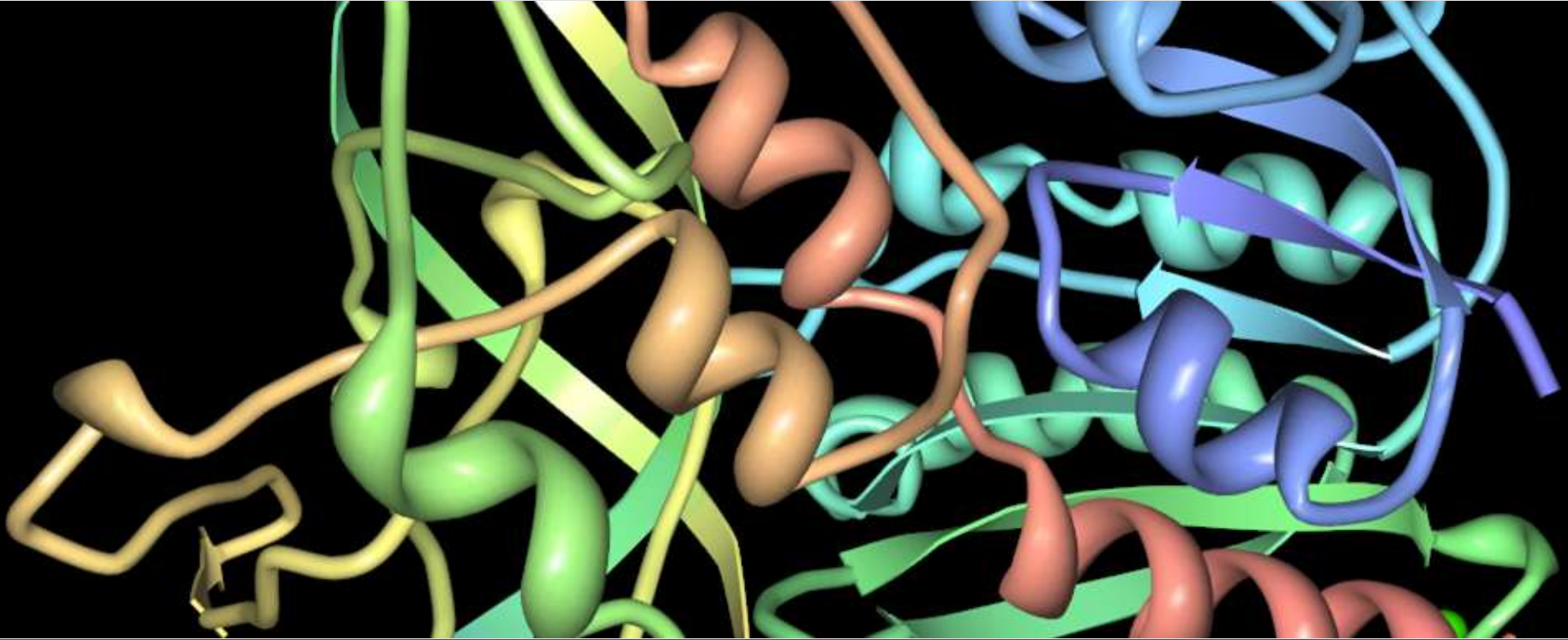


# Exploring Precision Medicine

- Chapter 1: What's the Right Medicine?
- Chapter 2: Is My Sense of Taste Controlled by my Genes?
- Chapter 3: Exploring Our DNA
- Chapter 4: How Is DNA Sequenced, and What Can We Learn?
- Chapter 5: Restriction Enzyme Digestion of TAS2R38 PCR Products
- Chapter 6: Gel Electrophoresis and Genotyping
- Chapter 7: SNPs and Drug Metabolism

