CHAPTER 6:
Gel Electrophoresis and Genotyping

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. For gel electrophoresis, it is submerged in a ***running buffer***, an electrolyte solution capable of forming ions and maintaining a stable pH. 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 (RM 6.2)**
- 1 lane of an agarose gel
- Student RE digest

For each team:

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

For the class:

- Restriction enzyme controls
- 2 lanes of an agarose gel to run the controls, plus one lane per any additional gel(s) for duplicate positive controls, as needed
- 1 transilluminator
- Camera

PROCEDURE (see **Figure 6.2** for flowchart)

Step	Notes
1. Ensure that your gel apparatus is set up properly. The gel should be positioned so that the wells are toward the anode (–). There should also be enough buffer in the running chamber to completely submerge the gel.	
2. Your teacher will assign you one lane into which to load your sample.	*Record your lane number in your lab notebook.*
3. Using a new pipette tip, load 10 µL of your restriction enzyme (RE) digest reaction into your assigned lane.	*Gently depress the micropipette button to the first stop to slowly expel the sample. Keep the micropipette button depressed at the first stop when removing the tip from the well, and release the button only after the pipette tip is in the air.*
4. If your teacher hasn't already done so, load 10 µL of the 100-bp ladder (M) into one outside well per row of samples.	
5. If your teacher hasn't already done so, load 10µL of the C+ and C– sample into their assigned wells.	
6. Run the gel at the voltage and for the amount of time directed by your teacher.	*If you run out of time during class, your teacher will turn off the unit and save your gel for the next class.*

While the gel is running . . .

Read the article "Genes Give Africans a Better Sense of Taste." Answer the following discussion questions in your science notebook and be prepared to discuss them with the class:

1. How were Kenyans' and Cameroonians' TAS2R38 genes different from those of Europeans and Asians?

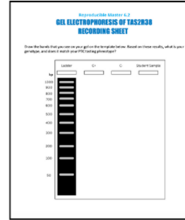
Step	Notes
2. One scientist speculated that iodine might play a role in natural selection of this gene. What did they suggest? 3. What is another reason that Africans have more genetic diversity than Europeans?	
Once the gel is complete . . .	
7. Place your gel on the transilluminator and observe the DNA bands in your sample.	*Your gels have been prepared with GelGreen dye, which attaches to DNA and fluoresces under blue light.* *Make sure you have the amber filter in place to view your gel on the transilluminator.*
8. Draw the bands that you find on the template on **Gel Electrophoresis of TAS2R38 Recording Sheet (RM 6.2).** Compare this to the predictions you made during the class discussion in Chapter 5 and discuss with your team.	*If your teacher loaded the positive (C+) and negative (C–) control samples on a different gel, review those results and include them in* ***RM 6.2.***

Figure 6.1: Materials for gel electrophoresis

For each student:

A pair of gloves

RM 6.2: Gel Electrophoresis of TAS2R38 Recording Sheet

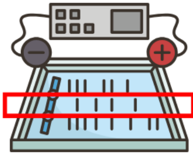


Reproducible Master 6.2

GEL ELECTROPHORESIS OF TAS2R38 RECORDING SHEET

Draw the bands that you see on your gel on the template below. Based on these results, what is your genotype, and does it match your PTC tasting phenotype?

1 lane of an agarose gel



Student restriction enzyme digest

For each team:

1 electrophoresis chamber and power supply

Gel running buffer

P-20 micropipette and tips

10 µL 100-bp ladder (M)

Waste container

Microfuge tube rack

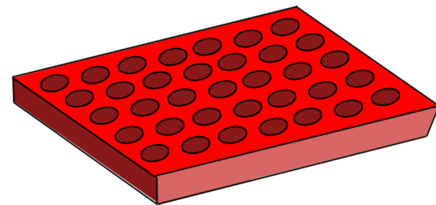
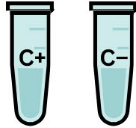


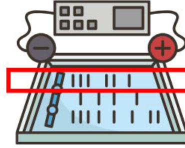
Figure 6.1: Materials for gel electrophoresis (continued)

For the class:

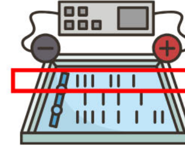
Control restriction enzyme digests, plus extra positive control digests as needed



