CHAPTER 5:
Restriction Enzyme Digestion of TAS2R38 PCR Products

INTRODUCTION

In the last chapter, you explored nucleotide sequences in a portion of the TAS2R38 gene to look for differences between people who are strong tasters, weak tasters, and nontasters. In this chapter, you will explore how you can use restriction enzyme digestion and gel electrophoresis to confirm your taster genotype.

ACTIVITY: Which Restriction Enzyme Should We Use?

Choosing the correct restriction enzyme is critical to ensure you can distinguish between strands of DNA amplified from the same region, with or without SNPs. In this activity, you will choose which restriction enzyme to use to distinguish between specific DNA sequences from strong tasters, weak tasters, and nontasters. To detect these differences, you will need to choose a restriction enzyme that cuts the DNA of one allele but not the other. In the previous activity, you identified the location of a SNP in the amplified fragment (145) and noted the sequences of both the tasting and non-tasting genotypes.

To determine which restriction enzyme to use, you will use an application called Sequence Extractor. This tool generates an interactive PCR primer map and restriction map of a DNA sequence. To use Sequence Extractor, paste the sequence of the TAS2R38 gene and the forward and reverse primers you used for PCR into the application. It will then produce a map of the DNA sequence showing the locations of your primers, as well as the sites where known restriction enzymes cut the TAS2R38 sequence.

MATERIALS

- Laptop or desktop computer with internet access
- 2 different-colored highlighters
- 1 copy of **Which Restriction Enzyme Should We Use? (RM 5.1)**

PROCEDURE

1. Watch the Sequence Extractor tutorial.
2. Go to the Sequence Extractor website.
3. Select all the text that appears in the box that says "Paste a sequence into the text area below. Accepted formats are: raw, GenBank, EMBL, and FASTA," and delete it. Copy the following sequence, which includes the region you amplified as well as the remainder of the TAS2R38 gene, and paste it into the box:

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  1 ATGTTGACTC TAACTCGCAT CCGCACTGTG TCCTATGAAG TCAGGAGTAC ATTTCTGTTC
 61 ATTTCAGTCC TGGAGTTTGC AGTGGGGTTT CTGACCAATG CCTTCGTTTT CTTGGTGAAT
121 TTTTGGGATG TAGTGAAGAG GCGGCCACTG AGCAACAGTG ATTGTGTGCT GCTGTGTCTC
181 AGCATCAGCC GGCTTTTCCT GCATGGACTG CTGTTCCTGA GTGCTATCCA GCTTACCCAC
241 TTCCAGAAGT TGAGTGAACC ACTGAACCAC AGCTACCAAG CCATCATCAT GCTATGGATG
301 ATTGCAAACC AAGCCAACCT CTGGCTTGCT GCCTGCCTCA GCCTGCTTTA CTGCTCCAAG
361 CTCATCCGTT TCTCTCACAC CTTCCTGATC TGCTTGGCAA GCTGGGTCTC CAGGAAGATC
421 TCCCAGATGC TCCTGGGTAT TATTCTTTGC TCCTGCATCT GCACTGTCCT CTGTGTTTGG
481 TGCTTTTTTA GCAGACCTCA CTTCACAGTC ACAACTGTGC TATTCATGAA TAACAATACA
541 AGGCTCAACT GGCAGAATAA AGATCTCAAT TTATTTTATT CCTTTCTCTT CTGCTATCTG
601 TGGTCTGTGC CTCCTTTCCT ATTGTTTCTG GTTTCTTCTG GGATGCTGAC TGTCTCCCTG
661 GGAAGGCACA TGAGGACAAT GAAGGTCTAT ACCAGAAACT CTCGTGACCC CAGCCTGGAG
721 GCCCACATTA AAGCCCTCAA GTCTCTTGTC TCCTTTTTCT GCTTCTTTGT GATATCATCC
781 TGTGTTGCCT TCATCTCTGT GCCCCTACTG ATTCTGTGGC GCGACAAAAT AGGGGTGATG
841 GTTTGTGTTG GGATAATGGC AGCTTGTCCC TCTGGGCATG CAGCCATCCT GATCTCAGGC
901 AATGCCAAGT TGAGGAGAGC TGTGATGACC ATTCTGCTCT GGGCTCAGAG CAGCCTGAAG
961 GTAAGAGCCG ACCACAAGGC AGATTCCCGG ACACTGTGCT GA
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4. Select all the text in the box that says "If there are primers you would like shown on the map, enter each primer as follows: the sequence of the primer, a blank space, and the name of the primer. Use commas to separate multiple primer entries," and delete it. Copy the following (the sequences of the forward and reverse primers) and paste it into the box.

