Introduction
(From Dolan DNA Learning Center)

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.

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

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 *Hae*III, 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.

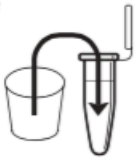
PCR Video: https://www.youtube.com/watch?v=eEcy9k_KsDI
Gel Electrophoresis Video: https://www.youtube.com/watch?v=XSO4ZBzu4jA

LAB FLOW

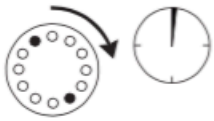
I. ISOLATE DNA BY SALINE MOUTHWASH

RINSE mouth with saline

TRANSFER saline



CENTRIFUGE



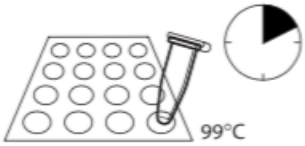
POUR OFF supernatant

RESUSPEND

ADD Chelex

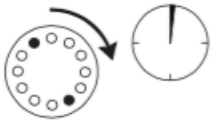
TRANSFER cell suspension

BOIL in thermal cycler



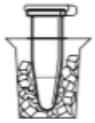
SHAKE vigorously

CENTRIFUGE



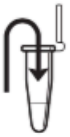
TRANSFER supernatant

STORE on ice



II. AMPLIFY DNA BY PCR

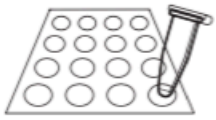
ADD primer/ loading dye mix



ADD DNA

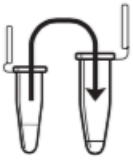
ADD mineral oil (if necessary)

AMPLIFY in thermal cycler



III. DIGEST PCR PRODUCTS WITH *Hae*III

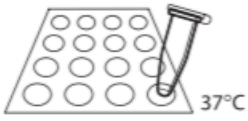
TRANSFER PCR product



ADD *Hae*III

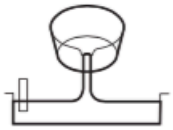
MIX

INCUBATE in thermal cycler



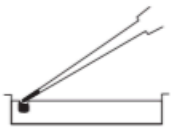
IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

POUR gel



SET

LOAD gel



ELECTROPHORESE 130V

PreLab Questions (refer to introduction, lecture, and google)

1. What does a polymerase chain reaction do?

    a) Name the three steps of a polymerase chain reaction.

    b) How many copies of DNA will you have after 3 cycles of PCR?

2. What is a restriction enzyme?

    a. What sequence does HaeIII cut?

3. What causes DNA fragments to migrate through the gel? (Hint: DNA is negatively charged)?

    a. What feature are DNA fragments are separated by?

    b. How can you determine the length of the DNA fragments in your sample?