AMGEN Biotech Experience

Scientific Discovery for the Classroom

Exploring Precision Medicine

STUDENT GUIDE

2026 Pilot

Contents

OVERVIEW

Welcome to the Amgen Biotech Experience module *Exploring Precision Medicine*! You are probably already familiar with how your genes affect your visible traits, such as your hair color and eye color, and you may have studied the heritability of disease. In this module, you will have a chance to explore how understanding genetics can help to personalize the medical treatments that we receive—which is the definition of ***precision medicine***. It's an approach to the prevention and treatment of disease that considers variability in genes, environment, and lifestyle. Throughout this module, you will:

- Review medical cases to learn how precision medicine works
- Explore how and why your DNA makes your sense of taste different from your peers
- Investigate how we know which genes control which traits
- Complete a laboratory experiment to extract your own DNA
- Explore your ability to taste a particular flavor via genetic sequence analysis and gel electrophoresis of polymerase chain reaction (PCR) products

CHAPTER 1:
What's the Right Medicine?

INTRODUCTION

What are traits? People often talk about *personality* traits, such as being funny, thoughtful, or quiet. In genetics, ***traits*** refer to characteristics of living organisms that can be described, quantified, or measured. Traits may be inherited or learned. ***Genotype*** is the genetic makeup of an individual, and ***phenotype*** is the set of observable characteristics of an individual based on how their genotype is expressed. While an individual's genetic makeup is the set of instructions on which their phenotype is based, environmental factors also influence their phenotype. For example, skin temperature affects the fur color of Siamese cats.

What determines our traits? For the activities in this chapter, you will think about the factors that control traits and discuss your ideas with your teammates.

First, you will start to explore traits by filling out a worksheet that challenges you to consider the influence of genetics and environment on particular traits.

Next, you will read an article from a webpage and answer some questions to think about the ways in which a patient's race and socioeconomic background might be important for individualized treatment.

Lastly, you will explore the case of a patient whose doctor proposes genotyping to reduce potential negative side effects from their necessary medication and review the science that makes this approach possible.

ACTIVITY: Genetics vs. Environment

Has anyone ever told you that you share facial features with a related family member? How about their personality? To what extent are your personal characteristics the result of your DNA versus your upbringing and lifestyle? Challenge yourself to consider the impact of genetics versus the environment on a number of human traits. Read and complete **What Controls Traits? (RM 1.1)**. Be prepared to discuss your answers with the class.

READING: Diversity and Inclusion in Clinical Trials

Read the article "Diversity and Inclusion in Clinical Trials" from the U.S. National Institute of Health's (NIH) National Institute on Minority Health and Health Disparities (NIMHD) to learn more about how medications have traditionally been tested and why and how that approach has changed in recent decades.

As you read, answer the questions on **Including Diverse Populations in Medical Studies (RM 1.2)**.

ARTICLE:

Diversity and Inclusion in Clinical Trials

Our health is a combination of physical and mental well-being, which is affected by our behavior, biology, environment, societal policies, and importantly, our lived experiences. The lived experiences of people in the United States vary based on their race and ethnicity, socioeconomic status (SES), geographic location, sexual orientation, gender identity, and other sociodemographic characteristics.

Lived experiences also need to be understood in the context of the individual and structural social determinants of health.

How and where we live, learn, work and play, and our access to high quality health care, healthy foods, and quality education can enhance our health outcomes.

Similarly, negative experiences and exposures, such as pollution, violence, and structural racism and discrimination, can negatively affect our health.

Our health status reflects the interwoven effects of such factors.

A clinical trial is a type of clinical research that evaluates the effects of intervention(s), including drugs, devices, surgeries, diets, behavioral approaches, and lifestyle interventions, on health-related biomedical or behavioral outcomes.

To account for the diverse lived experiences and exposures of various populations, clinical research should be appropriately inclusive of racial and ethnic minority groups, as well as other populations experiencing health disparities, including sexual and gender minority or socioeconomically disadvantaged populations.

Why Are Clinical Trials Important?

Clinical trials can:

- Determine if a new intervention is safe, works better, and/or has fewer side effects than an existing treatment or intervention.
- Examine ways to detect a disease early, when it is potentially more treatable, or ways to prevent a health problem altogether.
- Evaluate ways to improve the quality of life of people who have an illness or chronic medical condition.
- Include testing of behavioral, social, environmental, and structural interventions.

Participating in clinical trials is voluntary. People volunteer to participate in clinical trials for a variety of reasons.

- One of the most common reasons is altruism—the opportunity to contribute to science and the common good and/or help those with similar health issues.

- People may volunteer when it allows them to receive an experimental intervention for life-threatening or disabling disease where no standard treatments are available or were already tried without success.
- New interventions (e.g., weight loss or tobacco cessation interventions) that haven't yet been approved by the U.S. Food and Drug Administration (FDA) may be tested for common conditions to understand if the intervention might help a condition in situations where current treatments or interventions don't exist, don't work well, or have unwanted side effects, or provide symptomatic relief, but offer no cure.

The Importance of Diversity & Inclusion in Clinical Trials

People may experience the same disease differently. It's essential that clinical trials include people with a variety of lived experiences and living conditions, as well as characteristics like race and ethnicity, age, sex, and sexual orientation, so that all communities can benefit from scientific advances.

Factors that can influence the risk and likelihood of developing a disease, experiencing a long-term health outcome, and responding to treatment include (but are not limited to):

- Age
- Biological sex
- Pregnancy status
- Life experiences (negatives, such as psychosocial stress and lack of basic resources, or positives, such as educational and employment opportunities)
- Unhealthy behaviors (e.g., substance use, sedentary lifestyle, overeating, risky sexual activity)
- Health-promoting behaviors (e.g., adequate sleep, obtaining recommended preventive services, physical activity, healthy eating)
- Environmental conditions (e.g., pollution, access to health care or healthy foods, neighborhood segregation)
- Genetic variation and geographic ancestry
- Underlying medical problems or presence of comorbidities (i.e., additional diseases or conditions)

Historically, clinical trials did not always recruit participants who represented the individuals most affected by a particular disease, condition, or behavior. Often, these clinical trials relied almost exclusively on White male study participants. This shortcoming has created gaps in our understanding of diseases and conditions, preventive factors, and treatment effectiveness across populations. These gaps in knowledge can impede the quality of health care decision making, ability to counsel people on ways to reduce their risk, optimal treatment responses, and even the development of more effective medications or interventions.

Clinicians and researchers should carefully consider the inclusion or exclusion criteria for their clinical trials. For example, a clinical trial excluding participants with high blood pressure or other comorbidities may end up excluding many people over 65 years old, who are more likely to have these conditions. The trial may then underrepresent certain groups in the study and make the results less applicable to groups who may benefit the most from the findings.

Real-World Examples of the Need for Inclusion in Clinical Trials

Understanding COVID-19 Disparities

During the early stages of the pandemic, Coronavirus disease 2019 (COVID-19) disproportionately affected racial and ethnic minority populations, including African American, Hispanic/Latino, American Indian/Alaska Native, and Native Hawaiian and Pacific Islander population groups, with increased cases, hospitalizations and deaths.

It was critical that COVID-19 vaccine trials included sufficient representation across population groups to better understand vaccine effectiveness in populations who vary on environmental exposures and other lived experiences. By using inclusive recruitment practices in COVID-19 clinical trials, researchers have been able to show that vaccine safety and efficacy are similar across all racial and ethnic populations. Engaging diverse populations in planning and implementing such trials can also help increase public confidence that the vaccine is safe and effective.

Understanding Asthma Disparities

Asthma disparities are intricately linked with the environment. Living in a city may increase exposure to air pollution and risk for developing asthma. Exposure to tobacco smoke, chronic social stress, or unhealthy diets may also influence asthma risk or severity. Thus, it is vital for clinicians and researchers to consider where patients live, what they eat, and how they feel—as well as characteristics like race, ethnicity, socioeconomic status, and age—to get a more thorough understanding of their patients' experience with asthma symptoms and identify the best preventative strategies or treatment options.

Inclusive Participation in Clinical Trials Benefits Scientific Discovery

NIH is committed to inclusivity in clinical trial research. It is essential to have a wide range of people from different communities participate in clinical trials to reduce biases, promote social justice and health equity, and produce more innovative science. Below is a list of topics and examples to illustrate the important role of inclusive participation in clinical trial research.

Countering Mistrust in Clinical Research

Historical atrocities and incidents have engendered mistrust in clinical research and medical institutions.

