Using a Single-Nucleotide Polymorphism to Predict Bitter-Tasting Ability

1. The TAS2R38 protein has not been crystallized and there is no associated PDB number. (TAS2R38=PTC gene)
2. The nucleotide and putative amino acid sequence of the TAS2R38 gene is known. The GenBank Accession # is NM_176817.
Using restriction enzymes to cut DNA

Restriction Enzymes: cut covalent phosphodiester bonds at restriction / recognition sites (4 - 8 nucleotide sequence, often a palindrome); often in staggered fashion --> restriction fragments w/ “sticky ends”.

Naming: genus, species, strain, # discovered ex. ECoRI, HindIII

[Diagram of DNA cutting with restriction enzymes]

Restriction Enzyme cuts the sugar-phosphate backbones at each arrow

Sticky end

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Recognition and cleavage sequence</th>
<th>Cleavage pattern</th>
<th>Source organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI</td>
<td>GAATTC CTTAAG</td>
<td>G CTTAA AATTC G</td>
<td>E. coli</td>
</tr>
<tr>
<td>HindIII</td>
<td>AAGCTT TTCGAA</td>
<td>A TTCGA AGCTT A</td>
<td>Haemophilus influenzae</td>
</tr>
<tr>
<td>BamHI</td>
<td>GGATCC CCTAG</td>
<td>G CCTAG GATCC G</td>
<td>Bacillus amyloliquefaciens</td>
</tr>
<tr>
<td>Sau3A</td>
<td>GATC CTAG</td>
<td>CTAG GATC GATC</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>HaeII</td>
<td>GGCC CCGG</td>
<td>GG CC GG CC GG</td>
<td>Haemophilus aegypticus</td>
</tr>
</tbody>
</table>

http://www.dnai.org/b/index.html - Manipulation / Techniques/ Cutting & Pasting
Gel Electrophoresis

1. Make gel.
2. Obtain prepared DNA samples.
3. Load samples into gel.
4. Separate fragments by electrophoresis.
5. Stain DNA fragments and measure distances.
6. Prepare a standard curve. Determine fragment sizes.

Concentration of agarose gel can be increased for finer separation.

DNA = negatively charged
smaller fragments travel faster & therefore farther

correlate distance to size


www.dnai.org/index.htm  Manipulation / Sorting & sequencing

Digest with restriction enzymes
SNP’s & RFLP’s

SNP’s (single nucleotide polymorphisms) occur at a frequency of about 1 in every 1,000 nucleotides. Although some have a biological effect on the individual, most have no effect. However, they may be used as genetic markers in order to locate genes that cause or predispose to disease or influence other traits.

If you pick a restriction enzyme that recognizes the sequence TTAAA and cuts it between the T and A, then person 1’s DNA will be cut differently than person 2’s DNA.

PERSON 1

TTAAATCGTGCTGATATTGGCGATGATCGGGGGTTTAAACCGCTA

PERSON 2

TTAAATCGTGCTGATATTGGCGATGATCGGGGGTTTGAACCGCTA

RFLP’s - restriction fragment length polymorphisms ---> genetic markers (certain pattern of RFLP’s always associated with a particular disorder/trait)

Person 1 will have different length fragments cut by the restriction enzyme than person 2. If you analyze their DNA by gel electrophoresis, you will get different patterns due to the different length fragments of DNA ((RFLP’s).

Person 1 - fragment lengths of 2, 9, 34
Person 2 - fragment lengths of 2, 43

We will assume that each person has 2 of the same alleles for this trait. Two pieces of DNA of the same length stop on the gel at the same place and still appear as a single band. What would the banding pattern look like for someone that was heterozygous?

Hint: fragment lengths = 2, 9, 34, 43
**RFLP’s** - restriction fragment length polymorphisms --> **genetic markers**: certain pattern of RFLP’s always associated with a particular disorder/trait.

**SNP’s** - single nucleotide polymorphisms; occur at a frequency of about 1 in every 1,000 nucleotides. Although some have a biological effect on the individual, most have no effect. However, they may be used as genetic markers in order to locate genes that cause or predispose to disease or influence other traits.

**Haplotype** - a set of single nucleotide polymorphisms (**SNPs**) on a single chromatid that are statistically associated. This information is very valuable for studying similarities and differences in humans and investigating the genetics behind common diseases - see - International HapMap Project. [http://www.hapmap.org/thehapmap.html.en](http://www.hapmap.org/thehapmap.html.en) ([http://en.wikipedia.org/wiki/Haplotype](http://en.wikipedia.org/wiki/Haplotype))
DNA in the Cell

- Cell nucleus
- Chromosome
- Double stranded DNA molecule
- Target Region for PCR
- Individual nucleotides
PCR - Polymerase Chain Reaction

*Amplifies DNA segments to make unlimited quantities of sections of DNA / genes of interest

• DNA is heated, denatured to break hydrogen bonds
• Cool and add primers, DNA polymerase is added and DNA is synthesized
• Repeat; Amount of DNA doubles with each cycle

http://www.dnai.org/b/index.html - Manipulation/Amplifying
http://learn.genetics.utah.edu/content/labs/pcr/
The forward primer binds within the TAS2R38 gene, from nucleotides 101–144. There is a single mismatch at position 143, where the primer has a G and the gene has an A. This mismatch is crucial to the PCR experiment, because the A in the PTC sequence is replaced by a G in each of the amplified products. This creates the first G of the HaeIII recognition sequence GGCC (this is not naturally present in the TAS2R38 gene), allowing the amplified taster allele to be cut. The amplified nontaster allele reads GGGC and is not cut.

The following diagram shows how PCR amplification and restriction digestion identifies the G-C polymorphism in the TAS2R38 gene. The “C” allele, on the right, is digested by HaeIII and correlates with PTC tasting.

The following primer set was used in the experiment:
5'-CCTCGTTTTCTTGGTAATTGACATTATGATGAAGAGAGCGG-3' (Forward Primer)
5'-AGGTTGGCTTGGTTTCATCATC-3' (Reverse Primer)

TAS2R38 gene contains five SNPs, three of which particularly influence bitter taste perception. These SNPs are inherited as a unit, with one combination, or haplotype—proline/alanine/valine (PAV)—correlating most strongly with bitter-tasting ability.