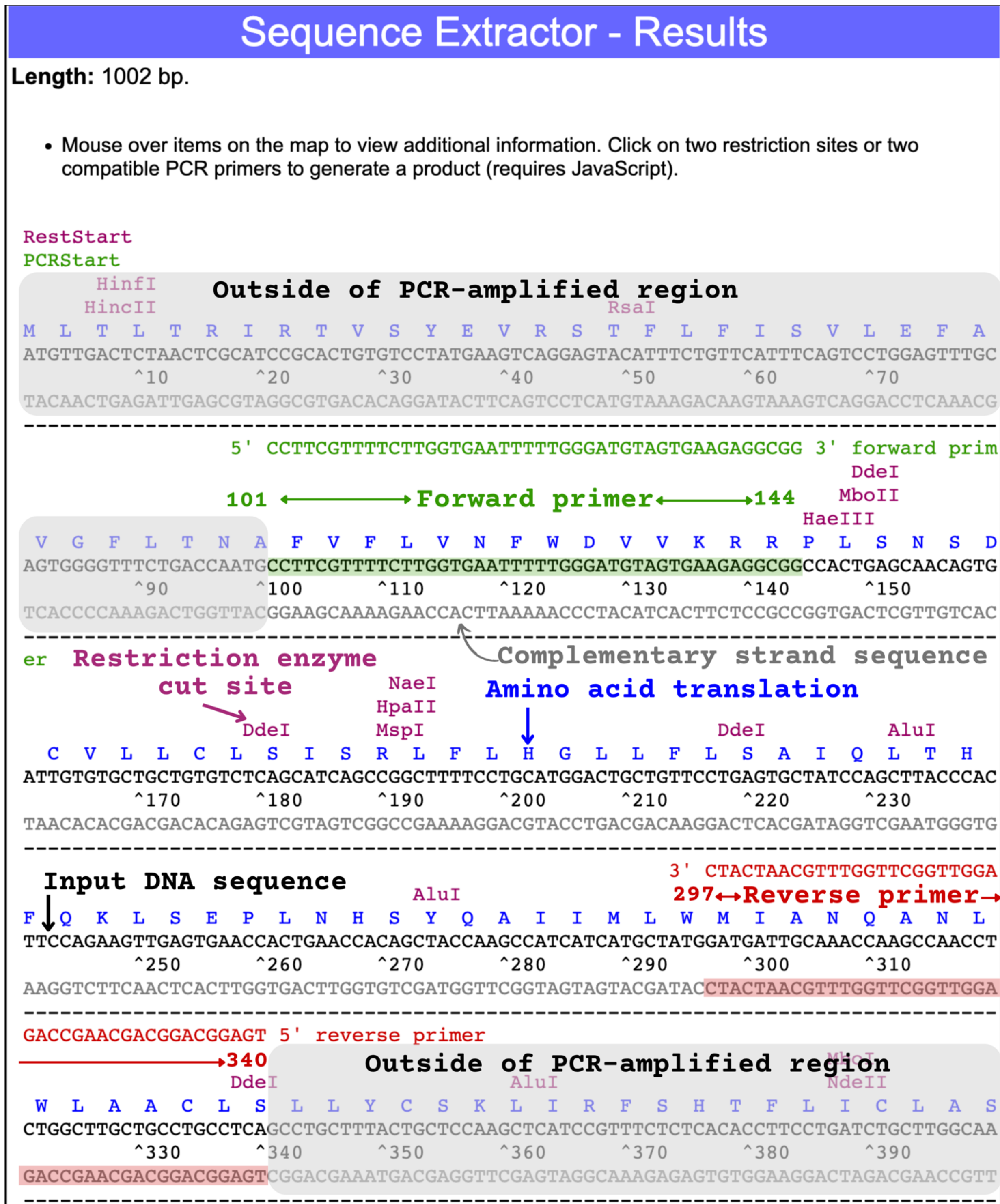
   Important: Be sure to separate the forward and reverse primers with a comma!

   CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG forward primer,
   TGAGGCAGGCAGCAAGCCAGAGGTTGGCTTGGTTTGCAATCATC reverse primer

5. Click "Submit." (Please note that you may receive an error message indicating that your information is not secure due to the website's configuration. It is OK to disregard this message and continue.)

NOTE: The results may be a little difficult to interpret at first. In addition to the tutorial, **Figure 5.1** can help you get oriented. Each segment of the strand appears between two dashed lines. Regions of the TAS2R38 gene outside the forward and reverse primers used for PCR are grayed out. The locations of the DNA sequence you entered, its complementary strand, restriction enzyme cut sites, and the amino acid translation are indicated, along with nucleotide start and end positions for the forward and reverse primers.

Figure 5.1: Location of primers and restriction sites in your sequence



6. After you've had a chance to examine the results of your submission, answer the questions on **Which Restriction Enzyme Should We Use? (RM 5.1)**.

LABORATORY: Restriction Digest of TAS2R38 PCR Products

In Chapter 3, you used PCR to amplify a portion of your TAS2R38 gene. This resulted in millions of copies of that DNA—enough to perform further experiments, which you can visualize through gel electrophoresis.

In this lab, you will use a restriction enzyme to cut the fragment you amplified, which will allow you to distinguish between TAS2R38 genotypes.

MATERIALS (see **Figure 5.2** for illustration)

For each student:

- Your PCR tube from Chapter 3
- 1 pair of gloves

For each team:

- 1 fine-point permanent marker
- 1 P-20 micropipette
- Micropipette tips
- Ice and ice bucket
- 1 0.2-mL microfuge tube containing HaeIII restriction enzyme
- 1 microcentrifuge tube rack
- Waste container

For the class:

- Microcentrifuge
- Thermocycler (PCR machine), heat block, or water bath
- Positive and negative control PCR products (C+ & C–) from Chapter 3

PROCEDURE (see **Figure 5.3** for flowchart)

Step	Notes
1. Put on gloves.	
2. Obtain your PCR tube from your teacher and place it in a microfuge rack to defrost.	
3. Centrifuge the PCR tubes, once fully thawed, to pool the liquid to the bottom of the tube.	*You will be digesting your entire sample.*

Step	Notes
4. Using a micropipette, add 2 µL of HaeIII restriction enzyme to your PCR tube and gently pipette up and down to ensure that the enzyme mixes with your sample.	
5. Using a waterproof marker in a different color from your initials, add a dot to the lid of your tube to indicate that HaeIII has been added.	
6. If your teacher has not done so, perform the restriction digest steps (2–5) for the positive and negative control PCR samples (C+ & C–).	*Depending on the number of gels required to run the student PCR digests for your classroom, you or your teacher may be performing restriction digests on additional positive control PCR samples.*
7. *Briefly c*entrifuge your sample to pool the reagents to the bottom of the tube.	
8. If your teacher has not pre-programmed your thermocycler, program the thermocycler to run at 37°C for 10 minutes and name it "HaeIII restriction digest."	
9. Place your tube in the thermocycler or heat block. Once all of your team's tubes and the control tubes are in the thermocycler, run the HaeIII restriction digest program.	*The digestion step is performed at 37°C because that is the optimal temperature for HaeIII enzyme activity.*
10. Optional Stop Point: Store your digest reaction at –20°C until the gel electrophoresis lab. Centrifuge the reaction tube before loading into a gel well.	

