Tt |
| 1/4 | tt |

Phenotype:

| | |
|---|---|
| 3/4 | dominant |
| 1/4 | recessive |

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# The Genetic Basis of the Bitter Tasting Ability

| 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 | Isoleucine > Valine |

- The *TAS2R38* gene is located on chromosome 7
- Ability to taste PTC is linked to Single Nucleotide Polymorphisms (SNPs) at three distinct locations
- Most common haplotypes are PAV and AVI, representing tasters and non-tasters, respectively.

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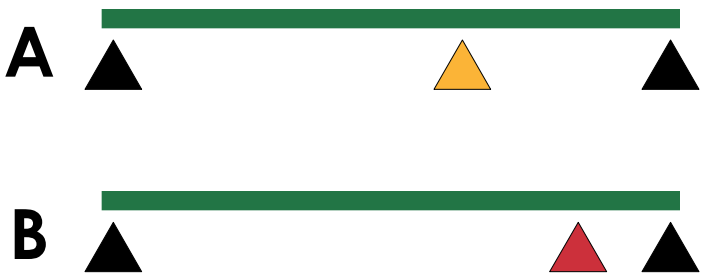
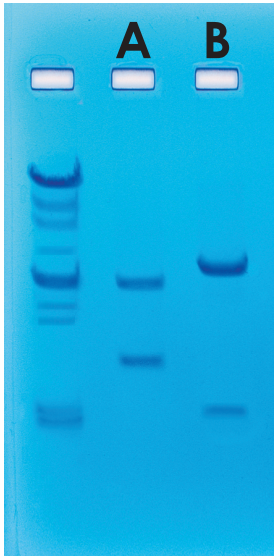
# Using the PCR-RFLP Method to Characterize *TAS2R38*




Cat. # 109

- The SNP at position 145 removes a restriction enzyme cut site to the DNA.
- This region of the *TAS2R38* gene can be amplified by PCR and characterized using Restriction Fragment Length Polymorphism (RFLP) analysis.

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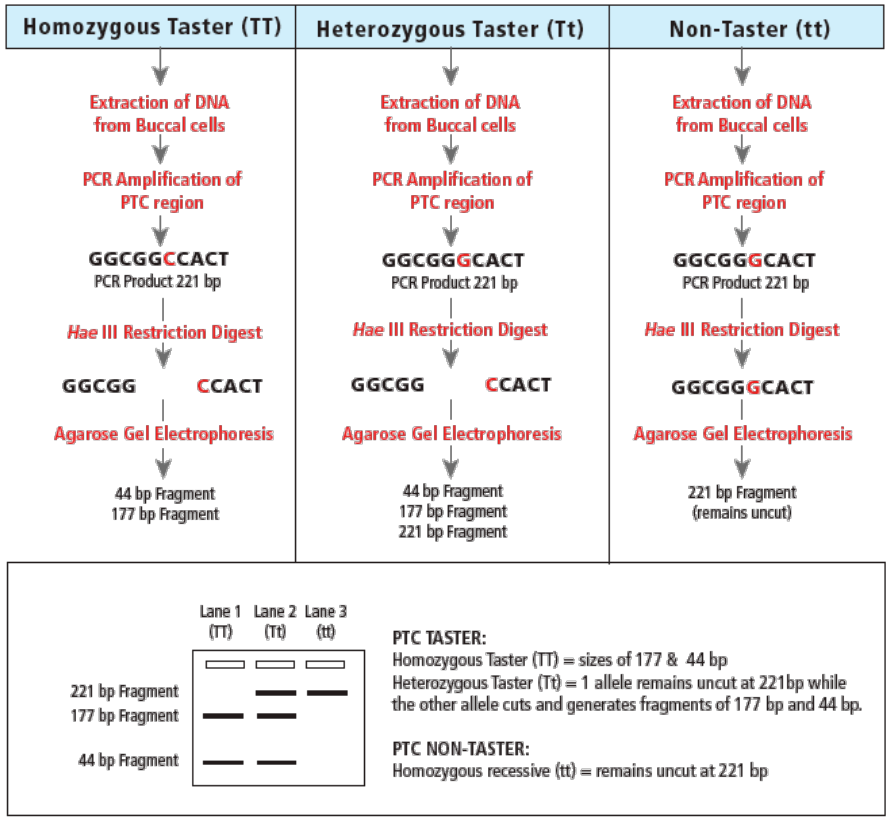
# Determining PTC Genotype using RFLP Analysis