Investigators conducting the U.S. Public Health Service Syphilis Study at Tuskegee between 1932 and 1972 did not explain the study's risks and obtain formal agreements (called informed consent) from the African American men who were its participants. The researchers wanted to study the effects of untreated syphilis and withheld penicillin treatment when it became available in 1945, which would have helped the 399 study participants with the disease. Only when news leaked of the study in 1972 did their unethical and discriminatory behavior come to light. Their actions caused preventable illness and death in study participants and their families.

In 2003, members of the Havasupai Tribe in Northern Arizona learned that DNA samples given in the early 1990s for a diabetes research study were later being used for additional research on ethnic migration, schizophrenia, and other unrelated genetic studies. The informed consent form from the original study did not ask participants for their permission to use these samples for these other

analyses. The researchers failed to obtain their consent for use of their data and specimens for other research purposes.

The failings of the Syphilis Study at Tuskegee contributed to the creation of the Belmont Report in 1976, which addresses ethical issues in research with human participants. It outlines basic ethical principles and essential guidelines to protect human research participants and ensure safety in clinical trial research.

Today, Institutional Review Boards are responsible for reviewing all studies involving humans for compliance with these guidelines and reports of any study protocol violations. In recent years, people from racial and ethnic minority communities and other populations experiencing health disparities have become more willing to participate in clinical research. Developing trust with communities who have been marginalized is best achieved through meaningful partnerships between researchers and community members in planning and carrying out studies with their input.

Inclusion of Women and People from Racial and Ethnic Minority Groups in Clinical Trials

The NIH Revitalization Act of 1993 was signed into law, authorizing NIH to continue its mission and importantly establishing guidelines for the inclusion of women and persons from racial and ethnic minority populations in clinical research. The goal of this law, and other guidelines, is for clinical trial participants to adequately reflect the diversity of the real-world population, so that researchers can determine whether the variables being studied affect women or members of any racial and ethnic population group. This helps ensure that research findings are generalizable to the entire population.

NIH efforts toward research inclusion remain at the forefront of clinical research policy. Recent activities include the publicly available NIH Research, Conditions and Disease Categorization Inclusion Statistics Report, which provides data on human research participation in NIH clinical research studies by race, ethnicity, and sex/gender. Additionally, in 2017, NIH updated its policy on the inclusion of women and people from racial and ethnic minority populations with a requirement that "recipients conducting applicable NIH-defined Phase III clinical trials ensure results of valid analyses by sex/gender, race, and/or ethnicity are submitted to Clinicaltrials.gov." See NIH Inclusion Outreach Toolkit: How to Engage, Recruit, and Retain Women in Clinical Research for more information.

Inclusion of Sexual and Gender Minority Populations

Until recently, health care systems and epidemiological surveys often didn’t ask sexual orientation and gender identity questions to consider inclusion of sexual and gender minority (SGM) persons. This has made it difficult to know if individuals within SGM populations are represented in clinical research studies in significant numbers to make results representative for them. This lack of knowledge can influence patient-clinician communication and can result in fewer health screening or treatment opportunities.

Inclusion by Socioeconomic Status (SES)

An individual’s SES is a major predictor of health outcomes, because it can impact access to health care, nutritious foods, prescription medications, and other resources for healthy living. Yet, SES measures (i.e., education and income level) are not collected routinely and reported in clinical trials.

In an analysis of all randomized clinical trials published in 2015 and 2019 in the Journal of the American Medical Association, The Lancet, and the New England Journal of Medicine, study

investigators reported that less than 15% of studies reported on the SES of trial participants. Lack of data collection and reporting on SES measures make it difficult to generalize research findings to all SES groups or to tailor interventions (e.g., new medications or other treatment interventions) to people with lower SES who may not be able to access or maximize the benefits of clinical trial outcomes. In addition, limited access to socioeconomic resources may pose a barrier to participation in clinical trials.

To ensure the inclusion and representation of participants across different SES levels in clinical trials, researchers should use appropriate data collection and reporting protocols. For example, NIMHD supported a social determinants of health collection in the PhenX Toolkit that includes established instruments for conducting research with human participants, such as clinical trials.

Researchers should also design their studies and provide resources to make it easier for people with lower SES to participate in clinical trials, such as offering convenient locations and hours of operation, childcare services, and transportation vouchers.

Data Collection and Reporting: Unmasking Hidden Truths

When scientists combine information from individual research participants, this is called data aggregation. Data aggregation is an important part of the research process that protects the anonymity of research volunteers and strengthens the statistical analysis of the study. However, aggregation of demographic data, including race and ethnicity, can also mask important differences in health risks or outcomes for specific subpopulations.

For example, many prior studies on the health of Asian Americans have not always examined differences by nationality. A recent study found that among Filipino, Vietnamese, Chinese, Japanese, and Korean American adults living in California, categorizing all participants as "Asian American" masked at least one health disparity for each subpopulation.

Clinicians and researchers must take care to define as best as possible the clinical trial sample in their studies and consider whether their findings can be generalized across population groups, including consideration for differences in lived experiences.

Source: NIH National Institute on Minority Health and Health Disparities (NIMHD), https://www.nimhd.nih.gov/ (accessed December 2024; hyperlinks removed)

READING: Applying Personalized Medicine

No two patients are alike, which makes the practice of medicine challenging. For example, no two surgeries are the same due to people's individual variations. Also, in many cases, an effective dosing of medication depends on an individual's genetic makeup and lifestyle.

When caring for a patient, a medical professional must come up with a diagnosis, determine appropriate treatments, properly administer medication or therapy, monitor the patient's progress, and adjust the course of treatment as needed—and each step must take the patient's medical history into account while respecting the patient's wishes.

It is crucial to provide patients with the right medication at the right dosage, but this is not as simple as it seems, as you have learned from reading the NIMHD article on diversity in clinical research. Numerous factors, including life stage, pre-existing conditions, and genetics, can result in patients responding quite differently to the same medication. Depending on the medication, some people may need larger or more frequent doses, and others, less frequent or smaller doses.

The enormous variety of medications currently on the market creates a large pool of options from which physicians and pharmacists can choose. However, this adds another layer of troubleshooting when prescribing medications. Sometimes even very subtle differences between multiple formulations of a single drug—such as generic versus brand name—can influence a patient's better response to one form over another.

So, how do practitioners know which medication to use? They rely on guidelines from groups of medical professionals who conduct clinical research trials to determine optimal treatments.

Below, read about a patient who needs a lifesaving medication and learn how their doctor treats them based on their genetics. Then, answer the questions on **Reading Questions: Balancing Prevention and Risk (RM 1.3).**

READING: Balancing Prevention and Risk

Renee Jackson is a 64-year-old grandma and recreational pickleball player. Recently, she began having perplexing episodes of chest pain, even when at rest and not exercising. She let her primary care physician know and was diagnosed with ***angina*** (one cause of chest pain) and told to monitor her symptoms.

At a recent tournament, she had to stop playing mid-match due to severe chest pain and shortness of breath and was rushed to the nearest emergency department. Thankfully, they determined that she had not had a heart attack, but that several of her coronary arteries were almost completely blocked. That set the stage for small blood clots to form in her arteries, reducing blood flow and causing her symptoms, termed ***unstable angina***.

Ms. Jackson will need a ***percutaneous coronary intervention (PCI)***, also known as an angioplasty, to clear the blockages and to insert ***stents***, which are tiny, expanding metal cage-like structures that prop arteries open. These devices will work to reduce her chest pain. She will also need to make

some medication and lifestyle changes to manage her coronary artery disease to prevent her arteries from being blocked in the future.

Ms. Jackson has an appointment with her interventional cardiologist, Naomi Silva, for a pre-procedure consultation.

Dr. Silva tells Ms. Jackson that she wants to pre-treat her with ***antiplatelet therapy*** (medication that prevents blood clotting), since potential clotting from the procedure might lead to an increased risk of heart attack and stroke. Usually, she would prescribe the medication clopidogrel (brand name Plavix), which reduces blood clots by stopping platelets from clumping.

However, she tells Ms. Jackson that her health insurance has approved a genetic test to determine whether she is a carrier of an abnormal allele, or variant, of the CYP2C19 gene, which encodes a ***cytochrome P450*** enzyme. ***Enzymes*** are proteins that catalyze chemical reactions without being used up or altered themselves, so they can be reused repeatedly. Cytochrome P450 enzymes are expressed in the liver, where they carry out oxidation and reduction reactions on substrates that include medications.