2 lanes of one agarose gel to run the controls

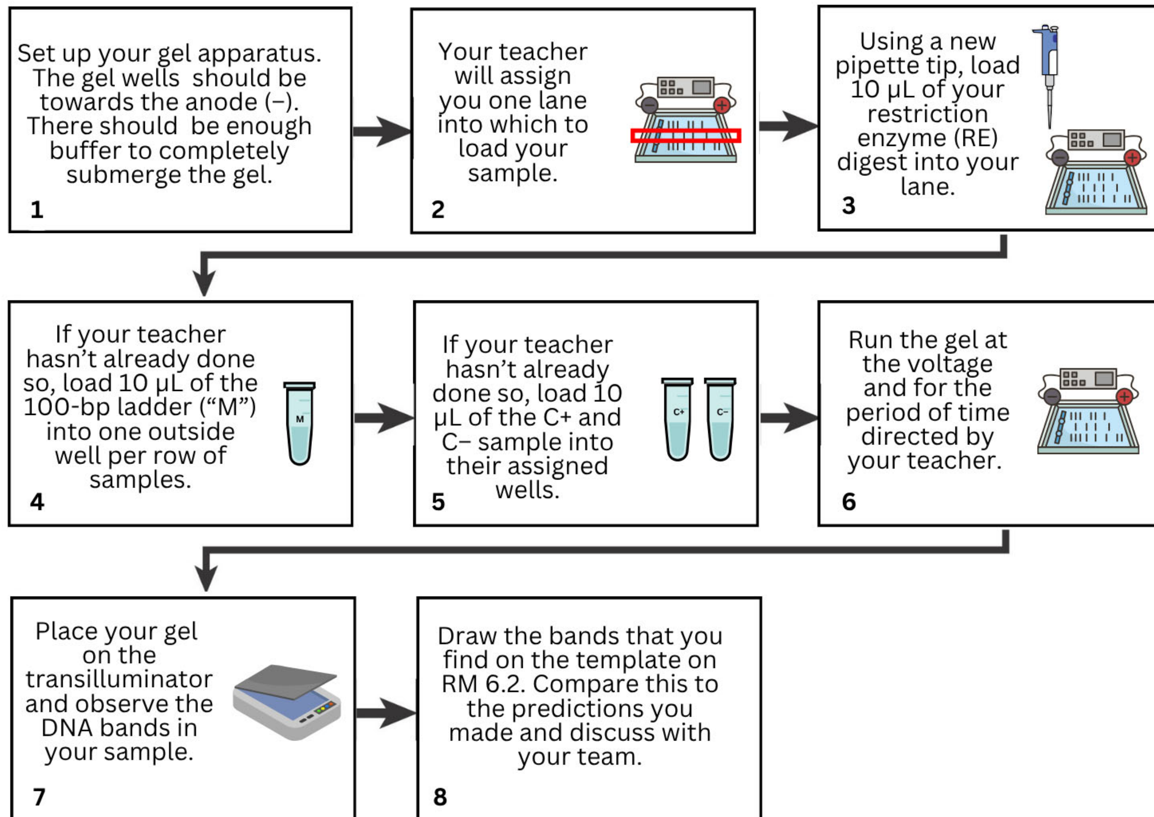


For every additional gel, 1 lane to run additional positive controls



Transilluminator

Figure 6.2: Flowchart procedure for gel electrophoresis



ACTIVITY: Restriction Digestion and SNP Genotyping

You just explored how you can use gel electrophoresis to visualize the results of your restriction digestion reaction to determine whether you are a PTC taster. The restriction enzyme you used selectively cut the DNA fragment you had amplified with PCR, but only when certain target sites were present.

In this activity, you will consider whether restriction digestion and gel electrophoresis can be used to determine whether a patient, like Ms. Jackson in Chapter 1, can safely take the antiplatelet medication clopidogrel before an angioplasty.

MATERIALS

- Laptop or desktop computer with internet access
- Files with the DNA sequence for Exons 5 and 4 of one wild-type ("normal") and two variants of CYP2C19, one with the loss-of-function SNP CYP2C19*2 and one with the loss-of-function SNP CYP2C19*3
- 1 copy of **Restriction Digestion and Genotyping (RM 6.3)**

BACKGROUND

In the Chapter 1 reading, "Balancing Prevention and Risk," you were introduced to a patient named Renee Jackson, who needs a percutaneous coronary intervention (PCI)—more commonly known as an angioplasty—to treat chest pain believed to be caused by blocked arteries. Patients like Ms. Jackson are typically prescribed antiplatelet therapy to prevent blood clotting from the procedure, which might otherwise lead to an increased risk of heart attack and stroke. One commonly prescribed medication for this is clopidogrel, which reduces blood clots by stopping platelets from clumping.