<table>
<thead>
<tr>
<th>Nucleotide Position</th>
<th>TASTER</th>
<th>NONTASTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>145</td>
<td>GCA</td>
<td>GCA</td>
</tr>
<tr>
<td>785</td>
<td>GCT</td>
<td>GCT</td>
</tr>
<tr>
<td>886</td>
<td>GTC</td>
<td>ATC</td>
</tr>
</tbody>
</table>

Amino Acid | proline | alanine | valine | isoleucine
One of the most important consequences of the mapping of TAS2R38 has been the change in perspective on the nontaster allele. Beginning with the very earliest findings, PTC sensitivity has been described in terms of “taster” and “nontaster” alleles, with little thought given to the molecular mechanisms underlying the differences between them. The tacit assumption has been that the nontaster allele is somehow broken, or nonfunctional. However, molecular studies of variation at TAS2R38 suggest that this assumption could be wrong. The major taster and nontaster haplotypes differ from each other by just three amino acid substitutions; no premature stop codons, frameshifts, insertions, deletions, or other obviously catastrophic mutations are present (Drayna et al. 2003; Kim et al. 2003; Wooding et al. 2004; Kim et al. 2005). Further, while haplotypes intermediate to the taster and nontaster haplotypes show attenuated response to PTC, response is not abolished completely (Bufo et al. 2005). Thus, the human nontaster allele may be a functional receptor for some family of compounds that does not include PTC. No specific ligand for the PTC nontaster allele has yet been described; however, two studies have reported that the fruits of the plant Antidesma bunius taste bitter to PTC nontasters, but sweet to PTC tasters, raising the possibility that it contains such a ligand (Henkin and Gillis 1977; Tharp et al. 2005). The molecular assays of Bufo et al. (2005) seem likely to resolve this problem soon.

Evidence that the TAS2R38 nontaster allele is functional suggests an immediate mechanism through which heterozygote advantage might arise at this locus. If the taster allele confers sensitivity to PTC and its chemical relatives, and the nontaster allele confers sensitivity to some other set of compounds, then heterozygotes should be able to taste both sets of compounds. Thus, they might garner a fitness advantage by being able to regulate the intake of a greater diversity of bitter compounds than can homozygotes.

TAS2R38 gene contains five SNPs, three of which particularly influence bitter taste perception. These SNPs are inherited as a unit, with one combination, or haplotype—proline/alanine/valine (PAV)—correlating most strongly with bitter-tasting ability.

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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Codon</td>
<td>Amino Acid</td>
</tr>
<tr>
<td>145</td>
<td>CCA</td>
<td>proline</td>
</tr>
<tr>
<td>785</td>
<td>GCT</td>
<td>alanine</td>
</tr>
<tr>
<td>868</td>
<td>GTC</td>
<td>valine</td>
</tr>
</tbody>
</table>
STUDENT LAB INSTRUCTIONS

INTRODUCTION

Mammals are believed to distinguish only five basic tastes: sweet, sour, bitter, salty, and umami (the taste of monosodium glutamate). Taste recognition is mediated by specialized taste cells that communicate with several brain regions through direct connections to sensory neurons. Taste perception is a two-step process. First, a taste molecule binds to a specific receptor on the surface of a taste cell. Then, the taste cell generates a nervous impulse, which is interpreted by the brain. For example, stimulation of “sweet cells” generates a perception of sweetness in the brain. Recent research has shown that taste sensation ultimately is determined by the wiring of a taste cell to the cortex, rather than the type of molecule bound by a receptor. So, for example, if a bitter taste receptor is expressed on the surface of a “sweet cell,” a bitter molecule is perceived as tasting sweet.

A serendipitous observation at DuPont, in the early 1930s, first showed a genetic basis to taste. Arthur Fox had synthesized some phenylthiocarbamide (PTC), and some of the PTC dust escaped into the air as he was transferring it into a bottle. Lab-mate C.R. Noller complained that the dust had a bitter taste, but Fox tasted nothing—even when he directly sampled the crystals. Subsequent studies by Albert Blakeslee, at the Carnegie Department of Genetics (the forerunner of Cold Spring Harbor Laboratory), showed that the inability to taste PTC is a recessive trait that varies in the human population.

Bitter-tasting compounds are recognized by receptor proteins on the surface of taste cells. There are approximately 30 genes for different bitter taste receptors in mammals. The gene for the PTC taste receptor, TAS2R38, was identified in 2003. Sequencing identified three nucleotide
positions that vary within the human population—each variable position is termed a single nucleotide polymorphism (SNP). One specific combination of the three SNPs, termed a haplotype, correlates most strongly with tasting ability.

Analogous changes in other cell-surface molecules influence the activity of many drugs. For example, SNPs in serotonin transporter and receptor genes predict adverse responses to anti-depression drugs, including PROZAC® and Paxil®.

In this experiment, a sample of human cells is obtained by saline mouthwash. DNA is extracted by boiling with Chelex resin, which binds contaminating metal ions. Polymerase chain reaction (PCR) is then used to amplify a short region of the TAS2R38 gene. The amplified PCR product is digested with the restriction enzyme HaeIII, whose recognition sequence includes one of the SNPs. One allele is cut by the enzyme, and one is not—producing a restriction fragment length polymorphism (RFLP) that can be separated on a 2% agarose gel.

Each student scores his or her genotype, predicts their tasting ability, and then tastes PTC paper. Class results show how well PTC tasting actually conforms to classical Mendelian inheritance, and illustrates the modern concept of pharmacogenetics—where a SNP genotype is used to predict drug response.


TASTE

adapted from notes by

DR. DANI REED

MONELL CHEMICAL SENSES CENTER
The inability to taste the bitterness of PTC must be a specific deficit rather than a general one, since one might not taste PTC but can still taste other bitter compounds. This observation led investigators studying taste to speculate that there might be different receptors for different bitter compounds on the human tongue: if one receptor did not function, specific compounds could not be tasted but the bitter taste sense as a whole was preserved. This prediction was found to be true – when the human genome was fully sequenced in 2000, about 25 different genes were found with characteristics that likely made them bitter taste receptors.

These genes have a particular name: the *TAS2R family*. The “TAS” means they are taste genes, the “2” means they are the second family of taste genes discovered (the first being the sweet receptor genes), and the “R” means the gene is a receptor (see Figure 5). The term “receptor” has the same meaning in molecular biology as it might have in general use – a receptor is the first point of contact with the signal and relays information downstream, in this case to the inside of the cell, to the nerves, and ultimately to the brain, where we ascribe quality and intensity to the stimuli.