- The restriction enzyme recognition site for *HaeIII* is present in the taster allele.
- The "non-taster" polymorphism changes a single nucleotide in the recognition site, rendering it unable to be digested by *HaeIII*
- After digestion, the samples are analyzed by Agarose Gel Electrophoresis

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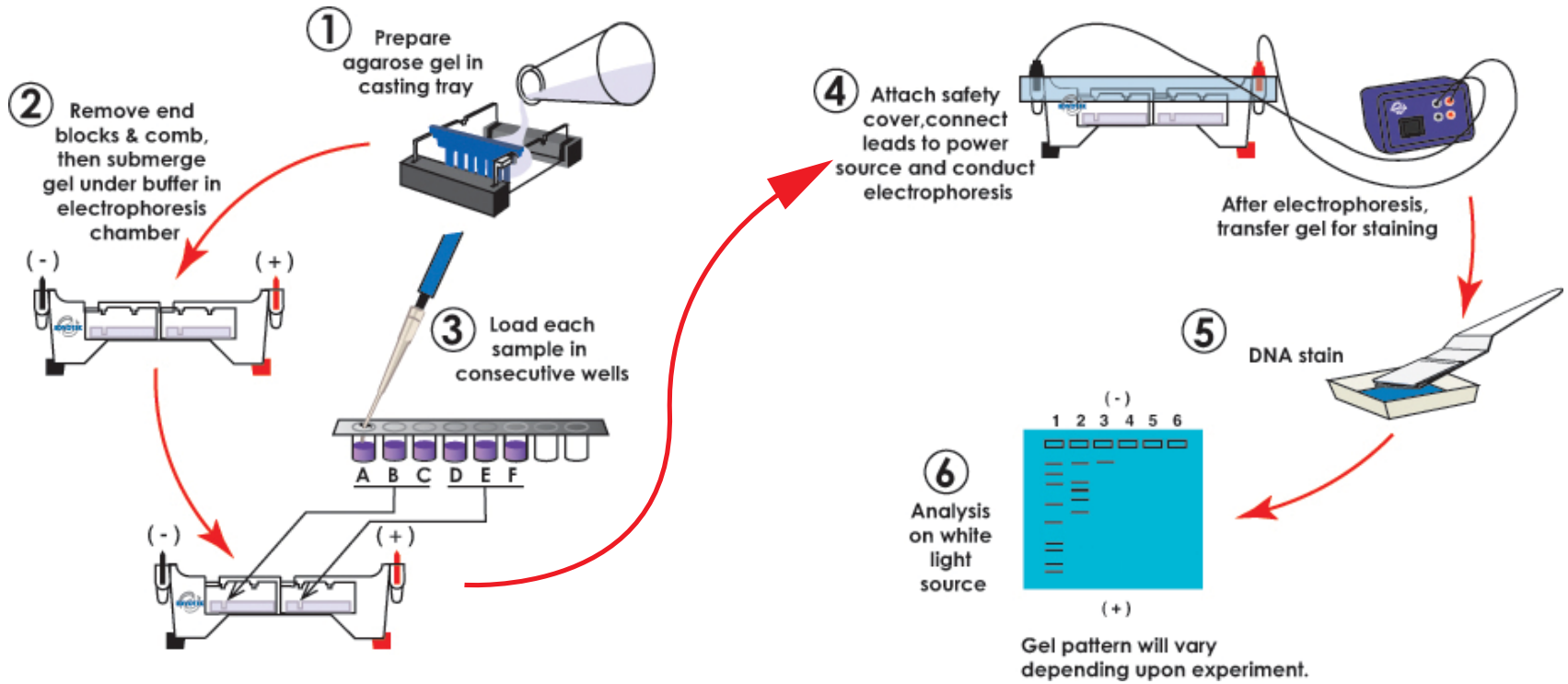
# Electrophoresis at a Glance