An ***allele*** is one of two or more alternative forms of a gene. Some alleles alter the form or function of the protein the gene encodes. Mutant alleles of CYP2C19 could put Ms. Jackson at additional risk for complications if Dr. Silva prescribes clopidogrel. If Ms. Jackson carries one or more of these alleles, she can still have the PCI, but will be prescribed a different antiplatelet medication instead, to minimize her risk of heart attack or stroke from the angioplasty.

Dr. Silva asks Ms. Jackson if she would be willing to share her self-identified race, in addition to providing details of her medical history.

Ms. Jackson understands that she needs to have the procedure as soon as possible but is worried about it and the possibility of complications. She's also concerned about the cardiologist's request to learn about her racial background. She has questions for the doctor before she consents to genetic testing.

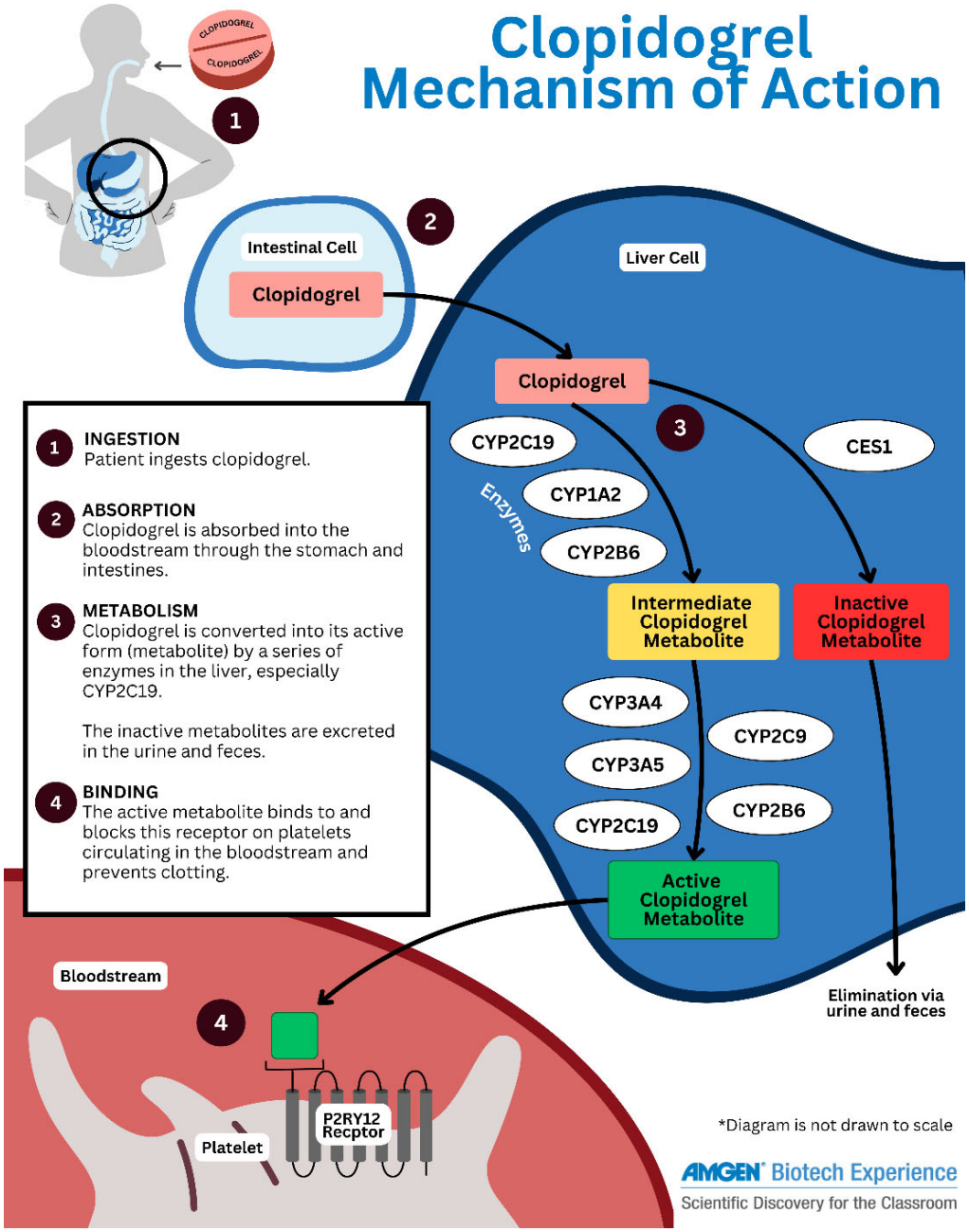
What should Dr. Silva tell her?

BACKGROUND INFORMATION

See **Figure 1.1** below to learn about the crucial role of the cytochrome P450 enzyme family in drug metabolism, and review Ms. Jackson's medical and family history. Then, view the video *Simple Genetic Test Shows Promise for Better Outcomes in Heart Stent Patients* to learn what researchers found about the effect genetic variation in CYP2C19 has on metabolism of antiplatelet medication.

Next, use the two tables from the Clinical Pharmacogenetics Implementation Consortium guidelines for the use of clopidogrel versus other antiplatelet agents to treat cardiovascular conditions, along with a table of phenotype frequency below, to answer the questions on **RM 1.3**.

Figure 1.1: Clopidogrel mechanism of action



Renee Jackson's medical history

Age: 64

Race: Mixed race (Black and White).

Family history: Her father (Black) had heart disease and diabetes, and his ancestry was West African. Her mother (White) had high cholesterol and metabolic syndrome, and her ancestry was European (British Isles).

Health conditions: Metabolic syndrome, arthritis, glaucoma, angina, high cholesterol

Table 1.1: Assignment of predicted CYP2C19 phenotype based on genotype

Predicted phenotype	Genotype	Examples of *CYP2C19* diplotypes[a]
CYP2C19 ultrarapid metabolizer	An individual carrying two increased function alleles	**17/*17*
CYP2C19 rapid metabolizer	An individual carrying one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer	An individual carrying two normal function alleles	**1/*1*
CYP2C19 likely intermediate metabolizer[b]	An individual carrying one normal function allele and one decreased function allele or one increased function allele and one decreased function allele or two decreased function alleles	**1/*9, *9/*17, *9/*9*
CYP2C19 intermediate metabolizer	An individual carrying one normal function allele and one no function allele or one increased function allele and one no function allele	**1/*2, *1/*3, *2/*17, *3/*17*
CYP2C19 likely poor metabolizer[b]	An individual carrying one decreased function allele and one no function allele	**2/*9, *3/*9*
CYP2C19 poor metabolizer	An individual carrying two no function alleles	**2/*2, *3/*3, *2/*3*
Indeterminate metabolizer	An individual carrying one or two uncertain function alleles	**1/*12, *2/*12, *12/*14*

[a] Please refer to the *CYP2C19* Diplotype-Phenotype Table online for a complete list. For allele functions and population-specific allele and phenotype frequencies, please refer to the *CYP2C19* Allele Functionality Table and the *CYP2C19* Allele Frequency Table online.[8,9]
[b] There are limited data to characterize the function of decreased function alleles.

Table 1.2: Antiplatelet therapy recommendations based on CYP2C19 phenotype when considering clopidogrel for cardiovascular indications

CYP2C19 phenotype[a]	Implications for phenotypic measures	Therapeutic recommendation	Classification of recommendation[b] - ACS and/or PCI[c]	Classification of recommendation[b] - non-ACS, non-PCI cardiovascular indications[d]
CYP2C19 ultrarapid metabolizer	Increased clopidogrel active metabolite formation; lower on-treatment platelet reactivity; no association with higher bleeding risk	If considering clopidogrel, use at standard dose (75 mg/day)	Strong	No recommendation
CYP2C19 rapid metabolizer	Normal or increased clopidogrel active metabolite formation; normal or lower on-treatment platelet reactivity; no association with higher bleeding risk	If considering clopidogrel, use at standard dose (75 mg/day)	Strong	No recommendation
CYP2C19 normal metabolizer	Normal clopidogrel active metabolite formation; normal on-treatment platelet reactivity	If considering clopidogrel, use at standard dose (75 mg/day)	Strong	Strong
CYP2C19 likely intermediate metabolizer	Reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid standard dose clopidogrel (75 mg) if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong[e]	No recommendation[e]
CYP2C19 intermediate metabolizer	Reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid standard dose (75 mg) clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong	No recommendation
CYP2C19 likely poor metabolizer	Significantly reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong[e]	Moderate[e]
CYP2C19 poor metabolizer	Significantly reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong	Moderate

ACE, acute coronary syndrome; PCI, percutaneous coronary intervention.