Table 1. Hypothesis based on observations about taste abilities of families: genotype of parent, genotype of child

<table>
<thead>
<tr>
<th>Genotype of ↓ parent</th>
<th>T</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>TT</td>
<td>Tt</td>
</tr>
<tr>
<td>t</td>
<td>Tt</td>
<td>tt</td>
</tr>
</tbody>
</table>

T = taster allele; t = nontaster allele.
People who are “TT” or “Tt” can taste PTC, whereas those who are “tt” cannot.
Taste receptors in the TAS2R family are proteins that are about 350 amino acids long; they have a specific structure and are called G-protein-coupled receptors. The term “G-protein-coupled” refers to what happens inside the cell when the receptor is stimulated by the taste molecule. G-proteins are signaling molecules that set off a particular series of chemical reactions inside the cell. In molecular biology terminology, a taste molecule is called a ligand and fits into a pocket created on the surface of the receptor. Although the “lock-and-key” metaphor is often used to describe how ligands interact with receptors, it is only partially useful to understand the concept. When ligands interact with receptors, the receptor changes shape. It is this change of shape that sets off reactions inside the cell, and it can also change the external shape of the receptor, sometimes creating new nooks and crannies for other ligands to bind. Figure 6 shows a simplified, two-dimensional drawing of TAS2R38, the receptor that is stimulated by the ligand PTC.

Figure 6. Snake diagram of bitter receptor TAS2R38. Regions of the receptor where people genetically differ are indicated with arrows.
There are only 25 bitter receptors, and yet this receptor is named “38.” This is because when the human genome DNA sequence was being discovered, there was pandemonium as people raced to identify new protein families, and several investigators were working at the same time to characterize these receptors. One consequence was that the numbering system became disorganized because so many people were finding the same receptors at the same time but naming them independently. In earlier times in science, if you discovered something first, you got to name it. But this causes trouble because if two or more investigators discover the same thing at the same time, the discovery has two names. Now there are committees that decide scientific names, and the bitter receptor naming is undergoing revision to straighten the system out. But for the moment, the gene of interest is \( \text{TAS2R38} \). (Note that gene names are in italic type, e.g., \( \text{TAS2R38} \), and the receptor protein that they code for are in regular (Roman) type, e.g., \( \text{TAS2R38} \).)

Note that three of the amino acids in Figure 6 are indicted with arrows. The arrows indicate locations in the receptor where people differ. The genetic basis of individual differences lies in the fact that the gene sequence of one person differs in some locations from that of other people, and geneticists use many words to describe this phenomenon. You may be familiar with the term “allele,” which is an alternative form, or variant, of a gene. This concept is also known by many other names, for example, polymorphism ("many-bodied") and marker (because amid a uniform string of nucleotides, a difference between people marks the location). The particular type of variation shown in Figure 6 is often called a single-nucleotide polymorphism. A haplotype is the ordered configuration of alleles.

**A reminder about genetic variation:**

**Gene, allele, haplotype**

<table>
<thead>
<tr>
<th>Portion of a gene:</th>
<th>TCG TTG GCA</th>
<th>GCT GCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele of a gene:</td>
<td>TCG TTG [G/C]CA</td>
<td>GCT GCT</td>
</tr>
<tr>
<td>Common haplotypes:</td>
<td>TCG TTG GCA</td>
<td>GCT GCT</td>
</tr>
<tr>
<td></td>
<td>TCG TTG CCA</td>
<td>GCT G[T]T</td>
</tr>
<tr>
<td>Protein translation:</td>
<td>S   L   A</td>
<td>A   A</td>
</tr>
<tr>
<td></td>
<td>S   L   P</td>
<td>A   V</td>
</tr>
</tbody>
</table>

*Figure 7*
In the Figure 7, the first single-nucleotide polymorphism is A or P (alanine or proline) at position +49. But the variation that gives rise to this change in protein is at the DNA level.

Recall that all your chromosomes come in pairs. This gene is on chromosome 7, an 

\textit{autosome} (i.e., each member of the pair has a copy of each allele – all your chromosomes except your sex chromosomes are autosomes), so you have two copies of this allele. We refer to the configuration of alleles on one chromosome as a 

\textit{haplotype} (A or P), and the combination from both chromosomes in the pair as a 

\textit{diplotype}. If we were to examine your DNA at position +49, we would learn your genotype:

\begin{itemize}
  \item AA, AP, or PP
\end{itemize}

Notice also that there are a total of three places in this protein where people commonly differ, not only the A or P at position +49, but also V (valine) or A at position +262, and I (isoleucine) or V at position 296. Another way to express this is A49P, V262A, and I296V. With this nomenclature, the most common amino acid comes first, followed by the position where the difference occurs, and then the least common amino acid at the end.

Because there are three places that differ, your diplotype might be the following:

\begin{itemize}
  \item AVI/AVI
\end{itemize}

This means you are \textit{homozygous} (have the same allele) for the AA at position +49, VV at position +262, and II at positive +296. If you were unable to taste the PTC, then your diplotype is likely AVI/AVI.

Note all the different possible combinations of haplotypes:

\begin{itemize}
  \item AVI (common, nontaster)
  \item AVV
  \item AAI (rare, mostly found in African populations)
  \item AAV (rare, mostly found in European populations)
  \item PVI
  \item PVV
  \item PAV (common, taster)
  \item PAI
\end{itemize}

Not all of these combinations are found in human DNA.

In our hypothetical example, with TT as tasters and tt as nontasters, we now know the details: TT tasters have the diplotype PAV/PAV, whereas tt nontasters have the diplotype AVI/AVI. Some haplotypes and diplotypes have intermediate phenotypes, which means that people can taste the bitterness but not very well. So although PTC is interesting because it seems initially to be a black-and-white trait – people can taste it or they can’t – there are shades of gray, which are now understood at the molecular level.

Also note that because the first amino acid at +49 predicts the haplotype, then for convenience, if the first genotype is measured, the remaining genotypes and the haplotype can be guessed. This guessing is called \textit{imputation} in genetics (e.g., an A at +49 imputes to AVI haplotype). Also, note the introduction of the term \textit{genotype}, which means the combination of alleles at a \textit{locus}, or location in a chromosome.

Now that we have tasted PTC and learned about the molecular basis of this trait in humans, we can also guess at our own genotype, haplotype, and diplotype. We could, if we were so inclined, genotype people and predict whether they can taste PTC. This demonstration was the basis of a PBS NOVA program filmed in this school, which is accessible from the Internet:

\begin{itemize}
  \item \url{http://www.pbs.org/wgbh/nova/sciencenow/0404/01.html}
\end{itemize}

We have learned that there are 25 bitter receptors, so we might logically wonder whether PTC is the only example of bitter blindness and \textit{TAS2R38} the only broken receptor, or whether other examples may exist. We think we may have
found another example of this situation for the bitter drug quinine and alleles of TAS2R19. The differences among people in quinine perception are not as dramatic as that for PTC, but they are still evident.

