PTC PCR II: Restriction Enzymes & Gel Electrophoresis

Objective
To apply what we’ve learned about genetics, molecular biology, and recombinant DNA to a specific human genetic trait.

Background
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; see structure at right), 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.

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.

**Overview**

In the first of two lab sessions, a sample of human cells is obtained by saline mouthwash from each student in the lab. DNA is extracted by boiling with Chelex resin, which binds contaminating metal ions. Polymerase chain reaction (PCR) is then used to amplify a 221 base-pair (bp) region of the TAS2R38 gene.

In the second session, The amplified PCR product is digested with the restriction enzyme HaeIII, whose recognition sequence includes one of the SNPs. The taster allele is cut by HaeIII to give a 44bp and a 177bp fragment, while the non-taster allele is not cut at all—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.

The PCR reaction is shown schematically below:

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**WARNINGS:**

1. In general, the lab is unforgiving of mistakes like using the wrong solution or taking the wrong amount. The construction folks at “This Old House”, say “Measure twice; cut once.” We’ll adapt this to “Check twice; pipette once”.

2. Although the chemicals we use are almost harmless, you should be careful with them. Always wear gloves, don’t eat or drink in lab, and wash your hands thoroughly when you are all done.

3. Always have a tip on the pipetman!

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Procedure I. Digest PCR products with \textit{HaeIII}

1. Obtain your frozen PCR product from the previous PTC lab.

2. Label a 1.5-mL tube with your assigned number and with a “U” (undigested).

3. Use a P20 with a fresh tip to transfer 10μL of your PCR product to the “U” tube. Store this sample on ice until you are ready to begin Part II.

4. Have your TA add 1μL of restriction enzyme HaeIII directly into the PCR product remaining in the PCR tube. Label this tube with a “D” (digested).

5. Mix and pool reagents by pulsing in a microcentrifuge or by sharply tapping the tube bottom on the lab bench or by vortexing. Then, spin briefly in the microfuge to bring all the drops together.

6. 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°C 30 minutes
Procedure II: Analyze Digested PCR Products by Gel Electrophoresis

1. We will prepare a 2% agarose gel in TBE buffer for you.

2. Loading a gel can be challenging, so you should practice loading a sample into the practice gel before loading your gel.

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

4. 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.

5. 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.

![Diagram of gel loading and electrophoresis](image-url)
6. A chemical stain that binds specifically has been mixed into the gel. This stain fluoresces under UV light when it is bound to DNA.

7. View the gel using transillumination, and photograph it using a digital or instant camera. Typical results are shown below:

![Gel Image]

Procedure III: Determine your PTC Genotype
1. 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.

2. 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.)

3. 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.

4. To “score” your alleles, compare your digested PCR product (D) with the uncut control. Record your genotype here:__________________

Procedure IV: 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? Compare this with the phenotype you’d expect based on your genotype.
**GFP Transformation follow-up**

In this last part of the lab, you will look at the plates you made in the GFP Transformation lab. By now, the bacteria that can grow will have grown to colonies and those that can produce GFP will be fluorescent under ultra-violet light.

1) What results should you expect? Discuss this as a class and fill in the table.

<table>
<thead>
<tr>
<th>Plate</th>
<th>Growth?</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None/Colonies/”Lawn”</td>
<td>None/some colonies/all colonies/”Lawn”</td>
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<tr>
<td>+pGLO LB/amp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+pGLO LB/amp/ara</td>
<td></td>
<td></td>
</tr>
<tr>
<td>–pGLO LB/amp</td>
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<tr>
<td>–pGLO LB</td>
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2) Obtain your plates from your TA. Fill in your actual results below:

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<td>–pGLO LB</td>
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3) Do they differ, why or why not?
Lab Report
There is no lab report for this lab.