PTC PCR I:
DNA Extraction & PCR

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.
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 short 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. 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.

As with the first GFP lab, we have not covered all of this material yet in class. However, you should be able to make sense of what we’re doing based on your understanding so far in the class. As the lectures catch up, it will be more clear what we did today.

Procedure I: Pipette Practice
In order that your experiment work, it is very important to measure the quantities of various reagents carefully. Although the “pipettemen” we will be using look easy to use, you will need to learn several tricks to using them properly. For this reason, we will start with a little practice.

The pipetteman you will be using. It measures volumes from 100µl (micro-liter; 1 millionth of a liter) to 1000µl or 1ml (milli-liter; 1 thousandth of a liter). It is calibrated with numbers near the top that are adjusted by turning the knob.

To use one, you must always put a fresh tip on the pipetman! You must never use a pipetman without a disposable tip! Pipetmen are expensive and the easiest way to wreck one is to use it without a tip!

Once you’ve put on a tip, you squeeze out the air by pushing down the plunger to the first stop with your thumb before you put it in the liquid (otherwise you make bubbles, mix your sample, and cause chaos). You then put it in the liquid and release your thumb slowly to draw up the desired amount of liquid. You then gently squeeze the button to release the measured volume of liquid. You should do the following until all the members of your group can do this with ease.

a) One person puts 500µl of water in a microtube.
b) Another person takes out 250µl, discards it, and takes the remaining 250µl. There should be no bubbles in the pipette (indicating that less than 250µl remained) nor should there be any liquid left (indicating that more than 250µl remained).

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!
Using Micropipettors

Introduction: Frequently in genetics, molecular biology, or cell biology labs, we need to measure or transfer very small volumes of liquids. Micropipettors are precise measuring devices used for this purpose. There are many different brands of micropipettors. Although the details of various models differ, the basic components of micropipettors are similar; these are depicted in Figure 1.

The tiny volumes we typically measure often range from less than 1 to 1,000 microliters (written as µL). Recall that 1 microliter (µL) is a millionth of a liter (L) and one thousandth of a milliliter (mL). Table 1 shows the three Pipetman models that we will be using and their recommended ranges of volumes.

<table>
<thead>
<tr>
<th>Model of Micropipettors</th>
<th>Recommended Range of Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>P20</td>
<td>2 to 20 µL</td>
</tr>
<tr>
<td>P200</td>
<td>20 to 200 µL</td>
</tr>
<tr>
<td>P1000</td>
<td>200 to 1000 µL</td>
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</tbody>
</table>

Table 1. Pipettors models used in this lab. Many more models are available.
Prior to using a micropipettors, it is critical to know what model you are using, how to set the volume, how to read the volume, and how to attach a tip. Micropipettors usually contain a label at the top of the device indicating the model (Figure 2).

Figure 2. Top images of the P20, P200, and P1000:

![Micropipettors Image]

**HOW TO READ THE VOLUME:**

**Figure 3. Volume Indicator Dials:** The volume indicators are shown for a P20, P200, and P1000.

- The **P20**, which has a range of 2 to 20 µL, is set to measure 6.86 µL. The dial contains 3 slots for numbers, with the bottom slot in red. The first slot, which is set to zero, sets the tens place. The second slot, which is set to 6, sets the ones place. The red slot indicates the tenths (whole number) and hundredths place (notches). Each red notch measures 0.02 µL.
- The **P200**, which has a range of 20 to 200 µL, is set to measure 132.4 µL. The dial contains 3 slots for numbers. The first slot, which is set to 1, sets the hundreds place. The second slot, which is set to 3, sets the tens place. The third slot sets the ones (whole numbers) and tenths place (notches). Each notch measures 0.2 µL.
- The **P1000**, which has a range of 200 to 1000 µL, is set to measure 262 µL. The dial contains 3 slots for numbers, with the top slot in red. The red slot, which is set to zero, sets the thousands place. This slot should never be set to a number other than 0 or 1! The second slot, which is set to 2, sets the hundreds place. The third slot sets the tens place (whole number) and the ones place (notches). Each notch measures 2 µL.