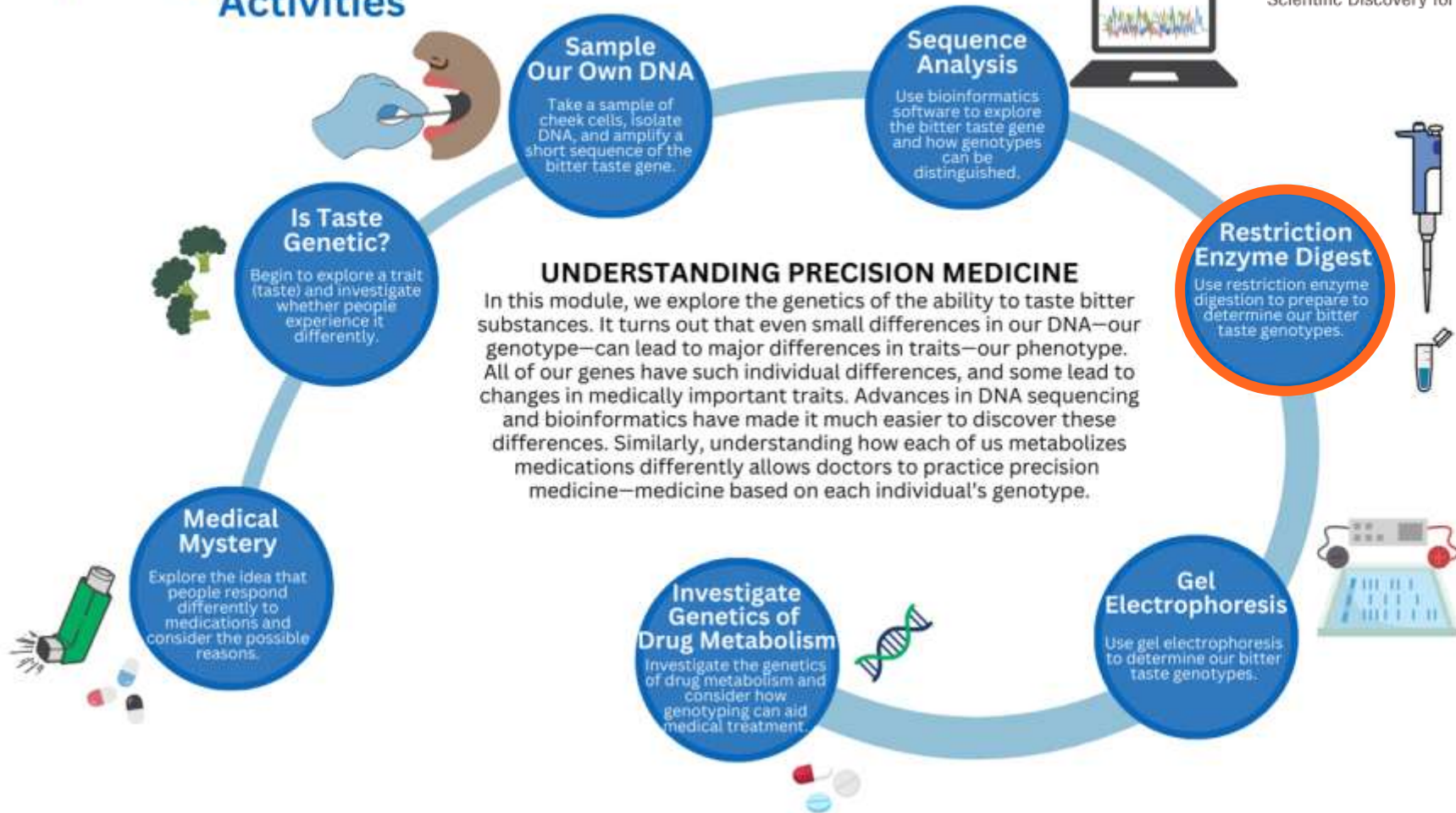
# Chapter 5: Restriction enzyme digestion of TAS2R38 PCR products