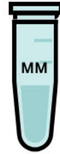
Figure 5.2: Materials for restriction digest

MATERIALS

For each student:

A pair of gloves

PCR tube

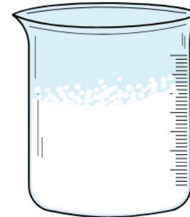


For each team:

Permanent fine-point marker

P-20 micropipette and tips

Ice and ice bucket



0.2-mL microfuge tube containing HaeIII restriction enzyme

Waste container

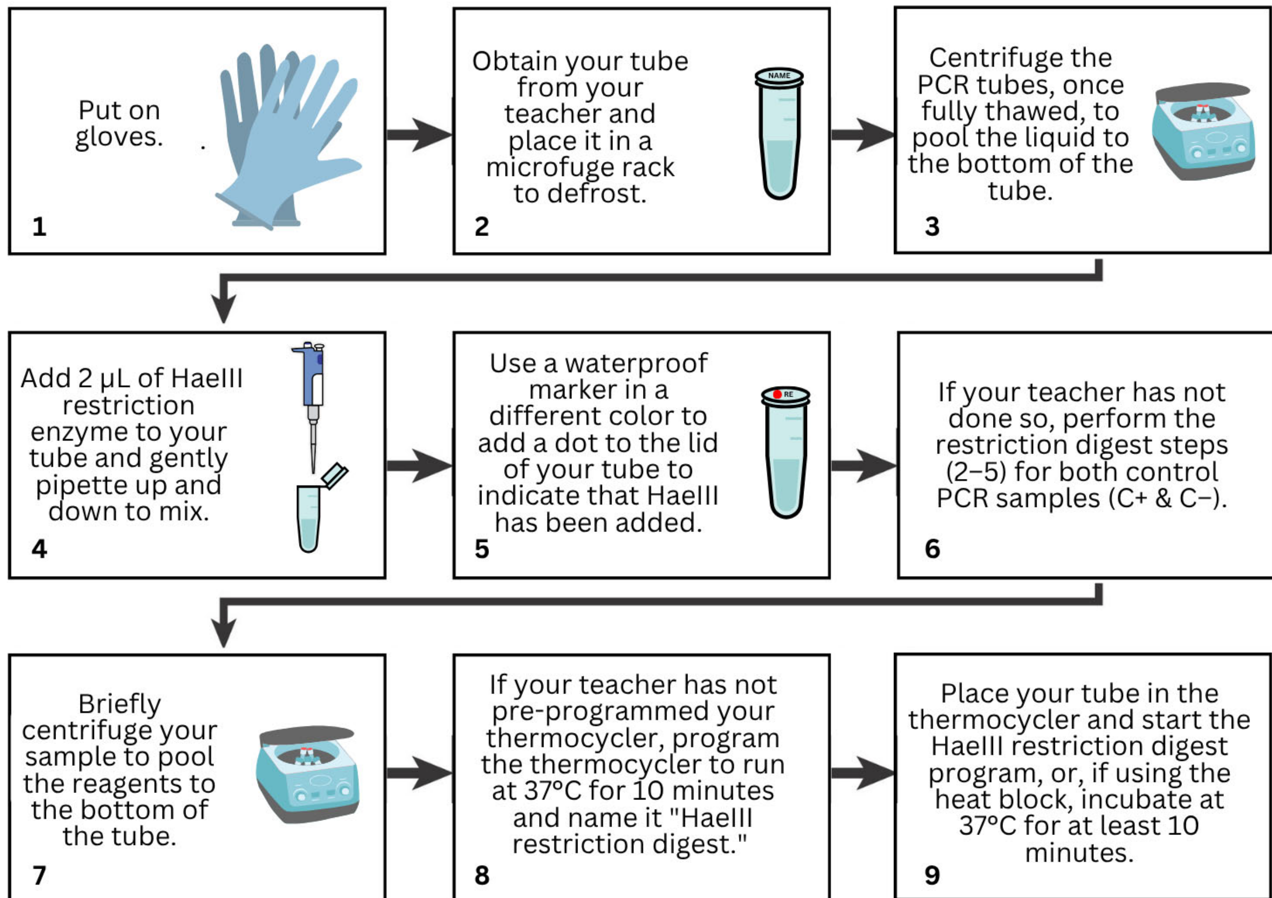
For the class:

Microcentrifuge

Thermocycler

Positive and negative control PCRs (C+ & C–) from Chapter 3, plus additional positive controls as needed

Figure 5.3: Procedure flowchart for restriction digest



Optional stop point: Store your digest reaction at −20°C until the gel electrophoresis lab. Centrifuge the reaction tube before loading into a gel well.