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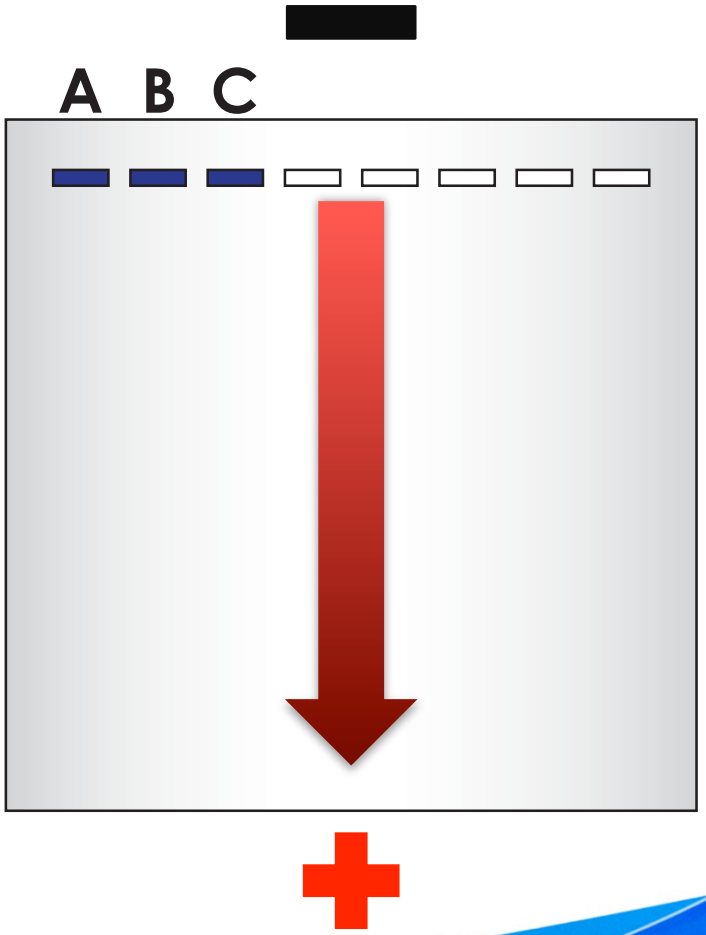
# Electrophoresis Separates DNA Fragments By Size

- The sugar-phosphate backbone of DNA has a strong negative charge.
- When an electrical current is passed through the gel, the current drives the DNA fragments through the gel towards the positive electrode.

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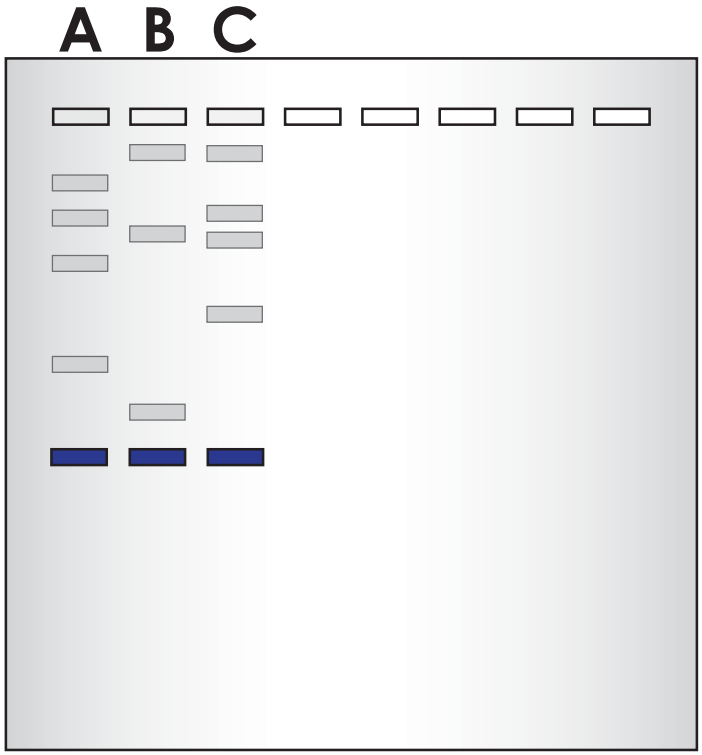
# Electrophoresis Separates DNA Fragments By Size

- The gel contains small channels through which the DNA can pass.
- Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels.

