Albert Blakeslee using a voting machine to tabulate results of taste tests at the AAAS Convention, 1938. (Courtesy Cold Spring Harbor Laboratory Research Archives)

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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.

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LAB FLOW

I. ISOLATE DNA BY SALINE MOUTHWASH

II. AMPLIFY DNA BY PCR

III. DIGEST PCR PRODUCTS WITH HaeIII

IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

– +

Your teacher may instruct you to collect a small sample of cells to observe under a microscope.

Before pouring off supernatant, check to see that pellet is firmly attached to tube. If pellet is loose or unconsolidated, carefully use micropipet to remove as much saline solution as possible.

Supplies and Equipment

Permanent marker Paper cup Micropipets and tips (10–1000 µL) 1.5-mL microcentrifuge tubes Microcentrifuge tube rack Microcentrifuge adapters Microcentrifuge Thermal cycler (or water bath or heat

block) Container with cracked or crushed ice Vortexer (optional)

Food particles will not resuspend.

The near-boiling temperature lyses the cell membrane, releasing DNA and other cell contents.

Alternatively, you may add the cell suspension to Chelex in a 1.5-mL tube and incubate in a boiling water bath or heat block.

METHODS

I. ISOLATE DNA BY SALINE MOUTHWASH

Reagents (at each student station)

0.9% saline solution, 10 mL 10% Chelex®, 100 µL (in 0.2- or 0.5-mL PCR

tube)

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.

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To use adapters, “nest” the sample

11. Place your tube, along with other student samples, in a balanced tube within sequentially larger tubes: 0.2 mL within 0.5 mL within 1.5 mL. Remove caps from tubes used as adapters.

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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

Reagents (at each student station)

*Cheek cell DNA, 2.5 µL (from Part I) *PTC primer/loading dye mix, 22.5 µL Ready-To-GoTM PCR beads (in 0.2-mL or 0.5-mL PCR tube)

Shared Reagent

Mineral oil, 5 mL (depending on thermal

cycler)

*Store on ice

1. Obtain a PCR tube containing a Ready-To-GoTM PCR Bead. Label with

The primer/loading dye mix will turn

your assigned number.

purple as the PCR bead dissolves.

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 CarolinaBLUTM.

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.

Supplies and Equipment

Permanent marker Micropipet and tips (1–100 µL) Microcentrifuge tube rack Thermal cycler Container with cracked or crushed ice

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.

If your thermal cycler does not have a heated lid: Prior to thermal cycling, you must add a drop of mineral oil on top of your PCR reaction. Be careful not to touch the dropper tip to the tube or reaction, or the oil will be contaminated with your sample.

III. DIGEST PCR PRODUCTS WITH HaeIII

Reagents (at each student station)

*PCR product (from Part II), 25 µL

Shared Reagent

*Restriction enzyme HaeIII, 10 µL

*Store on ice

1. Label a 1.5-mL tube with your assigned number and with a "U" (undigested).

2. Use a micropipet with a fresh tip to transfer 10 µL 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 µL of restriction enzyme HaeIII 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°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

1. Seal the ends of the gel-casting tray with masking tape, and insert a well-forming comb.

Supplies and Equipment

Permanent marker 1.5-mL microcentrifuge tubes Microcentrifuge tube rack Micropipet and tips (1–20 µL) Thermal cycler (or water bath or heat block) Container with cracked or crushed ice

The DNA in this tube will not be digested with the restriction enzyme HaeIII.

If you used mineral oil during PCR, pierce your pipet tip through the mineral oil layer to withdraw the PCR product in Step 2 and to add the HaeIII enzyme in Step 3.

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.

Reagents (at each student station)

*Undigested PCR product (from Part III), 10 µL *HaeIII-digested PCR product

(from Part III), 16 µL

Shared Reagents

*pBR322/BstNI marker 2% agarose in 1x TBE, 50 mL 1x TBE, 300 mL Ethidium bromide (1 µg/mL), 250 mL or CarolinaBLUTM Gel and Buffer Stain, 7 mL CarolinaBLUTM Final Stain, 375 mL

*Store on ice

Supplies and Equipment

Micropipet and tips (1–20 µL) Microcentrifuge tube rack Gel electrophoresis chamber Power supply Staining trays Latex gloves UV transilluminator (for use with

ethidium bromide) White light transilluminator (for use with

CarolinaBLUTM) Digital or instant camera (optional) Water bath (60°C) Container with cracked or crushed ice

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Avoid pouring an overly thick gel,

2. Pour 2% agarose solution to a depth that covers about 1/

3

the

height which is more difficult to visualize. The gel will become cloudy as it

of the open teeth of the comb.

solidifies.

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

minutes.

Do not add more buffer than

4. Place the gel into the electrophoresis chamber, and add enough 1x necessary.

Too much buffer above the gel channels electrical current

TBE buffer to cover the surface of the gel.

over the gel, increasing running

5. Carefully remove the comb, and add additional 1x TBE buffer to

just time.

cover and fill in wells—creating a smooth buffer surface.

100-bp ladder may also be used as

6. Use a micropipet with a fresh tip to load 20 µL of pBR322/BstNI

size a marker.

markers into the far left lane of the gel.

If you used mineral oil during PCR,

7. Use a micropipet with a fresh tip to add 10 µL of the undigested (U) pierce your

pipet tip through the mineral oil layer to withdraw the PCR products. Do not pipet any

and 16 µL of the digested (D) sample/loading dye mixture into different wells of a 2% agarose gel, according to the diagram below.

mineral oil.

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

MARKER STUDENT 1 STUDENT 2 STUDENT 3 pBR322/ U D U D U D

BstNI

bottom of the sample well.

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.

Destaining the gel for 5–10

9. Stain the gel using ethidium bromide or CarolinaBLUTM: minutes in tap water

leeches unbound ethidium bromide from

a. For ethidium bromide, stain 10–15 minutes. Decant stain back into the gel, decreasing background

the storage container for reuse, and rinse the gel in tap water. Use and increasing contrast of the stained DNA.

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 CarolinaBLUTM, follow directions in the Instructor Planning

section.

Transillumination, where the light

10. View the gel using transillumination, and photograph it using a source is below the gel, increases brightness and contrast.

digital or instant camera.

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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 HaeIII and correlates with PTC tasting.

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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.)

1857 1058 929 bp bp bp 383 bp

121 bp

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MARKER tt nontaster TT taster Tt taster MARKER pBR322/ U D U D U D 100 bp BstNI ladder

221 bp 177 bp

44 bp primer dimer (if present)

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.

Phenotype

Genotype	**Strong taster**	**Weak taster**	**Nontaster**
TT (homozygous)			
Tt (heterozygous)			
tt (homozygous)			

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.

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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?

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