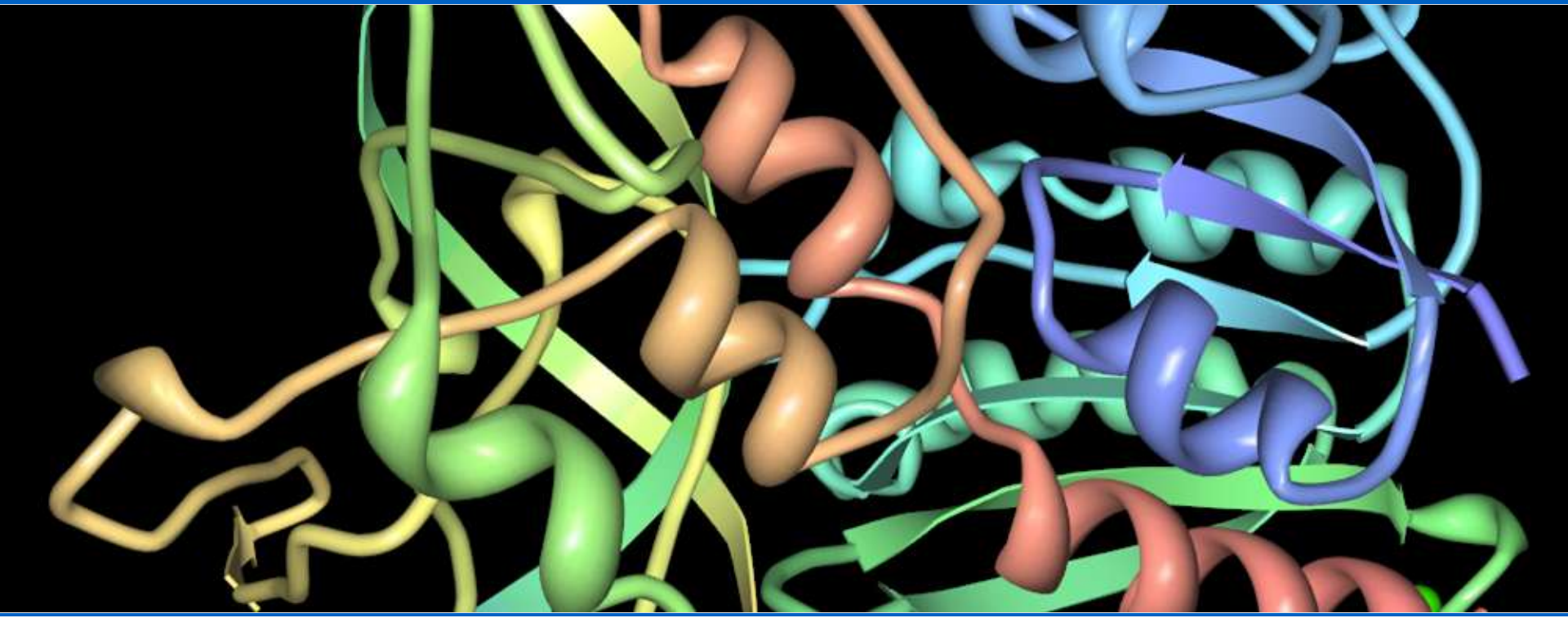
Exploring Precision Medicine

- 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

Sample Our Own DNA

Take a sample of cheek cells, isolate DNA, and amplify a short sequence of the bitter taste gene.

Sequence Analysis

Use bioinformatics software to explore the bitter taste gene and how genotypes can be distinguished.

Restriction Enzyme Digest

Use restriction enzyme digestion to prepare to determine our bitter taste genotypes.

Gel Electrophoresis

Use gel electrophoresis to determine our bitter taste genotypes.

Investigate Genetics of Drug Metabolism

Investigate the genetics of drug metabolism and consider how genotyping can aid medical treatment.

Medical Mystery

Explore the idea that people respond differently to medications and consider the possible reasons.

Is Taste Genetic?

Begin to explore a trait (taste) and investigate whether people experience it differently.

