4. Restriction Enzyme Digest

Overview

You may have heard of **DNA fingerprinting**, which is used in forensics to look for unique patterns in an individual's DNA. Many of these patterns exist because of minor differences in **nucleotide sequences** (the "letters" of your DNA "code") from one person to another. If these differences occur within a gene and result in changes to the gene's protein products, people with these variants may have different physiology from each other as well. You have carried out PCR to make multiple copies of a portion of your bitter taste receptor gene, TAS2R38. Now, you will use a special bacterial protein, a **restriction enzyme** (molecular "scissors" that bacteria use to cut harmful viral DNA), to analyze the DNA you copied.

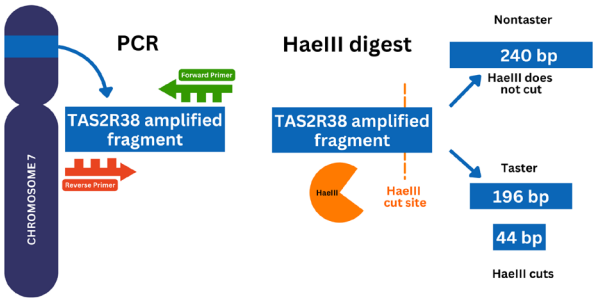
How it works

Bacteria use restriction enzymes to destroy the DNA of **bacteriophages (or phages)**, which are tiny viruses that attack bacteria to replicate. Scientists have identified thousands of these enzymes from different bacterial species. Each restriction enzyme cuts DNA at a distinct sequence of nucleotides, making them quite useful in molecular biology. If researchers know a DNA sequence (the readout of a DNA code), they can cut that DNA wherever the specific sequence of nucleotides—called a **restriction site**—occurs. Then they can use the cut DNA in a variety of important molecular biology techniques.

When you use a restriction enzyme, you mix it with template DNA and a buffer tailored to produce ideal conditions for that enzyme's cutting activity. The mixture is referred to as a **restriction digest (or digestion)** and is incubated in a heat block at 37°C (typical human body temperature!). Restriction enzymes are formulated to cut DNA rapidly and precisely. The longer the digest proceeds, the more thoroughly the DNA will be cut into fragments. If you know the DNA sequence of your template, you can predict the length of each fragment in your restriction digest.

What you will explore today

Because the TAS2R38 DNA of PTC tasters and nontasters differs, you can use a restriction enzyme to cut the DNA of the bitter taster variant, but not the nontaster variant. Researchers determined that the best restriction enzyme for this task is one called **HaeIII**.



Because HaeIII cuts DNA of bitter tasters but not nontasters, you will end up with different numbers and lengths of DNA fragments in your restriction digest depending on whether you are a strong taster, weak taster, or nontaster. Since you inherited two copies of the bitter taster gene from your two biological parents, you could have two taster copies (TT), one taster and one nontaster copy (Tt), or two nontaster copies (tt). To visualize these results, you will use gel electrophoresis.

RESTRICTION DIGESTION PROCEDURE

1. Put on gloves.

2. Centrifuge the PCR tubes to pool the liquid to the bottom of the tube.

3. Add 2 µL of HaeIII restriction enzyme to your tube and gently pipette up and down to mix.

4. Label the lid of your tube “RE” to indicate that HaeIII has been added.

5. Briefly centrifuge your sample to pool the reagents to the bottom of the tube.

6. Incubate in thermocycler or heat block at 37° C for 10 minutes.