The other question you might logically ask is whether blindness to PTC might have implications for taste, health, and nutrition. PTC is probably not found naturally in foods, but similar compounds are. Goitrin is found in many vegetables, such as cabbage and Brussels sprouts, and it has a similar chemical motif, N=C=S (nitrogen, carbon, and sulfur connected with double bonds), and also stimulates the PTC receptor (Woodying et al. 2010). Therefore, some people might experience vegetables containing goitrin as more bitter than do other people, depending on their TAS2R38 genotype (Sandell and Breslin 2006). Keep in mind that the concentrations of goitrins, like other nutrients and secondary plant chemicals, are not uniform in vegetables – different cultivars grown in different regions may have more or less goitrin.

Finally, why might these amino acid substitutions change the protein such that people cannot taste PTC and structurally related stimuli? The answer lies in the changes in chemical bonds that occur when the amino acids in the receptor change. For instance, at position +49, it changes from alanine (A; nontaster) to proline (P; taster).
LAB FLOW

I. ISOLATE DNA BY SALINE MOUTHWASH

- Rinse mouth with saline
- Transfer saline
- Centrifuge
- Pour off supernatant
- Resuspend
- Add Chelex
- Transfer cell suspension
- Boil in thermal cycler
- Shake vigorously
- Centrifuge
- Transfer supernatant
- Store on ice

II. AMPLIFY DNA BY PCR

- Add primer/loading dye mix
- Add DNA
- Add mineral oil (if necessary)
- Amplify in thermal cycler

III. DIGEST PCR PRODUCTS WITH HaeIII

- Transfer PCR product
- Add HaeIII
- Mix
- Incubate in thermal cycler

IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

- Pour gel
- Set
- Load gel
- Electrophorese 130V
METHODS

I. ISOLATE DNA BY SALINE MOUTHWASH

<table>
<thead>
<tr>
<th>Reagents (at each student station)</th>
<th>Supplies and Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% saline solution, 10 mL</td>
<td>Permanent marker</td>
</tr>
<tr>
<td>10% Chelex®, 100 µL (in 0.2- or 0.5-mL PCR tube)</td>
<td>Paper cup</td>
</tr>
</tbody>
</table>

1. Use a permanent marker to label a 1.5-mL tube and paper cup with your assigned number.

2. Pour saline solution into your mouth, and vigorously rinse your cheek pockets for 30 seconds.

3. Expel saline solution into the paper cup.

4. Swirl the cup gently to mix cells that may have settled to the bottom. Use a micropipet with a fresh tip to transfer 1000 µL of the solution into your labeled 1.5-mL microcentrifuge tube.

5. Place your sample tube, along with other student samples, in a balanced configuration in a microcentrifuge, and spin for 90 seconds at full speed.

6. Carefully pour off supernatant into the paper cup. Try to remove most of the supernatant, but be careful not to disturb the cell pellet at the bottom of the tube. (The remaining volume will reach approximately the 0.1 mark of a graduated tube.)

7. Set a micropipet to 30 µL. Resuspend cells in the remaining saline by pipetting in and out. Work carefully to minimize bubbles.

8. Withdraw 30 µL of cell suspension, and add it to a PCR tube containing 100 µL of Chelex®. Label the cap and side of the tube with your assigned number.

9. Place your PCR tube, along with other student samples, in a thermal cycler that has been programmed for one cycle of the following profile. The profile may be linked to a 4°C hold program. If you are using a 1.5-mL tube, use a heat block or boiling water bath.

   Boiling step:        99°C   10 minutes

10. After boiling, vigorously shake the PCR tube for 5 seconds.
11. Place your tube, along with other student samples, in a balanced configuration in a microcentrifuge, and spin for 90 seconds at full speed. If your sample is in a PCR tube, one or two adapters will be needed to spin the tube in a microcentrifuge designed for 1.5-mL tubes.

12. Use a micropipet with a fresh tip to transfer 30 µL of the clear supernatant into a clean 1.5-mL tube. Be careful to avoid pipetting any cell debris and Chelex® beads.

13. Label the cap and side of the tube with your assigned number. This sample will be used for setting up one or more PCR reactions.

14. Store your sample on ice or at –20°C until you are ready to continue with Part II.

II. AMPLIFY DNA BY PCR

1. Obtain a PCR tube containing a Ready-To-Go™ PCR Bead. Label with your assigned number.

2. Use a micropipet with a fresh tip to add 22.5 µL of PTC primer/loading dye mix to the tube. Allow the bead to dissolve for a minute or so.

3. Use a micropipet with a fresh tip to add 2.5 µL of your cheek cell DNA (from Part I) directly into the primer/loading dye mix. Insure that no cheek cell DNA remains in the tip after pipeting.

4. Store your sample on ice until your class is ready to begin thermal cycling.

5. Place your PCR tube, along with other student samples, in a thermal cycler that has been programmed for 30 cycles of the following profile. The profile may be linked to a 4°C hold program after the 30 cycles are completed. Complete 35 cycles if you are staining with CarolinaBLU™.

   - Denaturing step: 94°C 30 seconds
   - Annealing step: 64°C 45 seconds
   - Extending step: 72°C 45 seconds

6. After cycling, store the amplified DNA on ice or at –20°C until you are ready to continue with Part III.
III. DIGEST PCR PRODUCTS WITH \textit{HaeIII}

<table>
<thead>
<tr>
<th>Reagents (at each student station)</th>
<th>Supplies and Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>*PCR product (from Part II), 25 ( \mu )L</td>
<td>Permanent marker</td>
</tr>
<tr>
<td>Shared Reagent</td>
<td>1.5-\text{mL} microcentrifuge tubes</td>
</tr>
<tr>
<td>*Restriction enzyme \textit{HaeIII}, 10 ( \mu )L</td>
<td>Microcentrifuge tube rack</td>
</tr>
<tr>
<td>*Store on ice</td>
<td>Micropipet and tips (1–20 ( \mu )L)</td>
</tr>
</tbody>
</table>

1. Label a 1.5-\text{mL} tube with your assigned number and with a “U” (undigested).

2. Use a micropipet with a fresh tip to transfer 10 \( \mu \)L of your PCR product to the “U” tube. Store this sample on ice until you are ready to begin Part IV.

3. Use a micropipet with a fresh tip to add 1 \( \mu \)L of restriction enzyme \textit{HaeIII} \textit{directly} into the PCR product remaining in the PCR tube. Label this tube with a “D” (digested).