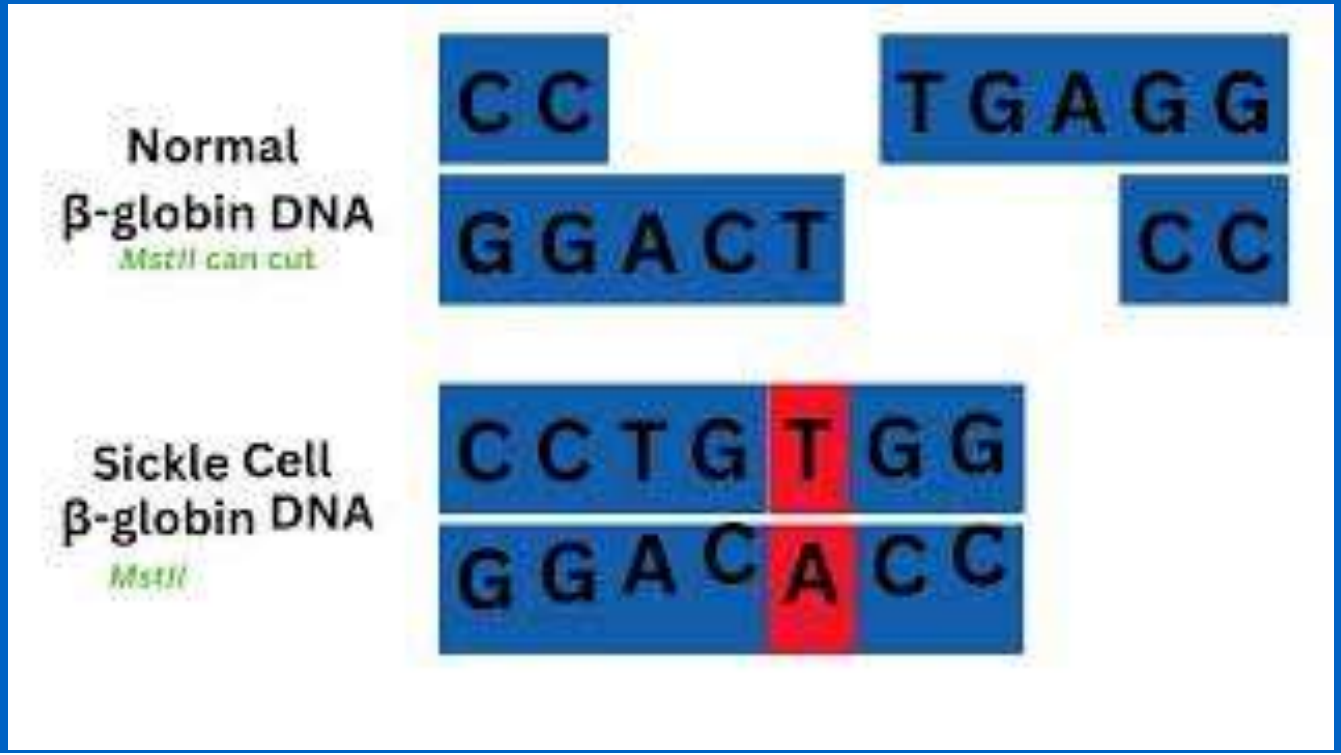
UNDERSTANDING PRECISION MEDICINE

In this module, we explore the genetics of the ability to taste bitter substances. It turns out that even small differences in our DNA—our genotype—can lead to major differences in traits—our phenotype. All of our genes have such individual differences, and some lead to changes in medically important traits. Advances in DNA sequencing and bioinformatics have made it much easier to discover these differences. Similarly, understanding how each of us metabolizes medications differently allows doctors to practice precision medicine—medicine based on each individual's genotype.

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!

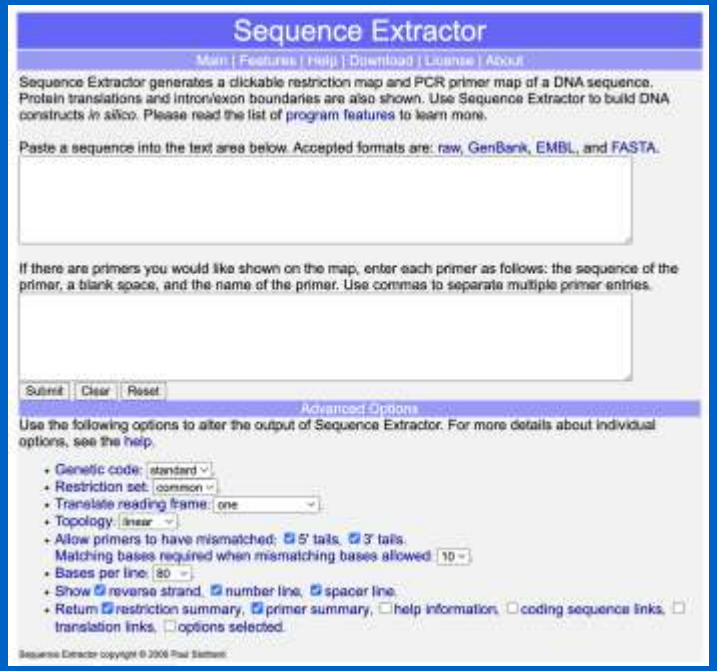
Video: Restriction enzymes in DNA testing