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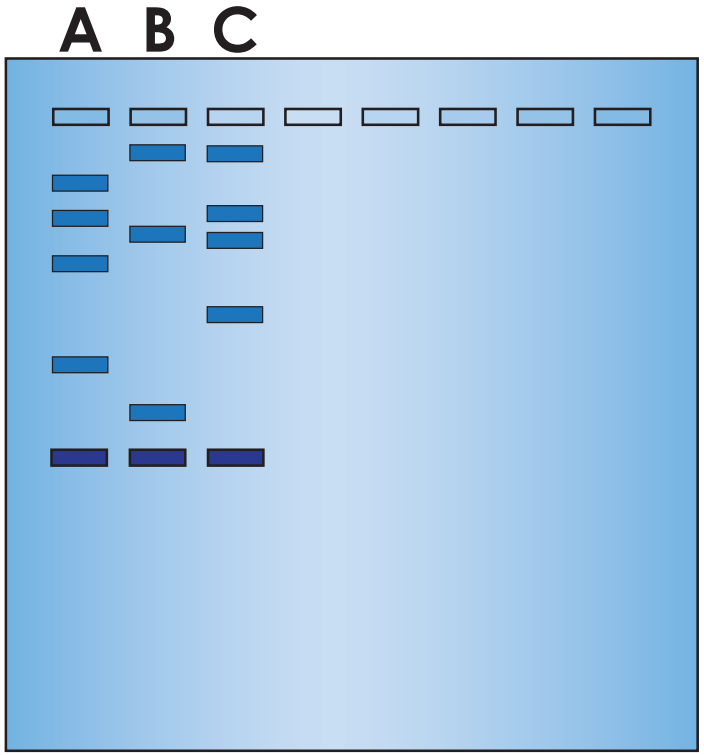
# Electrophoresis Separates DNA Fragments By Size

- Because molecules of different sizes travel at different speeds, discrete bands are formed.
- After the current is stopped, the bands can be visualized using a stain that sticks to DNA.
  - InstaStain ® EtBr
  - InstaStain ® Blue
  - FlashBlue™ Stain
  - Sybr® Safe stain

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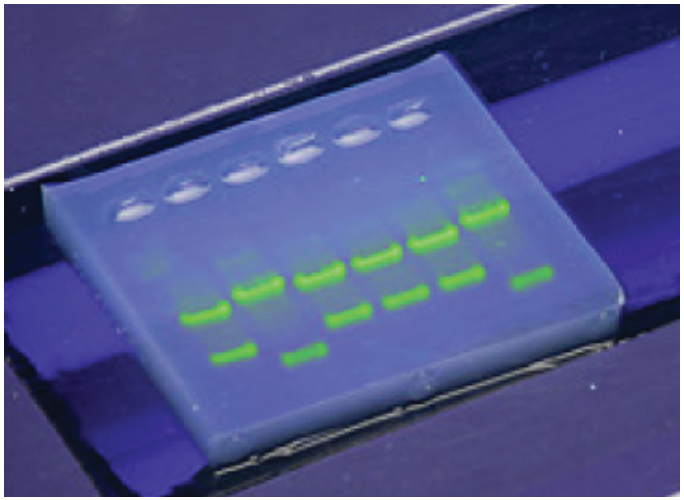
# SYBR® Safe DNA Stain

## In-gel Staining

1. Melt agarose and cool to 65°C.
2. Add concentrated Sybr® Safe stain to the molten gel at 1:10,000 dilution (5 µL per 50 mL agarose solution).
3. Run DNA samples through gel – no post staining or destaining necessary!