# Exploring Precision Medicine: Activities

AMGEN Biotech Experience

Scientific Discovery for the Classroom

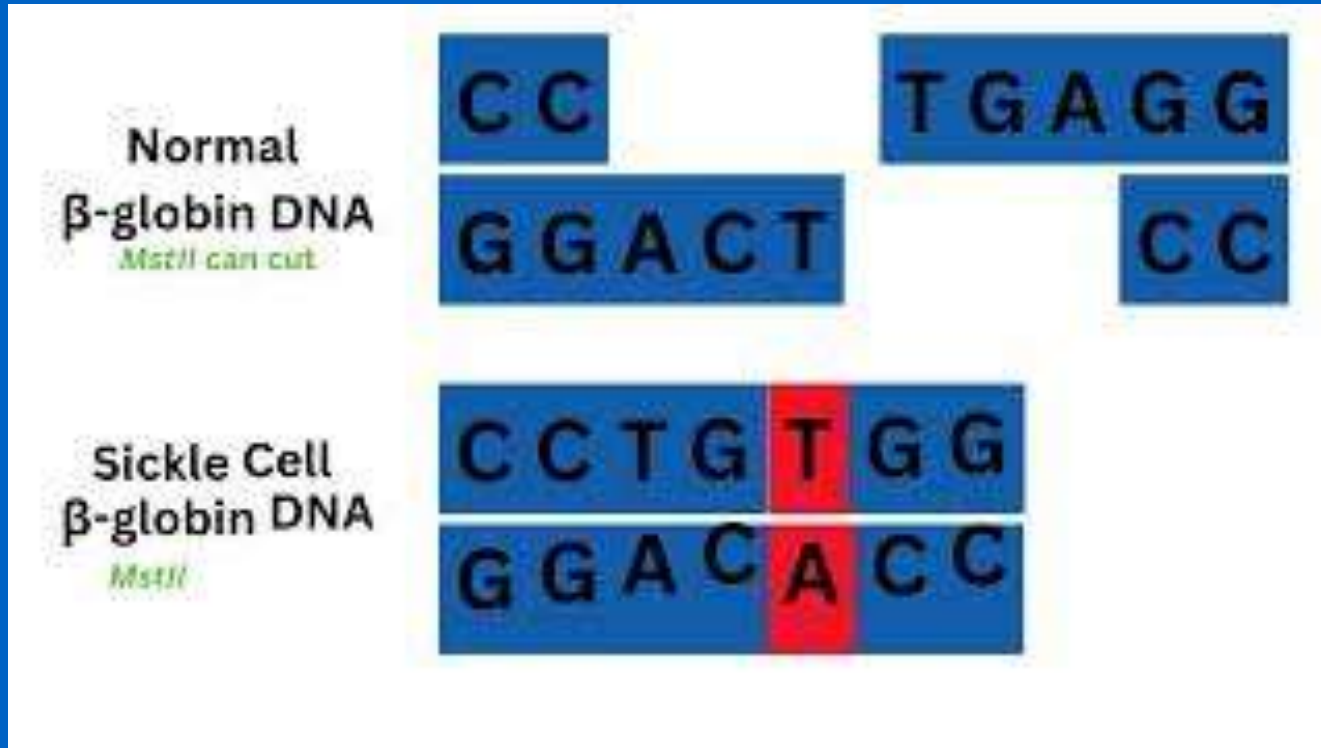


# We have amplified part of our TAS2R38 DNA

- We know our phenotypes now
- How can we distinguish between taster and nontaster alleles?
- Use restriction digestion and gel electrophoresis!



# Video: Restriction enzymes in DNA testing



# Discussion: genotyping sickle cell anemia

- **How did scientists isolate the patient's  $\beta$ -hemoglobin DNA?**
  - PCR of genomic DNA, with primers specific to the human hemoglobin  $\beta$  gene
- **Which restriction enzyme cuts normal  $\beta$ -globin DNA, but not the sickle cell allele?**
  - *MstII*
- **What was the patient's genotype, and how could you tell?**
  - They are a carrier (heterozygous) because their restriction digest produced 3 bands



# Activity: Which restriction enzyme should we use?

## Sequence Extractor

[Main](#) | [Features](#) | [Help](#) | [Download](#) | [License](#) | [About](#)

Sequence Extractor generates a clickable restriction map and PCR primer map of a DNA sequence. Protein translations and Intron/exon boundaries are also shown. Use Sequence Extractor to build DNA constructs *in silico*. Please read the list of program features to learn more.

