



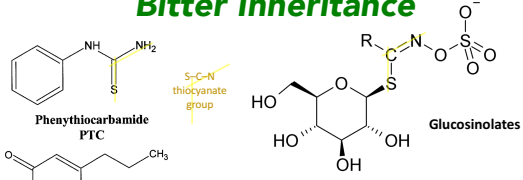
Bitter Inheritance

Many plants produce noxious alkaloid compounds to resist herbivory.
E.g., cruciferous vegetables — cabbage/mustard family — produce **glucosinolates**.
Humans perceive the taste of such compounds as **bitter**.

R-C(=O)-N(S(=O)(=O)[O-])

Bitter Inheritance



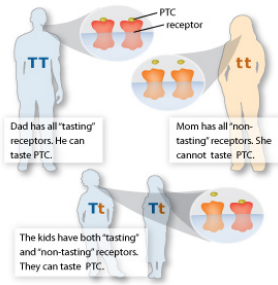
Two synthetic organic compounds, phenylthiocarbamide (**PTC**), and propylthiouracil (**PROP**) were found to stimulate the same mammalian taste receptors. So these compounds, esp. PTC, were used to study the perception of bitter flavor.

- However, it was observed that ~30% of humans tested could not taste bitter flavor from PTC.
- This ability/inability to taste PTC was heritable and attributed to variation in a single gene — the “**PTC gene**”

Bitter Inheritance

The inheritance of the PTC phenotype has been often used as an example of **simple dominance**.
With the “PTC-taster” allele (**T**) dominant to the recessive “PTC-nontaster” allele (**t**).

- **TT** or **Tt** genotype → “PTC-taster” phenotype
- **tt** genotype → “PTC-nontaster” phenotype



Mutation of the PTC gene

- “PTC gene” = gene **TAS2R38** on **chromosome 7**
- **T**: “taster allele” = **PAV** allele
- **t**: “non-taster” allele = **AVI** allele
- **3 single nucleotide polymorphisms (SNP)** → **3 amino acid substitutions**
 - Proline...Alanine...Valine → Alanine...Valine...Isoleucine
- **Primers** bracket **303 bp PCR product** *within* **TAS2R38** gene DNA
- **SNP** same size PCR product for **PAV & AVI** alleles
 - But one of the SNP is *within* the PCR product → disrupts a *SatI* restriction site
- **SatI** digest of **TAS2R38** DNA PCR product:
 - **PAC** PCR product cleaved by *SatI*
 - **AVI** PCR product not cleaved by *SatI*

TAS (PTC) PCR Cocktail

Reagent	Stock concentration	Reaction concentration	Volume per reaction	Volume per cocktail (n+1)
Water	—	—		
Buffer	5x	1x		
MgCl ₂	25 mM	1.5 mM		
Nucleotides	10 mM	200 µM		
PTC Forward Primer	10 µM	1 µM		
PTC Reverse Primer	10 µM	1 µM		
Taq polymerase	5 U/µl	1.25 U		
Total Cocktail			40 µl	
DNA template			10 µl	
Reaction Volume			50 µl	

TAS (PTC) PCR Thermocycler Protocol

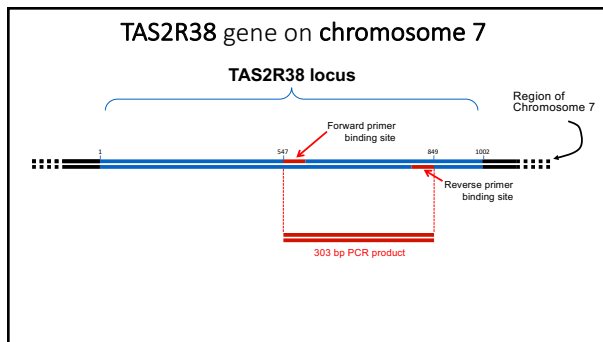
Step	Temperature (°C)	Time
Denature DNA Templates	95°	45 seconds
Anneal primers	55°	45 seconds
Product extension	72°	90 seconds
↻ Repeat for 40 cycles		
Final extension	72°	10 minutes
Final hold	4°	∞