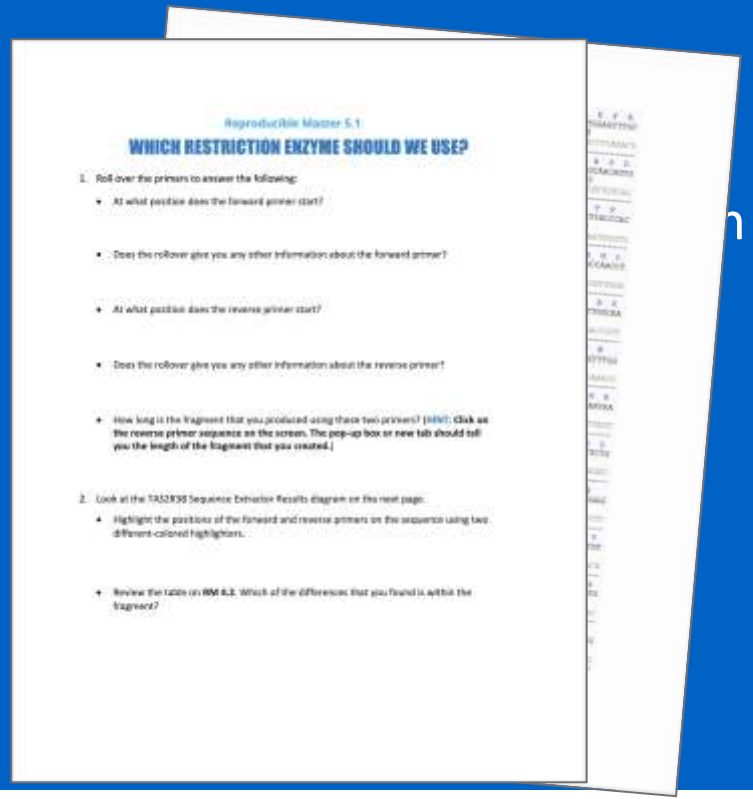


Discussion: genotyping sickle cell anemia

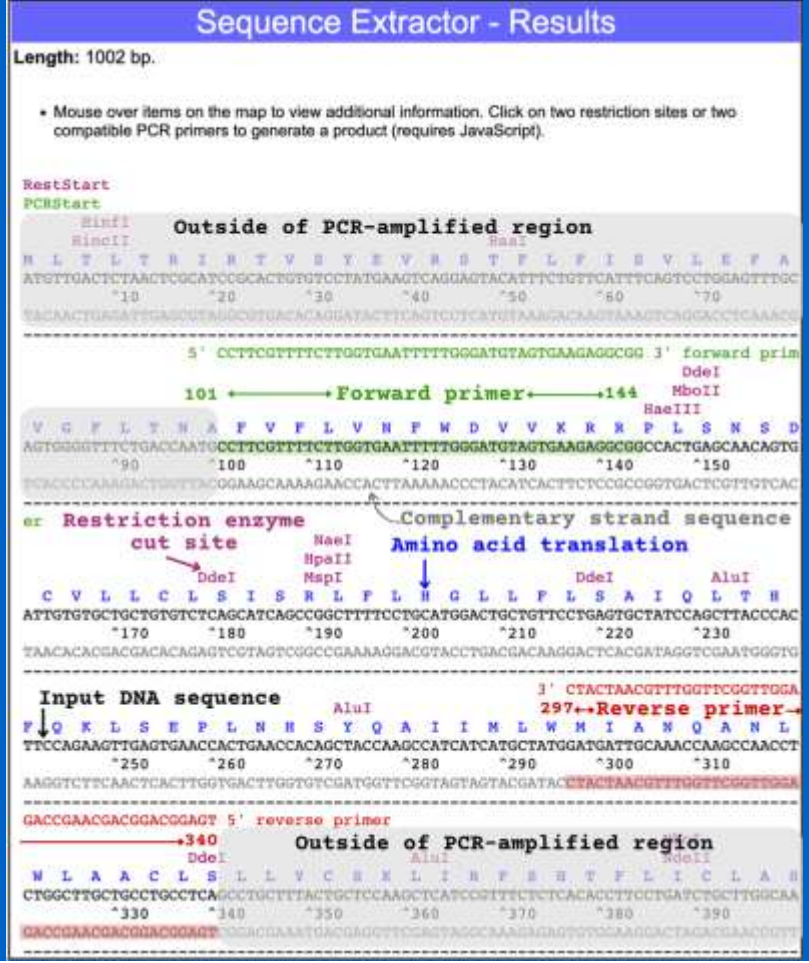
- **How did scientists isolate the patient's β-hemoglobin DNA?**
  - PCR of genomic DNA, with primers specific to the human hemoglobin β gene
- **Which restriction enzyme cuts normal β-globin DNA, but not the sickle cell allele?**
  - *MstII*
- **What was the patient's genotype, and how could you tell?**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

Activity: Which restriction enzyme should we use?