4. Mix and pool reagents by pulsing in a microcentrifuge or by sharply tapping the tube bottom on the lab bench.

5. Place your PCR tube, along with other student samples, in a thermal cycler that has been programmed for one cycle of the following profile. The profile may be linked to a 4°C hold program.

   Digesting step: \( 37^\circ C \) 30 minutes

6. Store your sample on ice or in the freezer until you are ready to begin Part IV.

IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

<table>
<thead>
<tr>
<th>Reagents (at each student station)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>*Undigested PCR product (from Part III), 10 ( \mu )L</td>
<td>Micropipet and tips (1–20 ( \mu )L)</td>
</tr>
<tr>
<td>*\textit{HaeIII}-digested PCR product (from Part III), 16 ( \mu )L</td>
<td>Microcentrifuge tube rack</td>
</tr>
<tr>
<td>Shared Reagents</td>
<td>Gel electrophoresis chamber</td>
</tr>
<tr>
<td>*pBR322/BstNI marker</td>
<td>Power supply</td>
</tr>
<tr>
<td>2% agarose in 1\times TBE, 50 mL</td>
<td>Staining trays</td>
</tr>
<tr>
<td>1\times TBE, 300 mL</td>
<td>Latex gloves</td>
</tr>
<tr>
<td>Ethidium bromide (1 ( \mu )g/mL), 250 mL</td>
<td>UV transilluminator (for use with etidium bromide)</td>
</tr>
<tr>
<td>or \textit{CarolinaBLU™} Gel and Buffer Stain, 7 mL</td>
<td>White light transilluminator (for use with \textit{CarolinaBLU™})</td>
</tr>
<tr>
<td>\textit{CarolinaBLU™} Final Stain, 375 mL</td>
<td>Digital or instant camera (optional)</td>
</tr>
<tr>
<td>*Store on ice</td>
<td>Water bath (60°C)</td>
</tr>
<tr>
<td>Container with cracked or crushed ice</td>
<td></td>
</tr>
</tbody>
</table>

Alternatively, you may incubate the reaction in a 37°C water bath or heat block. Thirty minutes is the minimum time needed for complete digestion. If time permits, incubate reactions for 1 or more hours.

1. Seal the ends of the gel-casting tray with masking tape, and insert a well-forming comb.
Avoid pouring an overly thick gel, which is more difficult to visualize. The gel will become cloudy as it solidifies.

Do not add more buffer than necessary. Too much buffer above the gel channels electrical current over the gel, increasing running time.

100-bp ladder may also be used as a marker.

If you used mineral oil during PCR, pierce your pipet tip through the mineral oil layer to withdraw the PCR products. Do not pipet any mineral oil.

Expel any air from the tip before loading. Be careful not to push the tip of the pipet through the bottom of the sample well.

2. Pour 2% agarose solution to a depth that covers about 1/3 the height of the open teeth of the comb.

3. Allow the gel to solidify completely. This takes approximately 20 minutes.

4. Place the gel into the electrophoresis chamber, and add enough 1× TBE buffer to cover the surface of the gel.

5. Carefully remove the comb, and add additional 1× TBE buffer to just cover and fill in wells—creating a smooth buffer surface.

6. Use a micropipet with a fresh tip to load 20 µL of pBR322/BstNI size markers into the far left lane of the gel.

7. Use a micropipet with a fresh tip to add 10 µL of the undigested (U) and 16 µL of the digested (D) sample/loading dye mixture into different wells of a 2% agarose gel, according to the diagram below.

8. Run the gel at 130 V for approximately 30 minutes. Adequate separation will have occurred when the cresol red dye front has moved at least 50 mm from the wells.

9. Stain the gel using ethidium bromide or CarolinaBLU™:
   a. For ethidium bromide, stain 10–15 minutes. Decant stain back into the storage container for reuse, and rinse the gel in tap water. Use gloves when handling ethidium bromide solution and stained gels or anything that has ethidium bromide on it. Ethidium bromide is a known mutagen, and care should be taken when using and disposing of it.
   b. For CarolinaBLU™, follow directions in the Instructor Planning section.

10. View the gel using transillumination, and photograph it using a digital or instant camera.

Destaining the gel for 5–10 minutes in tap water leeches unbound ethidium bromide from the gel, decreasing background and increasing contrast of the stained DNA.

Transillumination, where the light source is below the gel, increases brightness and contrast.

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<table>
<thead>
<tr>
<th>MARKER</th>
<th>STUDENT 1</th>
<th>STUDENT 2</th>
<th>STUDENT 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBR322/ BstNI</td>
<td>U</td>
<td>D</td>
<td>U</td>
</tr>
</tbody>
</table>

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BIOINFORMATICS

For a better understanding of the experiment, do the following bioinformatics exercises before you analyze your results.

Biological information is encoded in the nucleotide sequence of DNA. Bioinformatics is the field that identifies biological information in DNA using computer-based tools. Some bioinformatics algorithms aid the identification of genes, promoters, and other functional elements of DNA. Other algorithms help determine the evolutionary relationships between DNA sequences.

Because of the large number of tools and DNA sequences available on the Internet, experiments done in silico (in silicon, or on the computer) now complement experiments done in vitro (in glass, or test tube). This movement between biochemistry and computation is a key feature of modern biological research.

Point your browser to http://bioinformatics.dnalc.org/ptc/ for an onscreen version of the exercise below guided by David Micklos, founder and Executive Director of the Dolan DNA Learning Center at Cold Spring Harbor Laboratory.

In Part I, you will use the Basic Local Alignment Search Tool (BLAST) to identify sequences in biological databases and to make predictions about the outcome of your experiments. In Part II, you will find and copy the human PTC taster and non-taster alleles. In Part III, you will discover the chromosome location of the PTC tasting gene. In Part IV, you will explore the evolutionary history of the gene.