Figure 1. The Human PTC Gene. The gene that is primarily responsible for human PTC taste sensitivity is located on the long arm of chromosome 7. The **sequence of the nontaster allele is shown below** with attention drawn to common single nucleotide polymorphism sites (SNPs), Fnu4H1 restriction endonuclease sites, primer sites for gene amplification by PCR. The Figure also indicates the amino acid substitutions corresponding to the SNPs, the restriction digest fragment lengths obtained in RFLP analysis, and the amino acid sequence for the nontaster gene product. The amino acid sequence also indicates the initiation site for translation of the chimpanzee nontaster allele (light blue M). Note that the amino acid sequence given is still for the human nontaster allele, not the chimpanzee allele.



**Tasting Phenylthiocarbamide (PTC):
A New Integrative Genetics Lab with an Old Flavor**

From: The-American-Biology-Teacher
[https://bioone.org/journals/the-american-biology-teacher/volume-70/issue-5/0002-7685\(2008\)70%5b23%3aTPPANI%5d2.0.CO%3b2/Tasting-Phenylthiocarbamide-PTC--A-New-Integrative-Genetics-Lab-with/10.1662/0002-7685\(2008\)70\[23:TPPANI\]2.0.CO;2.full](https://bioone.org/journals/the-american-biology-teacher/volume-70/issue-5/0002-7685(2008)70%5b23%3aTPPANI%5d2.0.CO%3b2/Tasting-Phenylthiocarbamide-PTC--A-New-Integrative-Genetics-Lab-with/10.1662/0002-7685(2008)70[23:TPPANI]2.0.CO;2.full)



PCR x RFLP

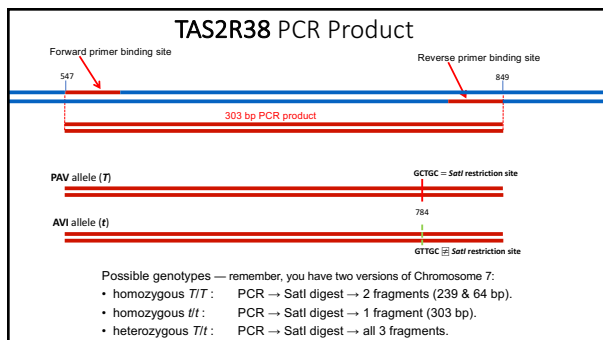
Cleaved Amplified Polymorphic Sequence (CAPS) Gel

SatI Digest

13.0 μ l TAS PCR reaction product
 1.5 μ l 10x buffer G
 0.5 μ l 5 Units/ μ l SatI restriction enzyme
 15.0 μ l total volume

1hr@37°C

2% agarose gel @ 80V



Cleaved Amplified Polymorphic Sequence (CAPS) Gel

Tt *tt* *tt* *Tt* *TT* *Tt* *tt* *Tt*

Bp ladder: 500, 400, 300, 200, 100

303, 239, 64

Genetic Penetrance and Expressivity

genotype \rightarrow phenotype

- Single genes do not function in isolation!
 - One step in multi-step pathways
 - Expression subject to pleiotropic, epistatic, epigenetic, & environmental modification
- Penetrance:** what fraction of the population exhibits the phenotype related to that allele
- Expressivity:** what variation in the population is there in how strongly the phenotype is demonstrated for that allele

Genetic Penetrance and Expressivity

PTC genotype \rightarrow PTC phenotype

- PTC / PROP Taster test
- 1. Rinse your mouth briefly with water.
- 2. Place a PTC test strip on your tongue. Leave it for 5 seconds.
- 3. Remove the strip. Wait 10 seconds. Rate how bitter the taste on a scale of 0 to 5:
 0 = tastes like plain paper; 1 = barely perceptible bitter; 5 = extremely bitter.
- 4. Record results in your notes and on the class data sheet.
- 5. Wait >10 minutes. Repeat above steps using PROP test strip.

PTC taste test		test					
0	1	2	3	4	5		

PROP taste test		test					
0	1	2	3	4	5		