Pharmacogenomics is the science of understanding how individuals' genotypes influence their response to medications. Due to decades of pharmacogenomics research, scientists now know that variation in a cytochrome P450 gene, called CYP2C19, can put patients at additional risk for complications when clopidogrel is prescribed. This is because the cytochrome P450 family of enzymes works in the liver to process medications, which then pass into the bloodstream where they can act on specific drug targets.

Certain alleles of CYP2C19 reduce the efficacy of this process, resulting in much lower levels of active antiplatelet medication in the bloodstream, and therefore an increased risk of continued blood clotting during medical procedures. If Ms. Jackson carries particular alleles of CYP2C19, she can still have the PCI but must be prescribed a different antiplatelet medication to minimize her risk of heart attack or stroke from the angioplasty.

You are going to use skills you developed in Chapter 4—first to analyze nucleotide differences in alleles of the CYP2C19 gene, then to select restriction enzymes that could be used in a diagnostic test to genotype patients.

PROCEDURE

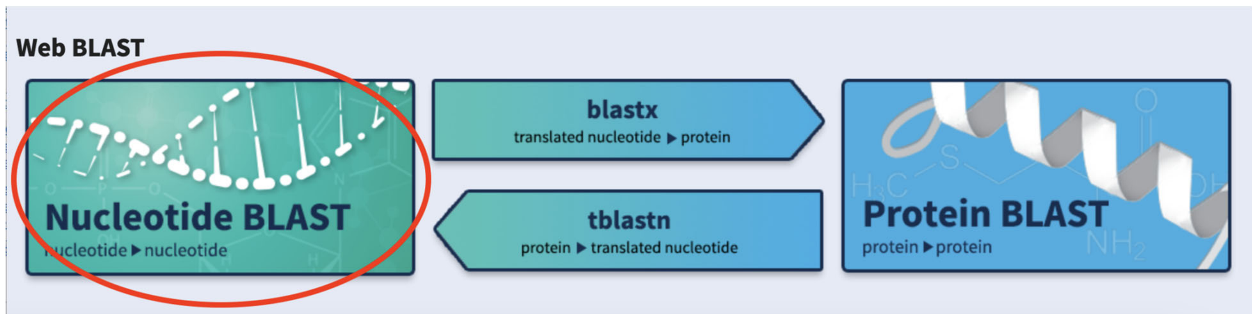
Research SNPs of CYP2C19

1. Go to SNPedia and type "CYP2C19" into the Search SNPedia box.
2. Record the information you find about the mutations which alter the function of this cytochrome P450 gene into the table titled "SNPs of the CYP2C19 gene and their effects on gene function" on **Restriction Digestion and Genotyping (RM 6.3)**. Only record those alleles that have known effects (refer to the "comments" column for details). Make sure to note the nucleotide positions within the gene where these SNPs are found.

Create an alignment between two nucleotide sequences from CYP2C19 Exon 5: wild-type and mutant

3. You will use NCBI BLAST to align two DNA sequences, just as you did in Chapter 4. This time, you will be comparing normal ("**wild-type**") DNA from Exon 5 of CYP2C19 to a mutant allele referred to as CYP2C19*2. The mutant allele results in reduced metabolism of medications.
4. Navigate to the NCBI BLAST homepage.
5. Under the header "Web BLAST," click on the "Nucleotide BLAST" box (see **Figure 6.3**).

Figure 6.3: Click on "Nucleotide BLAST"



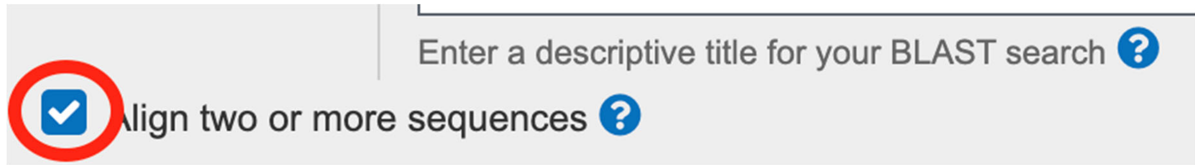
This time, you will create an alignment of a **selected range** of nucleotides from Exon 5 of CYP2C19. Please note that this is not the sequence of the entire exon—it is the sequence of a small portion of the DNA that contains a SNP of interest.