Discuss: RM 5.1



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?**

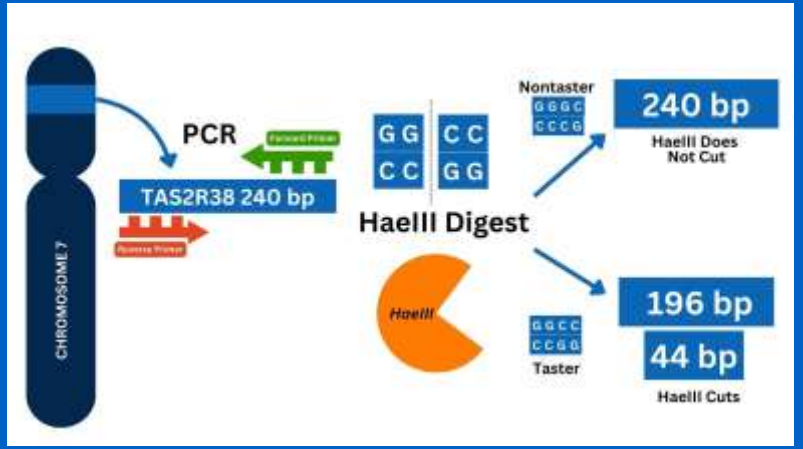
  196 bp and 44 bp

- **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?**

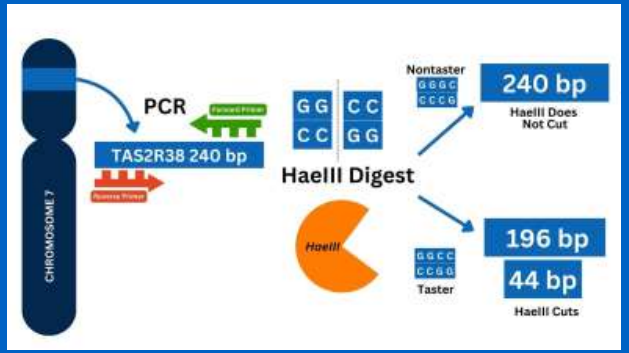
  All 3 lengths: 240 bp, 196 bp, and 44 bp



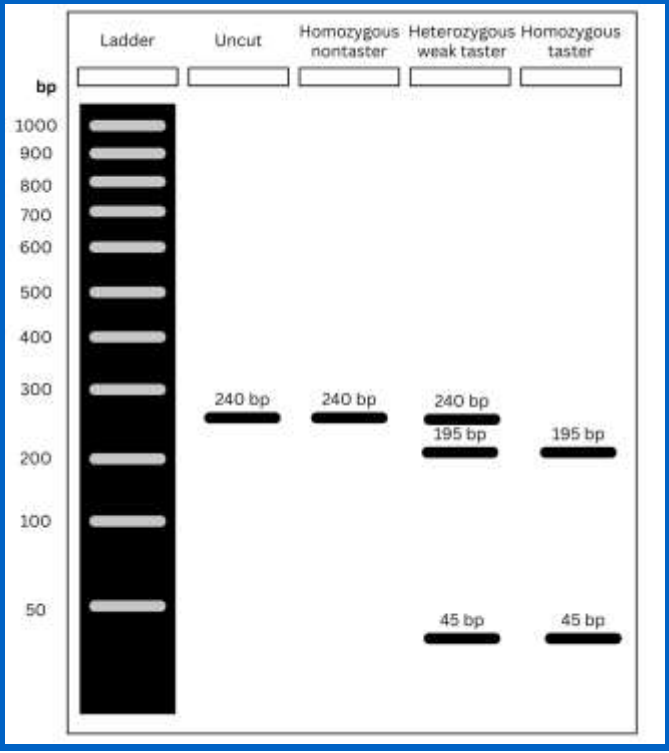
Discuss: RM 5.1 (continued)

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 β 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 taster 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 tasting 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 6.1** 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-bp ladder (M)
- 1 transilluminator
- Waste container

Set up your restriction digest