I. Use BLAST to Find DNA Sequences in Databases (Electronic PCR)

The following primer set was used in the experiment:

5’-CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG-3’ (Forward Primer)
5’-AGGTTGGCTTGGTTTGCAATCATC-3’ (Reverse Primer)

1. Initiate a BLAST search.
   b. Click on BLAST in the top speed bar.
   c. Click on Nucleotide-nucleotide BLAST (blastn).
   d. Enter the sequences of the primers into the Search window. These are the query sequences.
   e. Omit any non-nucleotide characters from the window, because they will not be recognized by the BLAST algorithm.
   f. Click on BLAST! and the query sequences are sent to a server at the National Center for Biotechnology Information in Bethesda, Maryland. There, the BLAST algorithm will attempt to match the sequences.
forward primer = 5'-
CCTCGTTTCTTGGTAATTTTGGATGTA
GTGGAAGGAGCGG-3'
reverse primer =
5'-AGGTTGGCTTTGCTTGCAATCATC-3'

Updated instructions for Performing the Bioinformatics Exercises for “Using a Single-Nucleotide Polymorphism to Predict Bitter-Tasting Ability”
(Cat. #’s 21-1376, 21-1377, 21-1378, 21-1379, 21-1380, and 21-1381)

Please note that NCBI has changed their website. Use the following updated instructions to perform the bioinformatics exercises.

The following instructions are to be used in place of those starting on page 12 of the manual.

I. Use BLAST to Find DNA Sequences in Databases (Electronic PCR)

5'-CCTCGTTTCTTGGTAATTTTGGATGTAAGTGAAGGAGCGG-3'
5'-AGGTTGGCTTTGCTTGCAATCATC-3'

The following primer set was used in the experiment:
5'-CCTCGTTTCTTGGTAATTTTGGATGTAAGTGAAGGAGCGG-3' (Forward Primer)
5'-AGGTTGGCTTTGCTTGCAATCATC-3' (Reverse Primer)

1. Initiate a BLAST search.


   b. Click on BLAST in the top speed bar.

   c. Click on the link to nucleotide blast (blastn) under the heading Basic BLAST.

   d. Enter the sequences of the primers into the Search window under the heading Enter Query Sequences. These are the query sequences.

   e. Omit any non-nucleotide characters from the window, because they will not be recognized by the BLAST algorithm.

   f. Under Choose Search Set, select the Nucleotide collection(nr/nt) database from the drop-down menu.

   g. Under Program Selection, optimize for somewhat similar sequences by selecting blastn. Click on BLAST! and the query sequences are sent to a server at the National Center for Biotechnology Information in Bethesda, Maryland. There, the BLAST algorithm will attempt to match the primer sequences to the millions of DNA sequences stored in its database. While searching, a page showing the status of your search will be displayed until your results are available. This may take only a few seconds, or more than a minute if a lot of other searches are queued at the server.

2. The results of the BLAST search are displayed in three ways as you scroll down the page:

   a. First, a graphical overview illustrates how significant matches, or hits, align with the query sequence. Matches of differing lengths are coded by color.
b. This is followed by a list of significant alignments, or hits, with their accession numbers and links to additional information. The accession number is in the first column and is the number by which the sequence is identified.

c. Next, is a detailed view of each primer sequence (query) aligned to the nucleotide sequence of the search hit (subject). Notice that a match to the forward primer (nucleotides 1–42), and a match to the reverse primer (nucleotides 44–68) are within the same hit. Also notice that position 43 of the forward primer is missing, and that nucleotide 44 of the forward primer appears as part of the reverse primer. What does this mean? What has happened here?

3. Determine the predicted length of the product that the primer set would amplify in a PCR reaction (in vitro):

a. In the list of significant alignments, notice the E-values in the column on the right. The Expectation or E-value is the number of alignments with the query sequence that would be expected to occur by chance in the database. The lower the E-value, the higher the probability that the hit is related to the query. What does an E-value of 6e-12 mean?

b. Note the names of any significant alignments that have E-values less than 0.1. Do they make sense? What do they have in common?

c. Scroll down to the Alignments section to see exactly where the two primers have landed in a subject sequence from Homo sapiens.

d. The lowest and highest nucleotide positions in the subject sequence indicate the borders of the amplified sequence. Subtracting one from the other gives the difference between the two coordinates.

e. However, the actual length of the fragment includes both ends, so add one nucleotide to the result from d. above to determine the exact length of the PCR product amplified by the two primers.

II. Find and Copy the Human (Homo sapiens) PTC Taster and Non-taster Alleles

1. In the list of significant alignments, select the hit containing the human taster allele from among those with the lowest E-values.

2. Click on the accession number link at the left to open the sequence datasheet for this hit.

3. At the top of the report, note basic information about the sequence, including its basepair length, database accession number, source, and references.

4. In the middle section of the report, note annotations of gene and regulatory features, with their beginning and ending nucleotide positions (xx .. xx). Identify the feature(s) contained between the nucleotide positions identified by the primers, as determined in 3.d in section I above.

5. The bottom section of the report lists the entire nucleotide sequence of the gene or DNA sequence that contains the PCR product. Highlight all the nucleotides between the beginning of the forward primer and end of reverse primer. Paste this sequence into a text document. This is the amplicon or amplified product.

6. Also copy the entire sequence and paste it into a text document. This is the entire human taster allele.

7. Repeat Steps 1–6 to copy the entire human non-taster allele and paste it into a text document. For the non-taster allele, copy just the entire allele sequence, not the region in between the two primers. Remember to indicate which sequence is which.
IV. Use Map Viewer to Determine the Chromosome Location of the TAS2R38 Gene

1. Return to the NCBI home page, then click on Map Viewer located in the Hot Spots column on the right.

2. Find "mono sam (human)" in the table, type in the number and click on the arrow under the Tools header. If more than one, build it up by selecting one in the first number, as this will be the most common version.

3. Enter the primer sequences into the search window. Omit any non-nucleotide characters from the window, or use "*". You will then be presented with the BLAST results.

4. Select BLASTN from the drop-down menu under Programs, click on Begin Search.

5. Click on View report to retrieve the results.

6. Click on the Genome View in the list of Other reports at the top of the page to see the chromosome location of the BLAST hit. On what chromosome have you landed? \textit{Note: You will see hits on the three different assemblies in the genome. Thus, you will see multiple hits-one for each of the 2 primers on each of the different genome assemblies.}

7. Click on the number of the marked chromosome number to view the TAS2R38 locus.

8. Click on the small blue arrow labeled Genes seq to display genes. The TAS2R38 gene occupies the whole field of the default view. Move the zoom out toggle on the left to 1/1000 to see the chromosome region surrounding TAS2R38 and its nearest gene "neighbors." What genes are found on either side of TAS2R38? How do their structures differ from TAS2R38? Click on their names and follow links for more information about them.

9. Click on the blue arrow at the top of the chromosome image to scroll up the chromosome. Look at each of the genes. Scroll up one more screen, and look at those genes. What do most of these genes have in common with TAS2R38, and what can you conclude?