The GenBank accession numbers for wild-type and mutant alleles of Exon 5 are:

- Wild-type: L31506
- Mutant (CYP2C19*2): L31507

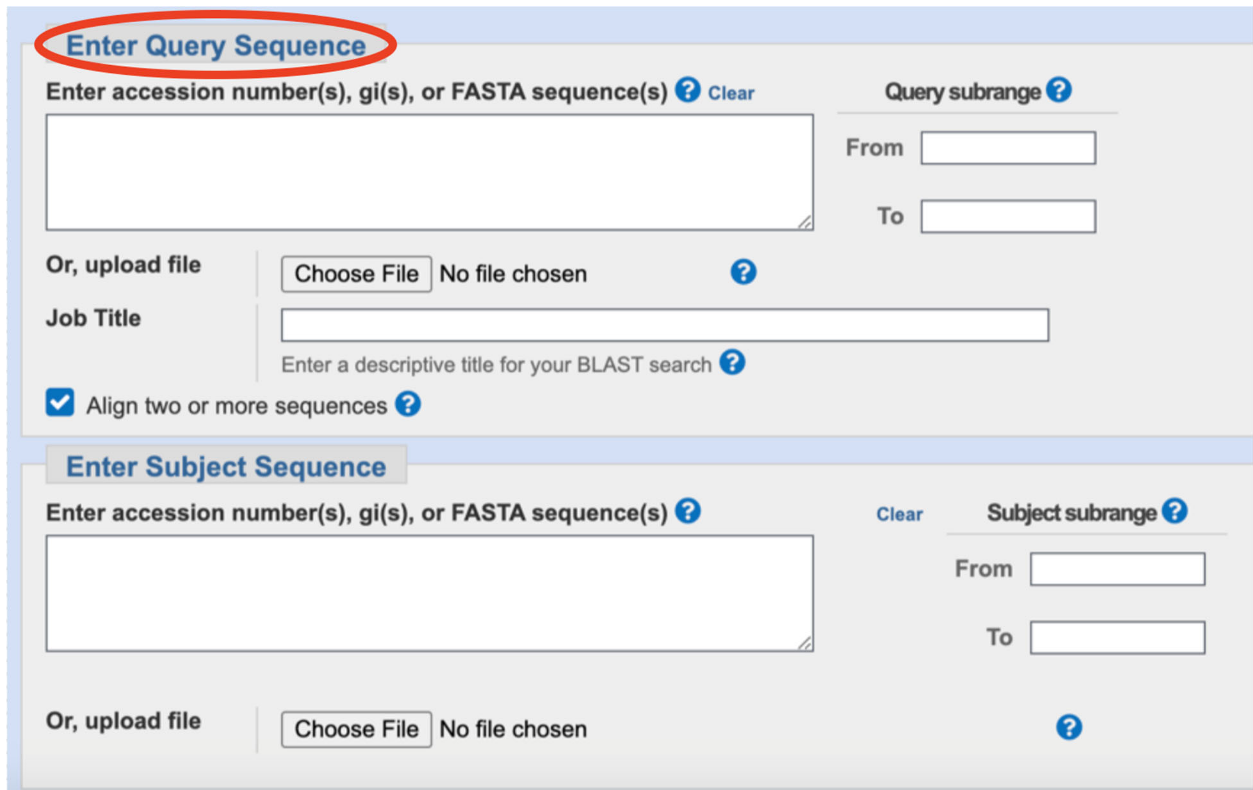
6. Under "Enter Query Sequence," check the "Align 2 or more sequences" box (see **Figure 6.4**).

Figure 6.4: Align two or more sequences



7. Now, you should see two boxes, one called "Enter Query Sequence" and one called "Enter Subject Sequence" (see **Figure 6.5**). Enter the wild-type CYP2C19 Exon 5 sequence reference number (L31506) into the Enter Query Sequence box.

Figure 6.5: Enter the sequence reference number into the Enter Query Sequence box



8. Specify the desired range of nucleotides to be analyzed in the Query Subrange box to the right of the Enter Query Sequence box. Enter 79 in the box labeled "From" and 129 in the box labeled "To" (see **Figure 6.6**).

Figure 6.6: Enter the range of nucleotides in the Query Subrange box

9. Enter the mutant CYP2C19*2 Exon 5 sequence reference number (L31507) into the Enter Subject Sequence box.
10. Once more, specify the desired range of nucleotides to be analyzed in the second sequence, this time in the Subject Subrange box to the right of the Enter Subject Sequence box. Enter 79 in the box labeled "From" and 129 in the box labeled "To" (see **Figure 6.7**).