Paste a sequence into the text area below. Accepted formats are: raw, GenBank, EMBL, and FASTA.

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.

### Advanced Options

Use the following options to alter the output of Sequence Extractor. For more details about individual options, see the help.

- Genetic code:
- Restriction set:
- Translate reading frame:
- Topology:
- Allow primers to have mismatched: ☒ 5' tails, ☒ 3' tails.  
Matching bases required when mismatching bases allowed:
- Bases per line:
- Show ☒ reverse strand, ☒ number line, ☒ spacer line.
- Return ☒ restriction summary, ☒ primer summary, ☐ help information, ☐ coding sequence links, ☐ translation links, ☐ options selected.

Sequence Extractor copyright © 2006 Paul Sittman

## Reproducible Master 5.1

### WHICH RESTRICTION ENZYME SHOULD WE USE?

1. Roll over the primers to answer the following:

- At what position does the forward primer start?
- Does the rollover give you any other information about the forward primer?
- At what position does the reverse primer start?
- Does the rollover give you any other information about the reverse primer?
- How long is the fragment that you produced using these two primers? (note: click on the reverse primer sequence on the screen. The pop-up box or new tab should tell you the length of the fragment that you created.)

2. Look at the TAS2R38 Sequence Extractor Results diagram on the next page:

- Highlight the positions of the forward and reverse primers on the sequence using two different-colored highlighters.
- Review the table on RM 6.3. Which of the differences that you found is within the fragment?

# Discuss: RM 5.1

## Sequence Extractor - Results

Length: 1002 bp.

• Mouse over items on the map to view additional information. Click on two restriction sites or two compatible PCR primers to generate a product (requires JavaScript).

RestStart  
PCRStart

**Outside of PCR-amplified region**

Restriction sites: *BlnI*, *BlnII*, *HaeI*

Sequence: N L T L T H I R T Y D Y E V R D T F L F I E V L E F A  
ATGTTGACTCTAACTCCGATCCGACTGTGTCTATGAAGTCAGAGTACATTTCTGTTCAITTCAGTCCGTGGAGTTTGC  
TACAACTGACATTCAGGCTGAGCTGACACAGGATCTTCAGTCTCATGTAAGACAGGTAAGCTCAGGACCTCAAAAG

5' CCTTCGTTTTCTTGGTGAATTTTGGGATGTAGTGAAGAGCGCG 3' forward prim

101 ← Forward primer → 144

Restriction sites: *DdeI*, *MboII*, *HaeIII*

Sequence: V G F L T N A F V F L V N F W D V V K R R P L S N S D  
AGTGGGGTTCTGACCAATGCCITTCGTTTTCTTGGTGAATTTTGGGATGTAGTGAAGAGCGCGCCACTGAGCAACAGTG  
TCACCCCAAGGCTGTTTCGGAAGCAAAGAACCACTTAAAGACCTACATCACTTCTCCGCCGGTGACTCGTTGTGCAC

**Complementary strand sequence**

**Restriction enzyme cut site**

**Amino acid translation**

Restriction sites: *DdeI*, *NaeI*, *HpeII*, *MspI*, *DdeI*, *AluI*

Sequence: C V L L C L S I S R L F L H G L L F L S A I Q L T H  
ATTGTGTGCTGCTGTCTCTCAGCATCAGCGGCTTTTCTGTCATGACTGCTGTTCTCGAGTGCTATCCAGCTTACCCAC  
TAAACACGACGACACAGAGTGTAGTGTGGCCGAAAGGACGTACCTGACGACAGGACTACGATAGGTGGAATGGGTG