## Post-electrophoresis Staining

1. Dilute concentrated stain to 1:20,000 (5 µL per 100 mL distilled water).
2. After electrophoresis, place gel in tray. Cover gel with diluted Sybr® Safe stain.
3. Stain gel for 10 – 15 minutes.



**Kit #109**
**Transilluminator #558**
**SybrSafe® Stain #608**



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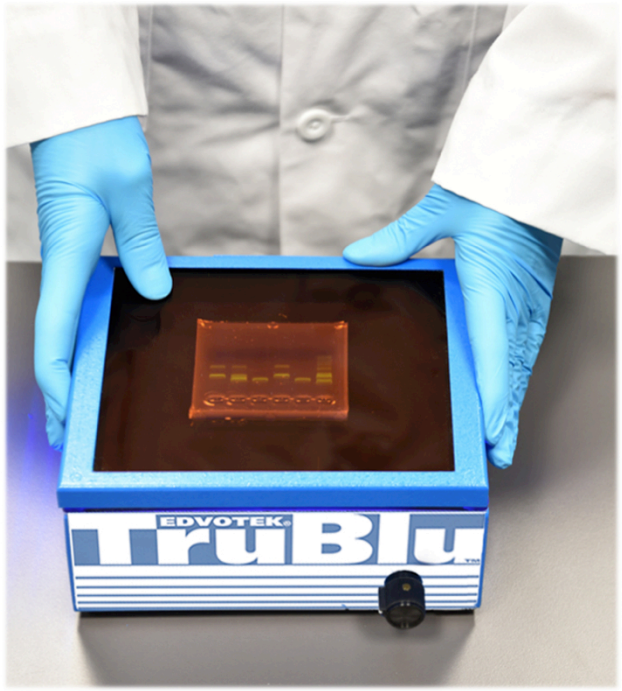
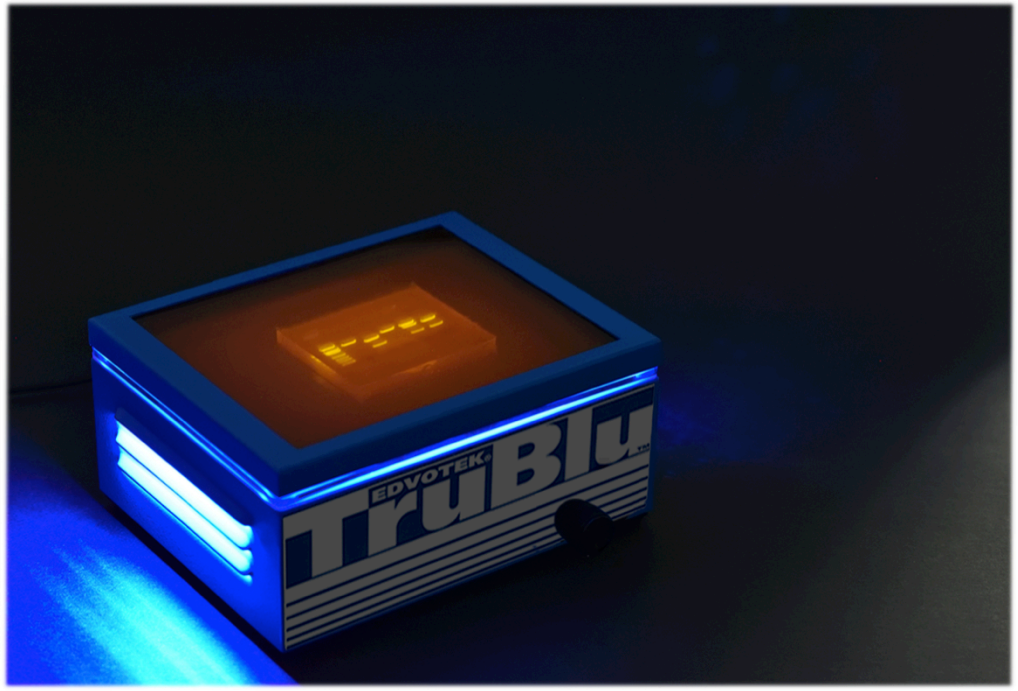
# **TruBlu™** Bluelight Transilluminator