[a] The online *CYP2C19* Allele Frequency Table provides phenotype frequencies for major race/ethnic groups, and the online *CYP2C19* Diplotype-Phenotype Table provides a complete list of possible diplotypes and phenotype assignments.[8,9]

[b] Rating scheme described in the **Supplementary Material** online.

[c] ACS and/or PCI includes patients undergoing PCI for an ACS or non-ACS (elective) indication.

[d] Non-ACS, non-PCI cardiovascular indications include peripheral arterial disease and stable coronary artery disease following a recent myocardial infarction outside the setting of PCI.

[e] The strength of recommendation for the "likely" phenotypes are the same as their respective confirmed phenotypes. "Likely" indicates the uncertainty in the phenotype assignment, but it is reasonable to apply the recommendation for the confirmed phenotype to the corresponding "likely" phenotype.

Tables 1.1 and 1.2 reproduced with permission from "Clinical Pharmacogenetics Implementation Consortium Guideline for *CYP2C19* Genotype and Clopidogrel Therapy: 2022 Update (January 2022)."

Table 1.3: Frequencies of CYP2C19 phenotypes in biogeographical groups*

Phenotype	African American/Afro-Caribbean	American*	Central/South Asian	East Asian	European*	Latino	Near Eastern	Oceanian	Sub-Saharan African
Ultrarapid Metabolizer	0.04294	0.00741	0.02916	0.00042	0.04641	0.02774	0.03664	0.00325	0.03005
Rapid Metabolizer	0.23738	0.13638	0.18567	0.02534	0.27118	0.24136	0.25737	0.02133	0.21081
Normal Metabolizer	0.32806	0.62756	0.29553	0.38055	0.39612	0.52498	0.45192	0.03501	0.36977
Likely Intermediate Metabolizer	0.02779	0	0	0.00077	0.00112	0.00371	0	0	0.04286
Intermediate Metabolizer	0.31399	0.21383	0.40807	0.45928	0.26109	0.19036	0.23548	0.36903	0.29946
Likely Poor Metabolizer	0.00709	0	0	0.00043	0.0002	0.00044	0	0	0.01033
Poor Metabolizer	0.04051	0.01482	0.08157	0.12979	0.02388	0.01141	0.01858	0.57139	0.03671
Indeterminate	0.00224	0	0	0.00341	0	0	0	0	0

Table 1.3 adapted and reproduced with permission from "Supplement to: Clinical Pharmacogenetics Implementation Consortium Guidelines for *CYP2C19* Genotype and Clopidogrel Therapy: 2022 Update (January 2022)."

*In this table, the "American" biogeographical group refers to people of Indigenous ancestry, such as Native Americans and Indigenous people of Mexico. White Americans are included in the "European" group.

OPTIONAL READING: How Your Genes Influence What Medicines Are Right for You

If your teacher has assigned the optional reading "How Your Genes Influence What Medicines Are Right for You" by Julie A. Johnson (2015), you can access it at the link in the Student Resources document.

CHAPTER 2:
Is My Sense of Taste Controlled By My Genes?

INTRODUCTION

As you learned in your exploration of traits in Chapter 1, many of your features are at least partially controlled by your genes, including your eye color, hair color, and height. But what about things that you can't easily see, such as your blood type or the way you react to blood thinners or pain medication? How do your genes factor into these unseen traits?

Genes play a role in many aspects of your health. For example, scientists recognize that different medicines affect people in different ways, based in part on their genetics. To better meet individual needs, researchers are working to create precision medicine tailored to each patient's genetics and lifestyle. By exploring how genes influence our health, scientists hope to help doctors keep their patients healthy and create better treatment plans for those who are ill.

ACTIVITY: Rate the Bitterness of These Foods

To explore how genes affect hidden traits like how we react to medicines, you're going to examine a different "hidden" trait—our sense of taste. You probably like some foods and dislike others. Some of your friends might love olives while you despise them, or vice versa.

Have you ever thought about why? Are "picky eaters" simply unadventurous types who don't want to try new foods, or do they actually taste things differently from other people? You may love some foods that taste bitter—such as coffee, olives, or bitter melon—or you may absolutely hate them.

In this activity, you will explore one aspect of your sense of taste—your perception of bitter tastes—and consider how your genes might influence this trait. You will also make an inventory of how various foods taste to you.

MATERIALS

For each student:

- 1 copy of **My Bitterness Ratings (RM 2.1)**

PROCEDURE

As your teacher shows each slide depicting a food, use the table on **My Bitterness Ratings (RM 2.1)** to rate the bitterness of that food on a scale of 1 to 3, with 1 being "Very bitter" and 3 being "Not at all bitter." When rating each food, imagine you are consuming it raw and without any condiments. After you've rated the bitterness of each food, decide which taste word you would use to describe it: salty, sweet, sour, bitter, or umami (savory).

ACTIVITY: Can You Taste It?

In 1931 in a laboratory at the DuPont chemical company, chemist Arthur Fox accidentally spilled a powdered chemical called ***phenylthiocarbamide (PTC)***. As the powder swirled in the air, a nearby scientist complained that the dust tasted bitter. However, Fox didn't taste anything. Fox was curious as to why he couldn't taste the PTC and his colleague could, so he began to run tests on others. Fox asked his friends and family members to taste the chemical and tell him how it tasted. He found that some people didn't taste it at all, some had a mild sensation of bitterness, and others found the taste unbearably bitter.

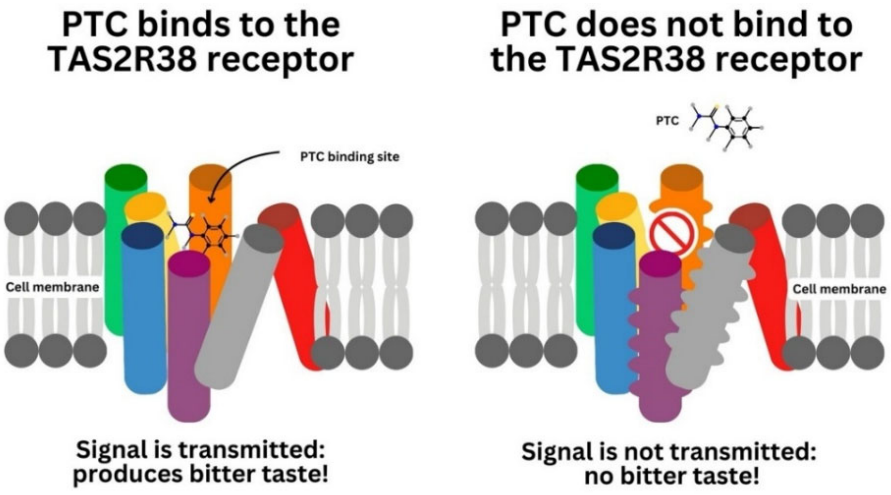
Bitter is only one of five basic tastes that humans recognize. The remaining four are:

- Salty
- Sweet
- Sour
- Umami

But how do you perceive these tastes? When food enters your mouth, the taste chemicals interact with receptors on your tongue. Each taste has its own type of receptor cell. Taste chemicals bind to the receptor cells on your tongue, and these cells send signals to your brain. Then your brain interprets the signal, and you taste the corresponding flavor. If the taste chemical doesn't bind to the receptor cell, you won't taste the chemical.

Today, scientists know that there is a connection between your genes and your ability to taste certain flavors. One of the taste genes—called Taste Receptor 2 Member 38 or TAS2R38—allows you to taste bitter foods. There are several versions of this gene; humans carry two variations (genotype), which influence their abilities to taste bitter compounds (phenotype). If you can taste bitter flavors, it's likely you can also taste PTC. If you are a bitter taster, PTC binds to the bitter-taste receptor on your tongue and sends a signal to your brain and YUCK! (See **Figure 2.1**.) While you will not know your genotype for certain until you complete the labs in Chapters 3–6, you will know your bitter-tasting phenotype after tasting PTC.

Figure 2.1: PTC tasting mechanism



Can you taste PTC? Let's find out!