10. Zoom out to view 1/100 for a better view of this region of the chromosome.

IV. Use Multiple Sequence Alignment to Explore the Evolution of the TAS2R38 Gene

1. Return to your original BLAST results, or repeat Part I above to obtain a list of \textit{significant alignments}.

2. Find sequences of the TAS2R38 gene from chimpanzee (\textit{Pan troglodytes}), bonobo (\textit{Pan paniscus}), and gorilla. Use only entries listed as "complete cds" (coding sequence). For each, click on its accession number link, copy its complete nucleotide sequence from the bottom of the datasheet, and paste the sequence into a text document. Remember to indicate which sequence is which.

3. Open the BioServers Internet site at the Dolan DNA Learning Center \url{www.bioservers.org}.

4. Enter the Sequence Server using the button in the left-hand column. (You can register if you want to save your work for future reference.)

5. Create PTC gene sequences for comparison:
   a. Click on \textit{Create Sequence} at the top of the page.
   b. Copy one of the TAS2R38 sequences (from Part II and from Step 2 above), and paste it into the Sequence window. Enter a name for the sequence, and click \textit{OK}. Your new sequence will appear in the workspace at the bottom half of the page.
c. Repeat Steps a. and b. for each of the human and primate sequences from Step 2. and Part II. Also create a sequence for the forward primer used in your PCR amplification, and for the amplicon.

6. Compare each of the following sets of sequences:
   - Human PTC taster vs. human PTC non-taster vs. 221 basepair amplicon
   - Human PTC taster vs. human PTC non-taster.
   - Human PTC taster vs. human PTC non-taster vs. chimpanzee vs. bonobo vs. gorilla.
   - Forward primer vs. human PTC taster vs. human PTC non-taster.

a. Click on the Check Box in the left-hand column to compare two or more sequences.

b. Click on Compare in the grey bar. (The default operation is a multiple sequence alignment, using the CLUSTAL W algorithm.) The checked sequences are sent to a server at Cold Spring Harbor Laboratory, where the CLUSTAL W algorithm will attempt to align each nucleotide position.

c. The results will appear in a new window. This may take only a few seconds, or more than a minute if a lot of other searches are queued at the server.

d. The sequences are displayed in rows of 25 nucleotides. Yellow highlighting denotes mismatches between sequences or regions where one sequence begins or ends before another.

e. To view the entire gene, enter 1100 as the number of nucleotides to display per page, then click Redraw.

f. Repeat Steps a–e for each of the four sets of sequences to be aligned.

g. **Human PTC taster vs. human PTC non-taster vs. 221 basepair amplicon**
   What does the initial stretch of highlighted sequences mean? Where does the amplicon line up with the two human alleles? At what position in the gene is the SNP examined in the experiment, and what is the difference between taster and non-taster alleles?

h. **Human PTC taster vs. human PTC non-taster.** List the nucleotide position(s) and nucleotide differences of any additional SNP(s). Count triplets of nucleotides from the initial ATG start codon to determine codon(s) affected by SNP(s). Use a standard genetic code chart to determine if an amino acid is changed by each SNP.

i. **Human PTC taster vs. human PTC non-taster vs. chimpanzee vs. bonobo vs. gorilla.** What is the ancestral (original) state of this gene at nucleotide positions 145, 785, and 886? Are other primates tasters or non-tasters, and what does this suggest about the function of bitter taste receptors? What patterns do you notice in SNPs at other locations in the gene?

j. **Forward primer vs. human PTC taster vs. human PTC non-taster.** Where does the primer bind? What discrepancy do you notice between the primer sequence and the TAS2R38 gene sequence? Of what importance is this to the experiment?
RESULTS AND DISCUSSION

The following diagram shows how PCR amplification and restriction digestion identifies the G-C polymorphism in the *TAS2R38* gene. The “C” allele, on the right, is digested by *Haell* and correlates with PTC tasting.
1. **Determine your PTC genotype.** Observe the photograph of the stained gel containing your PCR digest and those from other students. Orient the photograph with the sample wells at the top. Use the sample gel shown above to help interpret the band(s) in each lane of the gel.

   a. Scan across the photograph to get an impression of what you see in each lane. You should notice that virtually all student lanes contain one to three prominent bands.

   b. Locate the lane containing the pBR322/BstNI markers on the left side of the sample gel. Working from the well, locate the bands corresponding to each restriction fragment: 1857 bp, 1058 bp, 929 bp, 383 bp, and 121 bp. The 1058-bp and 929-bp fragments will be very close together or may appear as a single large band. The 121-bp band may be very faint or not visible. *(Alternatively, use a 100-bp ladder as shown on the right-hand side of the sample gel. These DNA markers increase in size in 100-bp increments starting with the fastest migrating band of 100 bp.)*

   c. Locate the lane containing the undigested PCR product (U). There should be one prominent band in this lane. Compare the migration of the undigested PCR product in this lane with that of the 383-bp and 121-bp bands in the pBR322/BstNI lane. Confirm that the undigested PCR product corresponds with a size of about 221 bp.

   d. To “score” your alleles, compare your digested PCR product (D) with the uncut control. You will be one of three genotypes:

   - **tt nontaster (homozygous recessive)** shows a single band in the same position as the uncut control.

   - **TT taster (homozygous dominant)** shows two bands of 177 bp and 44 bp. The 177-bp band migrates just ahead of the uncut control; the 44-bp band may be faint. *(Incomplete digestion may leave a small amount of uncut product at the 221-bp position, but this band should be clearly fainter than the 177-bp band.)*
Tt taster (heterozygous) shows three bands that represent both alleles—221 bp, 177 bp, and 44 bp. The 221-bp band must be stronger than the 177-bp band. (If the 221-bp band is fainter, it is an incomplete digest of TT.)

e. It is common to see a diffuse (fuzzy) band that runs just ahead of the 44-bp fragment. This is “primer dimer,” an artifact of the PCR reaction that results from the primers overlapping one another and amplifying themselves. The presence of primer dimer, in the absence of other bands, confirms that the reaction contained all components necessary for amplification.

f. Additional faint bands at other positions occur when the primers bind to chromosomal loci other than the PTC gene and give rise to “nonspecific” amplification products.

2. Determine your PTC phenotype. First, place one strip of control taste paper in the center of your tongue for several seconds. Note the taste. Then, remove the control paper, and place one strip of PTC taste paper in the center of your tongue for several seconds. How would you describe the taste of the PTC paper, as compared to the control: strongly bitter, weakly bitter, or no taste other than paper?