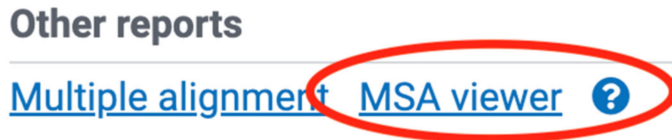
Figure 6.7: Enter the range of nucleotides in the Subject Subrange box

11. Select "Show results in a new window," then click on the "BLAST" button at the bottom of the page (see **Figure 6.8**). An alignment analysis will open on a new page.

Figure 6.8: Show results in a new window and BLAST

12. Click “MSA viewer” next to “Other reports” (see **Figure 6.9**). A graphical representation of your nucleotide sequence alignment will open in a new window.

Figure 6.9: MSA viewer

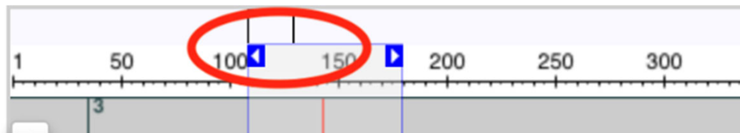


Analyze nucleotide differences between phenotypes

13. You should see three rows, labeled “consensus,” “L31506” (the wild-type [normal metabolizer] sequence) and “L31507” (the mutant [poor metabolizer] sequence). You will notice that the sequences of the wild-type and mutant alleles are mostly represented by dots, indicating that the sequences are identical to that of the consensus sequence. Only SNPs should be different. Above the sequences you will notice a horizontal gray bar. Any red vertical lines seen highlight the position of the SNPs.

14. Move the uppermost rectangular slider until it is centered on any SNP you see (see **Figure 6.10**).

Figure 6.10: Move slider



15. Record the nucleotide position of any SNPs, as well as any difference in nucleotide sequences between the wild-type and mutant CYP2C19 Exon 5 alleles, on **Restriction Digestion and Genotyping (RM 6.3)**. Next, you are going to look for a restriction enzyme that cuts only one of these two sequences.

Identify a diagnostic restriction enzyme for Exon 5 of CYP2C19

16. Download the CYP2C19 Exon 5 sequence files your teacher gave you access to.

17. Open and copy the entire wild type sequence, then go to the Sequence Extractor website and paste the sequence in the text area. Delete any text in the primers box, then click “Submit.”

18. Scroll down in the Sequence Extractor output until you find the variant nucleotide position you identified in your alignment. Look for any restriction enzymes within a few bases of that position. (Remember to look above the strand.) Hover over the restriction enzyme to see its restriction site.

19. Make note of any possible restriction enzymes in **RM 6.3,** in the **Restriction Enzymes and Restriction Sites in Exon 5 of the CYP2C19 Gene** table. The table may include more lines than you need. You should prioritize any restriction enzyme that cuts right at the SNP you identified. Circle the restriction enzyme that you think will be the best choice.

20. Repeat the Sequence Extractor restriction enzyme analysis with the sequence from the CYP2C19*2 allele. Did you find any restriction sites?

Repeat the analysis with Exon 4 of CYP2C19

21. Repeat **Steps 4–15** (creating a sequence alignment and identifying a SNP), this time for **Exon 4 of CYP2C19**. The GenBank accession numbers for wild-type and mutant alleles of Exon 4 of CYP2C19 are:

    - Wild type: L32982
    - Mutant (CYP2C19*3): L32983

    This time, the subject and query sequence subrange should be from 79 to 240.

22. Next, search for a diagnostic restriction enzyme to distinguish between wild-type and mutant alleles of Exon 4. Download the CYP2C19 Exon 4 sequence files your teacher provided access to.

23. As a reminder, this DNA fragment is from the 3' end of Exon 4, rather than the sequence of the entire exon. The wild-type sequence is titled "CYP2C19_Exon_4_WT.txt," and the sequence from the CYP2C19*3 allele is titled "CYP2C19_Exon_4*3.txt."

24. Repeat **Steps 17–20** above, this time using Sequence Extractor to compare the wild-type and mutant CYP2C19 alleles of Exon 4. Make note of any possible restriction enzymes in **RM 6.3**, in the table **Restriction Enzymes and Restriction Sites in Exon 4 of the CYP2C19 Gene.** The table may have more lines than you need. You should prioritize any restriction enzyme that cuts right at the SNP you identified. Circle the restriction enzyme that you think will be the best choice.

25. Finally, answer the questions on **RM 6.3**.