MATERIALS

For each student:

- Control taste paper
- PTC taste paper
- 1 cup of water (this should be drinkable water—you will use it to rinse your mouth)
- 1 copy of **Can You Taste It? (RM 2.2)**

PROCEDURE

1. Place a piece of control taste paper on the tip of your tongue. **Don't swallow it!** Record its taste on **Can You Taste It? (RM 2.2).**
2. Remove the control paper from your tongue and dispose of it in the solid waste container. Rinse your mouth with water and spit into your team's liquid waste container.
3. Place a PTC taste paper on the tip of your tongue. If you don't taste anything at first, leave the paper on your tongue for a few more seconds. **Don't swallow it!** If you sense a bitter taste, then you are a PTC taster. Due to genetics, there is a gradient of PTC tasting ability:
    - If the paper tastes awful, you are a strong taster.
    - If you perceive only a slight bitter taste, you are a weak taster.
    - If it tastes like the control paper, you are a nontaster.
4. Remove the PTC taste paper from your mouth and dispose of it in the solid waste container. Rinse your mouth, if needed. Spit your rinse water into your team's liquid waste container.
5. Record if you are a strong taster, weak taster, or nontaster in the table on your reproducible master.
6. Check with your teammates and record their statuses in the table as well.
7. As your teacher calculates the class totals, record them in your table. Calculate the percentage of students with each phenotype.
8. Make a bar chart showing the number of students with each phenotype on **RM 2.2**.

REVIEW: Genetics Concepts

MATERIALS

For each student:

- 1 copy of **Genes, Traits, and Environment (RM 2.3)**

Earlier, you considered the connection between our genes and how we respond to medications. Do all medications work for everyone in exactly the same way? The answer is no. A number of factors can influence how a patient responds to medications, just as various factors affect whether a person enjoys bitter foods.

Let's review some important genetics concepts:

1. Watch *What Is a Gene?* from Stated Clearly, starting at the 0:05 timestamp, and then answer the questions on **Genes, Traits, and Environment (RM 2.3)**.
2. If your teacher has assigned it, read the article "Mamas, Don't Let Your Babies Grow Up to Be Broccoli-Haters" by Lisa Bramen (*Smithsonian Magazine*), then answer the questions on **RM 2.3**.

READING: Prepare for the Next Lab

In the upcoming lab, you will extract your own DNA from your cheek cells. Then you will use a technique called ***polymerase chain reaction (PCR)*** to make many copies of a very small portion of your DNA to use later in the module. To help you prepare for the lab, read about what you will be doing and answer some questions.

DNA EXTRACTION

In the first part of the lab, you'll collect some dead skin cells from the inside of your cheek, then boil them. This will cause the breakdown of their cellular and nuclear membranes in a process known as ***lysis***. Once these membranes are broken down, components that are normally in the cytoplasm of the cell will mix with the DNA. These components include enzymes called ***DNases***, which break down DNA. DNases require metallic ions called ***cofactors*** to assist in their activities. These metallic ions are also found inside cells. In the lab, you will combine your DNA with Chelex, which is a ***chelating*** (binding) agent often used for DNA extraction. The Chelex resin will bind to these metal cofactors so they are not near the DNases.

POLYMERASE CHAIN REACTION (PCR)

WHAT IS PCR?

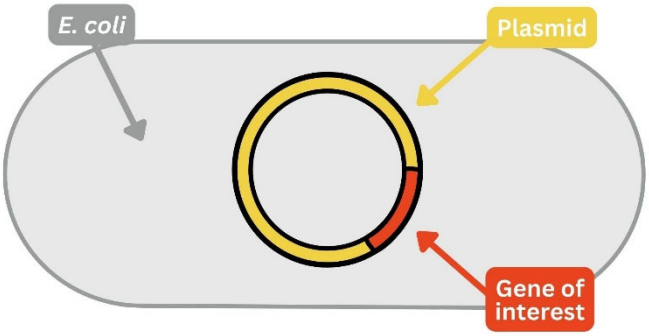
In the next part of the lab, you will use PCR to ***amplify***, or make many copies of, a specific region in your DNA. PCR is one of the most widely used techniques in biotechnology. Think of it as a molecular copy machine that can make multiple copies of genetic material (often several billion!).

PCR was first developed in 1983 by American biochemist Kary Mullis, who later received the Nobel Prize for his work. PCR has had a profound impact on biotechnology and is now used in many areas of research and applied biotechnology, including genetic engineering, forensics (the use of scientific tests or techniques in crime investigation), and medicine (such as in the laboratory tests used to detect COVID-19 infection).

Scientists are interested in making copies of specific genes—a process known as ***gene cloning.*** Two main methods for cloning are known as *in vivo* and *in vitro* cloning, each of which has different applications. Both methods use reagents isolated from living organisms to cut and copy DNA and/or to produce proteins, just as these living things do, but for different purposes.

Scientists clone genes ***in vivo*** (inside a living organism) by inserting a specific gene from one organism into another organism's DNA. This is often done using the gut bacterium *E. coli* by adding a gene to a small circular piece of DNA called a *plasmid.* ***Plasmids*** are circles of DNA found in bacteria and other microorganisms that are separate from chromosomal DNA and can replicate independently. This makes plasmids useful vectors for generating numerous copies of DNA encoding a specific gene. The process of introducing a plasmid to bacteria is called ***transformation.***

Figure 2.2: Bacterial transformation to produce protein from a gene of interest



To transform bacteria, scientists add a plasmid with a gene of interest to numerous identical bacterial cells. Then, each transformed bacterium begins replicating that gene of interest (**Figure 2.2**). As each transformed bacterium reproduces, its plasmid does as well, resulting in large colonies of bacteria, each containing the added plasmid. The added gene in each engineered bacterium is transcribed and translated to produce the encoded protein. Such proteins are ***gene products***. One gene product produced *in vivo* through this process is the human therapeutic protein, insulin.

While gene cloning *in vivo* produces a protein, gene cloning can also be carried out ***in vitro*** (outside of a living organism—for example, in a test tube) by using PCR. *In vitro* gene cloning uses the same enzymes and starting materials as living cells but produces many copies of a DNA fragment and no gene product. These fragments are just pieces of genetic material; they will not produce a protein unless they are introduced into a living cell.

NOTE: *In vitro* literally means "in glass," referring to the laboratory equipment originally used to carry out the necessary chemical reactions. Nowadays, much of *in vitro* research occurs in plastic labware, rather than glass test tubes!

PCR copies a specific region of DNA from a sample, then rapidly produces billions of copies of that specific region of DNA. Scientists call PCR "DNA amplification" because it makes many copies of a

small amount of DNA (almost like amplifying a quiet sound so that it can be heard). Those copies will vastly outnumber any other DNA fragments in a sample, making the sequence far easier to detect and analyze. Prior to the development of PCR, the only way to make multiple copies of a specific sequence of DNA was through biological amplification in bacteria, which was very expensive and took a long time. PCR is much less expensive and can be done very quickly in comparison.

PCR uses an enzyme—***DNA polymerase***—to replicate DNA. This is the same enzyme all organisms, whether unicellular or multicellular, use to assemble nucleotides into new strands of DNA prior to cell division. This process is known as ***DNA replication.***

Because most living organisms survive at temperatures between 25°C ("room temperature") and 37°C ("body temperature"), most enzymes are stable and active at these temperatures. To replicate DNA *in vitro*, first scientists must separate its two strands to expose the bases they want to copy. They do this by heating it, which creates a conundrum because heating DNA to separate it also destroys the enzymes necessary to replicate it. This fact made early PCR a long and laborious process, since researchers had to add fresh enzymes to the reaction after every heating cycle so the DNA could replicate.

However, in the late 1960s, microbiologists discovered new microorganisms called ***thermophiles*** (heat-loving). Thermophiles live at much higher temperatures than other organisms, ranging from 55°C to a sizzling 121°C (much higher than the temperature of boiling water!). Strains of these thermophiles can be found anywhere warm, from compost piles to thermal vents in the ocean floor to the boiling hot springs in Yellowstone National Park.

Taq polymerase, a type of DNA polymerase, was discovered in the thermophile bacterium ***T****hermus* ***aq****uaticus*, which uses it to replicate its own DNA at the high temperatures of its environment. *Taq* polymerase, therefore, can function at the higher temperatures required for PCR. Without it, modern PCR would not be possible.