3. Correlate PTC genotype with phenotype. Record class results in the table below.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (homozygous)</td>
<td>Strong taster</td>
</tr>
<tr>
<td>Tt (heterozygous)</td>
<td></td>
</tr>
<tr>
<td>tt (homozygous)</td>
<td></td>
</tr>
</tbody>
</table>

According to your class results, how well does TAS2R38 genotype predict PTC-tasting phenotype? What does this tell you about classical dominant/recessive inheritance?

4. How does the HaeIII enzyme discriminate between the C-G polymorphism in the TAS2R38 gene?

5. The forward primer used in this experiment incorporates part of the HaeIII recognition site, GGCC. How is this different from the sequence of the human TAS2R38 gene? What characteristic of the PCR reaction allows the primer sequence to “override” the natural gene sequence? Draw a diagram to support your contention.

6. Research the terms synonymous and nonsynonymous mutation. Which sort of mutation is the G-C polymorphism in the TAS2R38 gene? By what mechanism does this influence bitter taste perception?

7. Research other mutations in the TAS2R38 gene and how they may influence bitter taste perception.
8. The frequency of PTC nontasting is higher than would be expected if bitter-tasting ability were the only trait upon which natural selection had acted. In 1939, the geneticist R.A. Fisher suggested that the PTC gene is under “balancing” selection—meaning that a possible negative effect of losing this tasting ability is balanced by some positive effect. Under some circumstances, balancing selection can produce heterozygote advantage, where heterozygotes are fitter than homozygous dominant or recessive individuals. What advantage might this be in the case of PTC?

9. Research how the methods of DNA typing used in this experiment differ from those used in forensic crime labs. Focus on: a) type(s) of polymorphism used, b) method for separating alleles, and c) methods for insuring that samples are not mixed up.

10. What ethical issues are raised by human DNA typing experiments?

Conclusion:

1. How were you able to find and visualize (including knowing exact fragment lengths) your TAS2R38 genotype? Be sure to include the following in your explanation: primer, PCR, SNP, RFLP, HaeIII restriction enzyme, gel electrophoresis, DNA ladder/markers

2. What are haplotypes? What haplotypes are associated with TAS2R38 and tasters/non-tasters? (see notes and also website: http://www.ncbi.nlm.nih.gov/entrez/submit.cgi?id=607751 Look under Molecular Genetics


The TAS2R38 or PTC Gene  (7q35-q36)  Taster/nontaster FAV/AVI


One of the most important consequences of the mapping of TAS2R38 has been the change in perspective on the nontaster allele. Beginning with the very earliest findings, PTC sensitivity has been described in terms of “taster” and “nontaster” alleles, with little thought given to the molecular mechanisms underlying the differences between them. The tacit assumption has been that the nontaster allele is somehow broken, or nonfunctional. However, molecular studies of variation at TAS2R38 suggest that this assumption could be wrong. The major taster and nontaster haplotypes differ from each other by just three amino acid substitutions; no premature stop codons, frameshifts, insertions, deletions, or other obviously catastrophic mutations are present (Drayna et al. 2003; Kim et al. 2003; Wooding et al. 2004; Kim et al. 2005). Further, while haplotypes intermediate to the taster and nontaster haplotypes show attenuated response to PTC, response is not abolished completely (Bufo et al. 2005). Thus, the human nontaster allele may be a functional receptor for some family of compounds that does not include PTC. No specific ligand for the PTC nontaster allele has yet been described; however, two studies have reported that the fruits of the plant Antidesma bunius taste bitter to PTC nontasters, but sweet to PTC tasters, raising the possibility that it contains such a ligand (Henkin and Gillis 1977; Tharp et al. 2005). The molecular assays of Bufo et al. (2005) seem likely to resolve this problem soon.

Evidence that the TAS2R38 nontaster allele is functional suggests an immediate mechanism through which heterozygote advantage might arise at this locus. If the taster allele confers sensitivity to PTC and its chemical relatives, and the nontaster allele confers sensitivity to some other set of compounds, then heterozygotes should be able to taste both sets of compounds. Thus, they might garner a fitness advantage by being able to regulate the intake of a greater diversity of bitter compounds than can homozygotes.
Gel Analysis

Gel Electrophoresis is a method used to separate biological molecules based upon molecular charge (polarity), molecular mass, and molecular shape. This is particularly useful in separating charged biomolecules such as deoxyribonucleic acid, DNA, and ribonucleic acid, RNA.

The Logger Pro Gel Analysis feature calculates the number of base pairs of various molecules based on the distance traveled during electrophoresis.

Setup for Gel Analysis

Select Gel Analysis from the Insert menu of the program, and select either Take Photo or From File. Take Photo is a live method of capturing your gel results using a ProScope, Logitech, or other digital camera. Choose From File to select a gel photograph stored in your computer.
Analyzing the Gel Photograph

The following sequence of steps will guide you through analysis of your gel photograph:

1. Click on the Set Origin button and position your cursor to the left of the first well and click. A yellow origin will appear where vertical and horizontal lines intersect. Drag the origin up or down to position the horizontal line in the middle of all the wells. Use the dot on the horizontal line to rotate, if needed.

2. If your photo includes a reference to distance such as a ruler, click on the Set Scale button. This step is optional. If it is skipped, distances will be reported in pixels. Use your cursor to draw a line of known distance on the photo. A window will appear allowing you to input the distance of the line you draw.

3. Click on the Set Standard Ladder button. Click on the center of the first band of the Standard Ladder in the photo. Enter the number of base pairs in the window that appears. Click on the next band and repeat for all bands in the standard ladder.

4. Once the Standard Ladder has been set, click on the Add Lane button and on the Add Lane option. Click on the center of the first band of the first experimental lane. The number of base pairs will be calculated and entered in the data table and on the graph. Click on each of the remaining bands in this lane.

5. To register the bands of the next lane, click on the Add Lane button and on the Add Lane option that appears. Repeat procedure employed in step 4. This sequence is repeated for each additional lane being added to your analysis of this gel photograph.

Analysis of your gel is complete.

Note: Logger Pro will automatically name the first experimental lane "Lane 2." If you wish to change the name, you can do so by double-clicking on the data set name in the data set table and typing in the new name.
Completed Gel Analysis of plasmid pBR322 digested with three different restriction enzymes (RE’s). Lanes 6 & 7 exhibit the result of multiple RE’s acting on the plasmid. The central lane with five bands is the Standard Ladder lane.

The graph depicts the Standard Curve and the large point circles used to establish the curve. The solid circles represent the standard ladder while the other symbols indicate the different experimental bands. The graph is scaled to show millimeters (mm) migrated on the horizontal axis and number of base pairs along the vertical logarithmic axis.