PTC I - 4
HOW TO ATTACH A TIP PROPERLY:

i. Find the correct tips. Different models of the micropipettors require different sizes and/or types of tips. If you use the wrong size tip you may not measure the volume accurately. The P20 and P200 use small yellow tips, and the P1000 uses large blue tips.

ii. Leave the tips in their tip boxes.

iii. Firmly attach the tip to the shaft, without touching either the shaft or the tip, as depicted below in Figure 4.

To remove a tip, use the tip ejector button, as shown in Figure 4. Always use fresh clean tips for each sample in order to prevent contamination.

Figure 4 Attaching and removing tips: Left, the proper method for attaching a tip from a tip box. Right, the proper method for using the tip ejector button.

When using a micropipettor, there are a few guiding principles to keep in mind:

- Be consistent with speed and smoothness when using the plunger
- Hold consistent pressure on the plunger at the first stop
- Maintain vertical positioning of the micropipettor
- Avoid air bubbles
- Change tips in order to prevent contamination.
- NEVER drop a micropipettor.
- NEVER rotate the volume adjuster either below or above the range of the instrument.
- NEVER lay a filled micropipettor on its side. (This will contaminate the shaft.)
- NEVER immerse the barrel of a micropipettor in a liquid above the tip.
- NEVER allow the plunger to snap up when liquid is being drawn into the tip.
Procedure

**Step 1.** Push down plunger to first stop. Use your thumb!!

**Step 2.** Insert pipette tip into solution. (Make sure the tip is fully submerged in the solution.)

**Step 3.** Slowly release the plunger with your thumb. As you do this, you will see the solution rise up the pipette tip

**Step 4.** Remove the pipette tip from the solution. (Make sure you do this before step 5!)

**Step 5.** Dispense the fluid inside the pipette tip by pushing down on the plunger all the way (to the second stop).

![Figure 5. Using the Plunger: Left, the plunger is not depressed. Middle, the plunger is at the first stop. Right, the plunger is at the second stop.](image)

**Step 6.** Set up your pipetmen to get them ready:

- Set your P1000 to 1000ul: “1 0 0”
- Set your P200 to 30ul: “0 3 0”
- Set your P20 to 2.5ul: “0 2 5”
- Leave your pipetmen at these settings until you’re read to use them.

**References:**

This handout is based on materials from the University of Michigan-Dearborn (http://www.umd.umich.edu/casl/natsci/slc/slconline/MICRPIP/index.html) and from STL Biochimie Genie Biologique (http://stlbgb.apinc.org/spip.php?article8).
Procedure II. Isolate DNA by Saline Mouthwash

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 P1000 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 empty saline tube. 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.) Discard the paper cup.

7. Set a P200 to 30μL. Put a new tip on the pipetman. Resuspend cells in the remaining saline by pipetting in and out. Work carefully to minimize bubbles.

8. Using a P200, withdraw 30μL of cell suspension, and add it to a tube containing 100μL 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.

Boiling step: 99°C 10 minutes. This breaks open the cells and makes the DNA available for the later PCR reaction.

10. After boiling, vigorously shake or vortex 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. Save your tube.
Procedure III: Amplify the TAS2R38 Gene using the Polymerase Chain Reaction (PCR)

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

2. Change the setting on your P200 to 22.5μl (“2 2 5”). Use this pipetman with a fresh tip to add 22.5μL of PTC primer/loading dye mix to the tube. Allow the bead to dissolve for a minute or so. The primer/loading dye mix will turn purple as the PCR bead dissolves.

3. Use a P20 with a fresh tip to add 2.5μL of your cheek cell DNA (from the chelex tube in Part II step 11) directly into the primer/loading dye mix. Insure that no cheek cell DNA remains in the tip after pipetting. If the reagents become splattered on the wall of the tube, pool them by pulsing in a microcentrifuge or by sharply tapping the tube bottom on the lab bench.

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 to the following profile for 30 cycles if you use ethidium bromide or 35 cycles if you are using CarolinaBLU™. The profile may be linked to a 4°C hold program after cycling is completed.

   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 the next lab.

**Lab Report**
There is no lab report for this session.