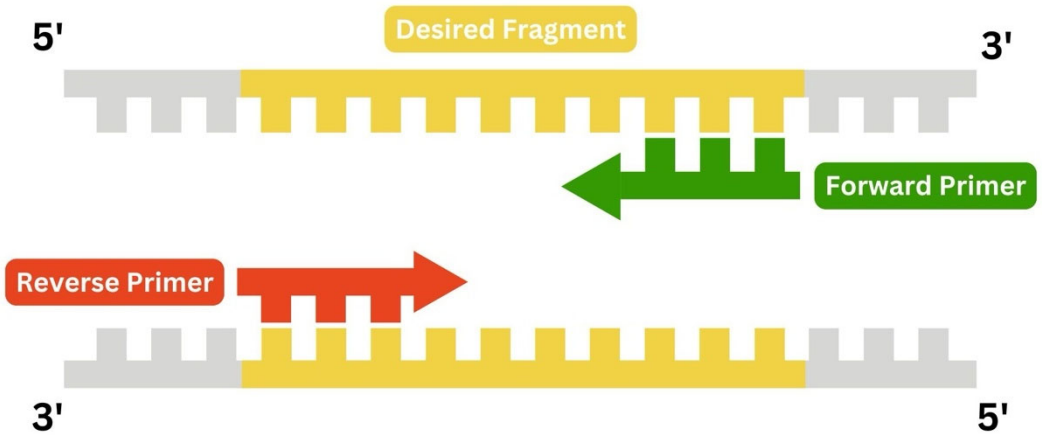
HOW DOES PCR WORK?

Like other biotechnology methods, PCR is based on basic scientific discoveries, in this case, DNA replication. PCR involves multiple rounds of DNA replication, resulting in the production of over 1 billion copies of a specific segment of DNA.

There are five ingredients required to set up a polymerase chain reaction:

1. ***Template DNA***—The pool of genomic DNA from which you will make copies
2. Forward and reverse primers—Short stretches of DNA designed to match the beginning and end of the section of genomic DNA that you want to copy (see **Figure 2.3**)
3. DNA nucleotide bases (dNTPs)—Loose "building bricks" used to build the new copies of DNA
4. *Taq* polymerase enzyme—A catalyst that aids in building the new DNA strands
5. A buffer—A chemical solution that creates optimal conditions for the reaction

Figure 2.3: Template DNA between the forward and reverse binding sites will be amplified by PCR



There are three phases of PCR:

1. **Denaturation Phase**

   At high temperatures, the hydrogen bonds between the bases in the two strands of DNA break, allowing the strands to separate, or ***denature*** (also known as melting). The melting temperature of DNA depends on its physical properties, but is generally above 70°C. In PCR, the mixture is heated to 94–95°C sufficiently to ensure that the DNA strands have separated completely.

2. **Annealing Phase**

   In this phase, the mixture is cooled, allowing the primers to ***anneal*** (attach) to the denatured single-stranded DNA. The annealing temperature is calculated based on the melting temperature of the primers being used in the PCR.

3. **Extension Phase**

   In this phase, the temperature is raised. *Taq* polymerase replicates the region of interest by adding dNTPs to the 3' end of the primers.

Did you know?

Commonly used formulas for approximating the melting temperature of primers are:

For sequences < 14 nucleotides:

Tm= (wA+xT)*2 + (yG+zC)*4

where w, x, y, z are the number of the bases A, T, G, C in the sequence, respectively.

For sequences > 13 nucleotides:

Tm= 64.9 +41*(yG+zC-16.4)/(wA+xT+yG+zC)

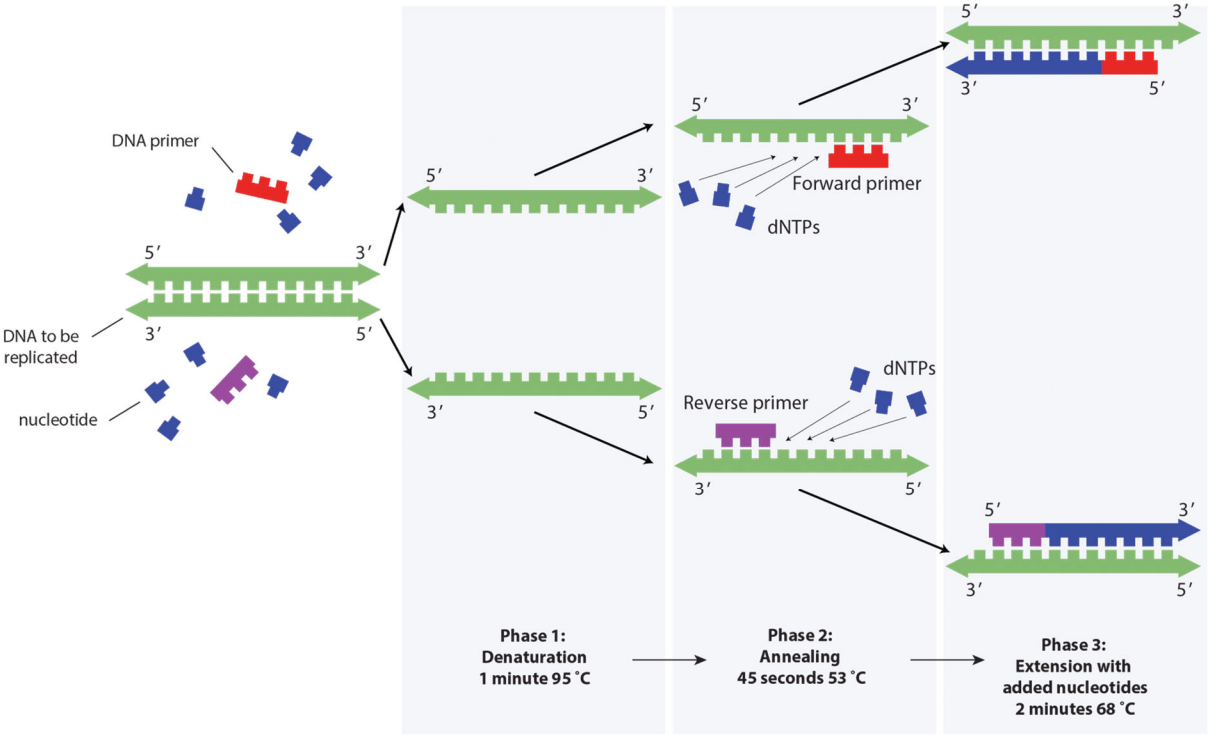
More complex algorithms exist for even more accurate melting point approximations.

These three phases complete one PCR cycle, and this cycle is then repeated numerous times to achieve amplification.

PCR is carried out in an instrument called a ***thermocycler***, which controls the temperature and length of time for each phase of the reaction. During each cycle, the number of copies of DNA from

the region of interest doubles. In the example that follows, one cycle would take approximately 4 minutes (see **Figure 2.4**). This cycle is then repeated to make more copies. A reaction that runs for 30 cycles can result in more than 1 billion copies.

Figure 2.4: A complete cycle of PCR



The success of the PCR is then determined by using ***gel electrophoresis*** to analyze the PCR products. (Gel electrophoresis is a laboratory technique for separating nucleic acids or proteins based on their relative size by applying an electric current that pulls materials through a gel in a buffer solution.) By comparing the PCR products to standard-sized pieces of DNA in a DNA ladder, it is possible to determine whether the reaction has been successful. For example, if a set of PCR primers were designed to bracket a single fragment of 1800 base pairs (bp) for copying, we would expect to see a single distinct band in the gel that is next to the location in the ladder that corresponds to 1800 bp.

Demonstrate your knowledge of PCR by completing **How PCR Works (RM 2.4)**.

CHAPTER 3:
Exploring Our DNA

INTRODUCTION

In this chapter, you will perform a process that medical laboratories use to collect, isolate, amplify, and examine a patient's DNA to determine which medication might be most effective for them.

ACTIVITY: Using Cheek Cells to Collect DNA

Your task is to extract genomic DNA from the nuclei of your cheek cells. The procedure described below and alternatively shown in a flowchart (**Figure 3.1**) will allow you to lyse (break open; related to the term *lysis* introduced in Chapter 2) the cheek cells, remove other cellular components, and isolate just the DNA from the cells.