**Input DNA sequence**

3' CTACTAACGTTTGGTTCGGTTGGA

297 → Reverse primer →

Sequence: F Q K L S E P L N H S Y Q A I I M L W H I A N Q A N L  
TTCCAGAGTTGAGTGAACCACTGAACACAGCTACCAAGCATCATCATGCTATGGATGATTGCAAAACCAAGCAACCT  
AAGGTCCTCAACTCACTTGGTGACTTGGTGTGATGGTTCGGTAGTAGTACGATACCTCAACGTTTGGTTCGGTTGGA

GACCGAACGACGGACGGAGT 5' reverse primer

→ 340

**Outside of PCR-amplified region**

Restriction sites: *DdeI*, *AluI*, *NdeI*

Sequence: W L A A C L S L L V C S E L I R F E H T F L I C L A B  
CTGGCTTGCTGCTGCTCAAGCTGCTTACTGTCTCAAGCTCATCCGTTTCTCTCACAGCTTCTGATGATCTGCTTGGCAA  
GACCGAACGACGGACGGAGT



## Discuss: RM 5.1 (continued)

- **How long is the fragment you amplified?**  
240 bp
- **At what position should you cut to differentiate between tasters and nontasters?**  
The SNP at position 145
- **Which restriction enzyme did you choose to do the job?**  
*HaeIII*

## Discuss: RM 5.1 (continued)

- Whose DNA is cut by *HaeIII*? Bitter taster or nontaster?

Bitter taster

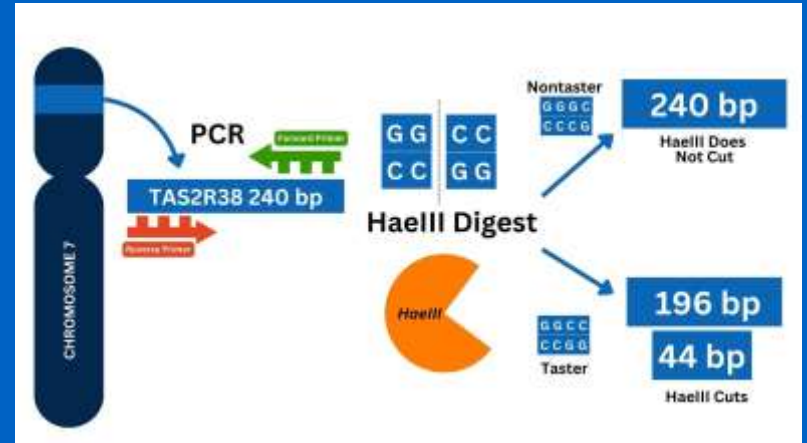
- What length would the cut fragments of the 240-bp DNA be?

196 bp and 44 bp

- How could a person end up with both alleles? They would inherit one from their biological father and one from their biological mother

- What length fragments would you expect after *HaeIII* digestion of a PCR from a heterozygote?

All 3 lengths: 240 bp, 196 bp, and 44 bp



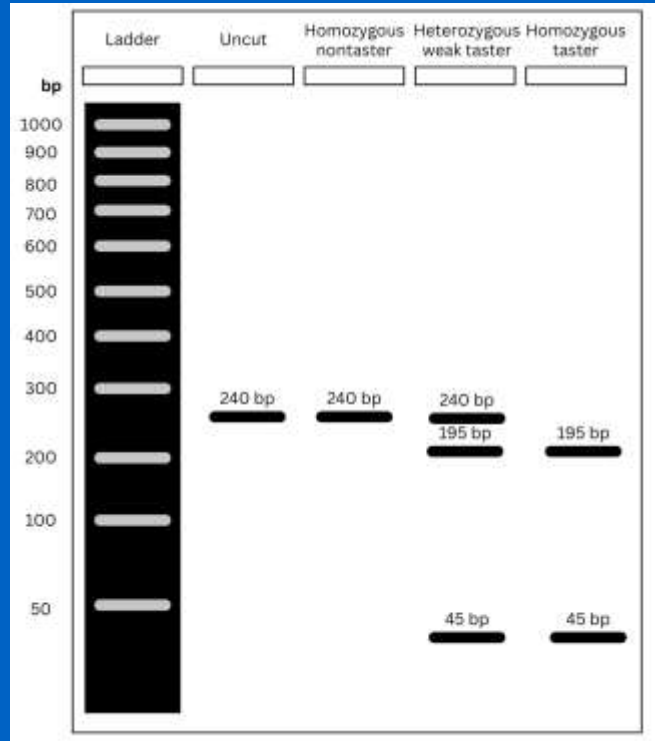
# Discuss: RM 5.1 (continued)