Optimized for **SYBR® Safe** stained gels • Large viewing area • No harmful UV

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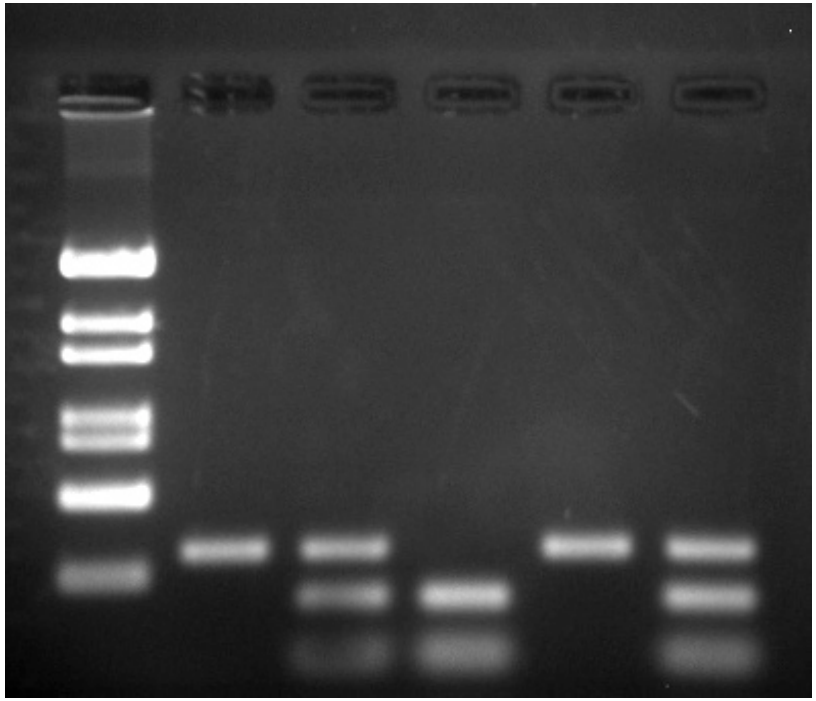
# Exploring Genetic Diversity with PCR-RFLP Analysis



What do these genotypes mean in terms of phenotype?

| Lane | Sample |
|---|---|
| 1 | EdvoQuick™ DNA Ladder |
| 2 | Undigested DNA |
| 3 | Student 1 DNA Sample |
| 4 | Student 2 DNA Sample |
| 5 | Student 3 DNA Sample |
| 6 | Student 4 DNA Sample |

EDVO-Kit #345

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# Characterization of the PTC Gene Using PCR-RFLP

- Step 1: DNA is extracted from cheek cells
- Step 2: Extracted DNA is amplified using PCR
- Step 3: PCR products are digested using restriction enzymes
- Step 4: Restriction digests are analyzed using electrophoresis
- **Step 5: Taste PTC paper to link genotype to phenotype**

| | T | t |
|---|---|---|
| T | TT | Tt |
| t | Tt | tt |

Genotype:

| | |
|---|---|
| 1/4 | TT |
| 1/2 | Tt |
| 1/4 | tt |

Phenotype:

| | |
|---|---|
| 3/4 | dominant |
| 1/4 | recessive |

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# Identification of Bitter Tasting Ability with PTC Paper

1. TASTE the Control strip of paper.
2. TASTE the PTC strip of paper.
3. Compare the taste of the two strips of paper.
   - What does the PTC paper taste like when compared to the Control paper: intensely bitter, somewhat bitter, or tasteless?

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# Exploring Genetic Diversity with PCR-RFLP Analysis

- **Genetic differences in the *TAS2R38* gene affects the way individuals can taste PTC.**
- **PCR-RFLP analysis of this gene allows us to determine our genotype.**
- **The genotype is linked to phenotype by determining whether the individual perceives PTC as bitter.**

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# Mistakes **Happen!**

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## We are ready to help!

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# Solar Eclipse viewing party

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**Monday – Friday**

**8AM-5:30PM EST**

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