MATERIALS (See **Figure 3.1** for illustration)

For each student:

- 1 pair of gloves
- 1 flat toothpick
- 100 µL of Chelex beads in a 0.2-mL microfuge tube
- 1 clean 0.2-mL microfuge tube

For each team:

- 1 P-20 micropipette and tips
- 1 fine-point permanent marker
- An ice bucket or a cup of crushed ice
- Microfuge tube rack

For the class:

- Microcentrifuge
- PCR machine (thermocycler) or heat block
- Waste container(s)
- Device with internet access and either USB or Bluetooth, such as a laptop computer or smartphone, with thermocycler software installed

PROCEDURE (see **Figure 3.2** for flowchart)

Step	Notes
1. Put on gloves.	
2. Turn on your thermocycler or heat block and set it to 99°C.	*This step can be done either as a class, using one large thermocycler, or with individual machines.*
3. Label the tube of Chelex beads with your initials.	*Record how you labeled your tube in your science notebook.*
4. Using a flat toothpick, gently swab the inside of your mouth for at least 30 seconds.	*Carefully scrape the inside of both cheeks. You do NOT need to apply a lot of pressure.*
5. Swirl the toothpick in the tube of the Chelex beads for at least 30 seconds to dislodge the cells.	*Ensure the toothpick touches the beads inside the tube. Swirling thoroughly will help dislodge as many cheek cells as possible.*
6. Dispose of the toothpick in the waste container.	
7. Incubate the Chelex bead tube containing cheek cells at 99°C for 10 minutes using the preheated thermocycler or the heat block you turned on in Step 2.	*Follow the steps in Programming the Thermocycler while samples are incubating at 99°C for 10 minutes.*
8. Spin the tube in the microcentrifuge at a minimum of 4,800 x *g* for 1 minute to pellet the cell debris and the Chelex beads.	*Insert the 0.2-mL tube into an adapter or an empty 0.5-mL tube so that it fits properly into the microcentrifuge.* *Make sure that you balance the microcentrifuge with other students' Chelex bead tubes or a balance tube of the same mass.*
9. Label a clean 0.2-mL microfuge tube with your initials and the letter "T."	*"T" stands for "template." This tube is the template tube, which will be used in the PCR.*
10. Set your P-20 pipette to 20 µL and add a clean tip.	

Step	Notes
11. Carefully transfer 20 µL of the supernatant (liquid above the beads) from the Chelex bead tube to your template tube ("T").	*Be very careful when removing the Chelex bead tube from the centrifuge and moving the tube to your station to not disrupt the Chelex bead bed.* ***DO NOT*** *transfer any Chelex beads to this new tube as it will interfere with the PCR and further analysis.*
12. Discard the used tip and tube in a designated waste container for proper disposal.	***Important:*** *Dispose of waste according to your teacher's instructions as the solution contains human body fluids and/or tissues.*
13. Optional stopping point: Store your template tube at –20°C to be used in the next lab.	

Figure 3.1: Materials for cheek cell DNA extraction

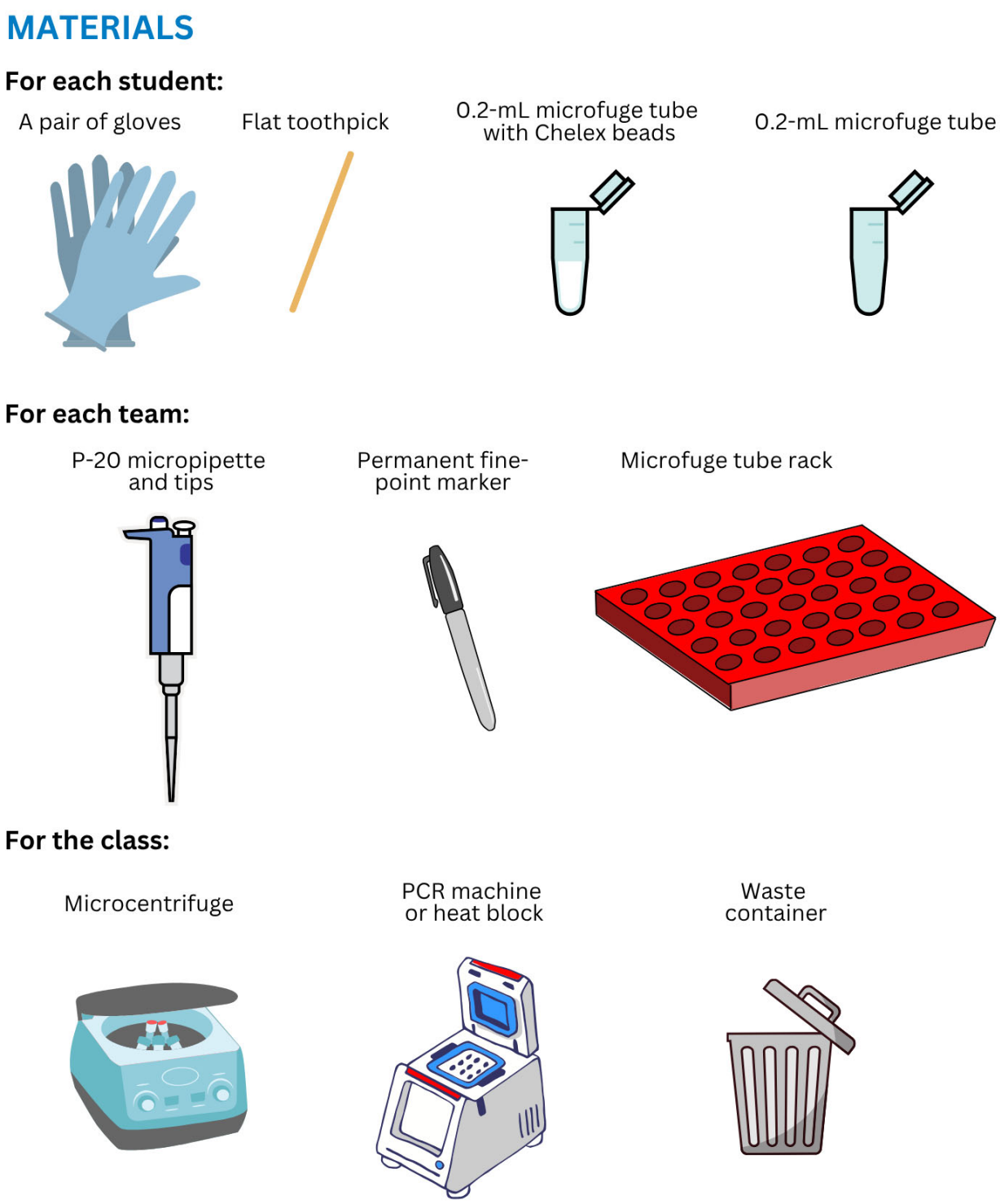
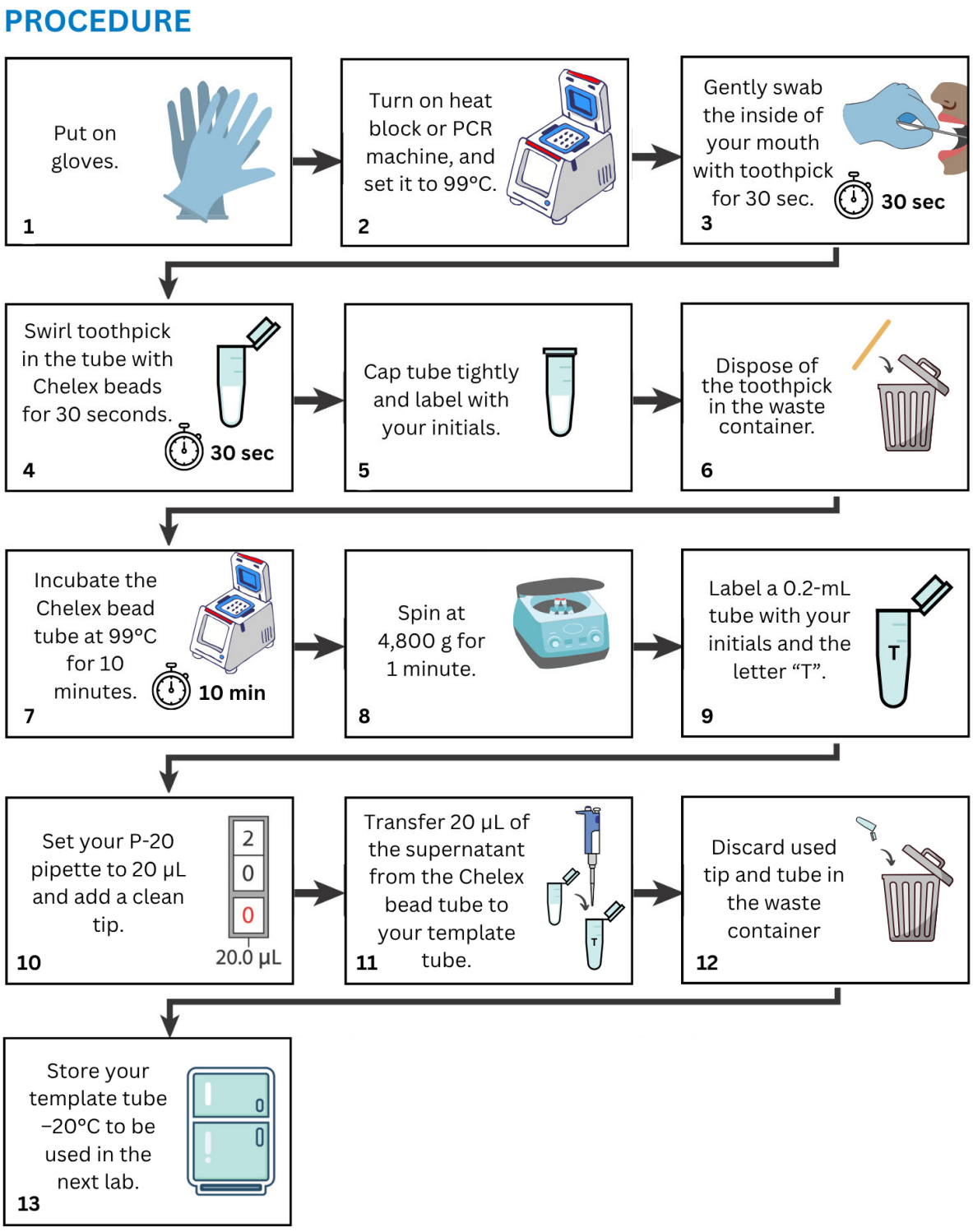