## What are the possible phenotypes for PTC tasting?

Phenotype	Genotype	Copy 1: Cut or uncut?	Copy 2: Cut or uncut?	DNA fragment length		
				44 bp	196 bp	240 bp
	TT	cut	cut	X	X	
weak taster	Tt	cut	uncut	X	X	X
nontaster	tt	uncut	uncut			X



# Predict the outcomes



# Why would a heterozygous “weak” taster have 3 bands on a gel?

- One allele is from a nontaster and is not cut by the restriction digest
- The other allele from a taster **is** cut into two pieces by the restriction digest
- Outcome: 3 fragments of DNA

# How is our analysis of TAS2R38 similar to the $\beta$ globin example?

- DNA sequences of alleles differ
- Researchers look for restriction enzymes which cut one allele but not the other
- Different cuts = different size fragments on a gel
- Can be used for genotyping



# Read the lab intro “Restriction Digest and Gel Electrophoresis of TAS2R38”

## INTRODUCTION

In this chapter, you will use gel electrophoresis to visualize the results of your restriction digest and compare your taste genotype to your PTC paper taste test.

## LABORATORY: Gel Electrophoresis of TAS2R38 Restriction Digest

Now that you have performed your restriction digest, you will use gel electrophoresis to see whether your DNA was cut by the restriction enzyme, which will tell you what your taste genotype is.

Although you are using gel electrophoresis to determine your TAS2R38 genotype, you can also use it as a quality-control measure for your PCR sample before you perform a more expensive procedure. Scientists and researchers use gel electrophoresis to ensure that their PCR was successful in amplifying the correct fragment—by checking whether a fragment of the proper size is present. It is wise to verify that a sample has been amplified and appears to be the correct size before proceeding to more time-consuming or expensive techniques.

An agarose gel, like the one you will use for this lab, is a porous matrix. Smaller DNA fragments move through the pores more easily, allowing them to travel faster and move farther than larger fragments. As a sample progresses through the gel, the fragments are sorted into distinct bands based on their sizes.

### Materials (see Figure 6.3 for illustration)

#### For each student:

- 1 pair of gloves
- 1 copy of Gel Electrophoresis of TAS2R38 Recording Sheet (RW 6.1)
- 1 lane of an agarose gel
- Student PE digest

#### For each team:

- Access to a shared electrophoresis chamber and power supply
- 1 microcentrifuge tube rack
- Gel running buffer
- 1 P-20 micropipette
- Micropipette tips
- 10 µL, 100-µg ladder (RW)
- 1 transilluminator
- Waste container

100

# Set up your restriction digest

## RESTRICTION DIGEST OF TAS2R38 PCR PRODUCTS

### MATERIALS

For each student:

A pair of gloves



For each team:

Fine point permanent marker



0.2-mL microfuge tube containing HaeIII restriction enzyme



For the class:

Microcentrifuge



P-20 micropipette and box



Waste container



Thermocycler



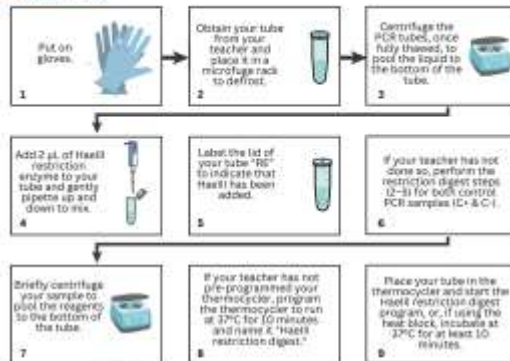
Ice and ice bucket



T2R control PCRs (C+ & C-) from Chapter 3



### PROCEDURE



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.