Figure 3.2: Flowchart for cheek cell DNA extraction



ACTIVITY: Programming the Thermocycler

Your next task is to amplify a specific section of your DNA using PCR. The process of PCR is controlled precisely by software. With your team, you'll create a program for your thermocycler that will use PCR to copy the desired DNA sequence.

MATERIALS

- Device with internet access and either USB or Bluetooth, such as a laptop computer or smartphone
- Software that controls your thermocycler

PROCEDURE

1. Using the software that controls your thermocycler, name the program that you are creating "Copy That DNA."
2. Set up the thermocycler program as shown in **Table 3.1**.

Table 3.1: "Copy That DNA" program for the thermocycler

Step	Number of cycles	Temperature	Time (seconds)
Initial Denaturation	1	94°C	300
Denaturation	35	94°C	30
Annealing		64°C	30
Extension		72°C	30
Final Extension	1	72°C	60

LABORATORY: Copy That DNA!

In this lab, you will use PCR to amplify (copy) a small fragment of DNA from your cheek cells. The area you wish to copy is called the ***template DNA***. In your body, every ***somatic cell*** (non-reproductive cell) that has a nucleus contains your full genome, but we're only interested in the gene associated with the ability to taste PTC. This gene is called TAS2R38, and it is located on chromosome 7. Rather than amplify your entire genome, which has over 3 billion base pairs, you will amplify only this small portion of your DNA—fewer than 300 base pairs. When you complete the lab, your sample will contain over a million copies of that small fragment of DNA!

In a medical context, a patient's DNA would be amplified in a very similar way to ensure that there is sufficient DNA for testing and sequencing. Technicians might amplify a patient's entire genome or only a small portion of particular interest. The patient's DNA sample would then be analyzed for the presence or absence of a specific allele (one of two or more alternative forms of a gene) or changes (mutations) to the DNA in question.

MATERIALS (see **Figure 3.3** for illustration**)**

For each student:

- 1 pair of gloves
- 1 0.2-mL template microfuge tube labeled "T"
- 1 0.2-mL microfuge tube containing 12.5 µL One*Taq* Hot Start 2X Master Mix (MM), labeled "MM" on the side of the tube (must be kept on ice)
- Access to an ice bucket or a cup of crushed ice

For each pair of students:

- 1 0.2-mL microfuge tube containing 11 µL TAS2R38 forward primer, labeled "FWD" (must be kept on ice)
- 1 0.2-mL microfuge tube containing 11 µL TAS2R38 reverse primer, labeled "REV" (must be kept on ice)

For each team:

- 1 fine-point permanent marker
- 1 P-20 micropipette and tips
- Access to an ice bucket or a cup of crushed ice

For the class:

- Microcentrifuge
- Thermocycler
- Laptop or desktop computer with internet access (if necessary)
- 1 0.2-mL microfuge tube containing 3 µL PCR positive control plasmid ("C+")
- 1 0.2-mL microfuge tube containing 3 µL ddH_2O ("C–")

- 1 0.2-mL microfuge tube containing 12.5 µL One*Taq* Hot Start 2X MM, labeled "MM" on the side of the tube (must be kept on ice)
- 1 0.2-mL microfuge tube containing 11 µL TAS2R38 forward primer, labeled "FWD" (must be kept on ice)
- 1 0.2-mL microfuge tube containing 11 µL TAS2R38 Reverse primer, labeled "REV" (must be kept on ice)

Note: Your teacher will designate one gel per class to run the negative control PCR. Depending on your class size and the number of gels your teacher has prepared, your teacher may opt to run several additional positive control PCRs so that each gel can have a positive control lane.

PROCEDURE (see **Figure 3.4** for flowchart)

Step	Notes
1. Put on gloves.	
2. Label the top of the MM tube with your initials or assigned number with a fine-point permanent marker.	*Master mix ("MM") contains Taq polymerase, cofactors, dNTPs and buffer.*
3. While keeping the MM tube on ice, add 5 µL of the forward primer into the MM tube. Using a new tip, add 5 µL of the reverse primer into the MM tube. Gently pipette up and down 3X to mix the solutions.	*It is OK if there is extra primer left in either tube after you and a partner have removed a total of 10 µL.*
4. Carefully transfer 2.5 µL from your template tube (T) into your labeled MM tube. Gently pipette up and down 3X to mix the solutions.	*Now this is your reaction tube, as it has the five components needed (listed earlier) for a PCR in addition to template DNA.*
5. Ensure that the tube is capped tightly.	*Store your sample on ice until you are ready to begin the reaction.*
6. Centrifuge the tubes for 5–10 seconds to bring the PCR solution to the bottom of the tubes. Place the tubes on ice until all samples are ready.	**NOTE:** *Your teacher has the materials for positive and negative control PCRs. They will either set these up or instruct you to do so.*
7. Add all sample tubes to the thermocycler(s). Run the "Copy That DNA" program.	*You created this program when you programmed your thermocycler (see Programming the Thermocycler).*

Step	Notes
 **Stop here! Your teacher will carry out the next step of the procedure.**	
8. Once the "Copy That DNA" program is finished, open the lid of the thermocycler and leave it for 15–30 seconds to cool before removing the tubes from the thermocycler and storing them at 4°C. (For longer storage, your teacher will store your samples at –20°C). **NOTE:** It would be OK to leave the tubes in the thermocycler at room temperature overnight if the PCR will not be complete before the end of the day.	*The thermocycler interior and/or tubes might be hot, so make sure to let them cool slightly before removing.*

Figure 3.3: Materials for PCR

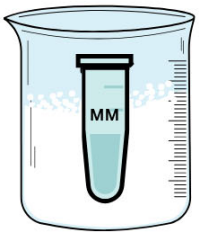
MATERIALS

For each student:

A pair of gloves

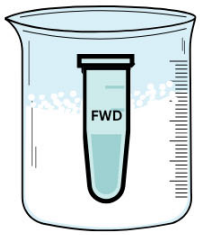
0.2-mL template microfuge tube ("T")

0.2-mL microfuge tube containing 12.5 µL PCR master mix ("MM") (must be kept on ice)



For each pair of students:

1 0.2-mL microfuge tube containing 11 µL TAS2R38 forward primer ("FWD"). (must be kept on ice)

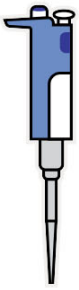


1 0.2-mL microfuge tube containing 11 µL TAS2R38 reverse primer ("REV") (must be kept on ice)

For each team:

Permanent fine-point marker

P-20 micropipette and tips



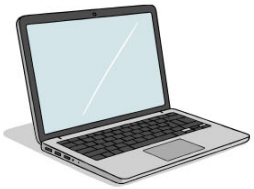
Cup of crushed ice

For the class:

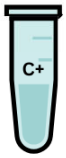
Microcentrifuge

Thermocycler

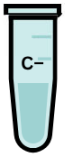
Computer or laptop (if necessary)



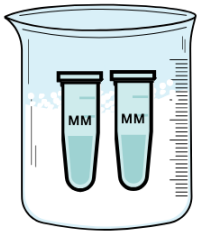
For every additional classroom gels, 1 0.2-mL microfuge tube containing 3 µL C+ control template DNA (add extras as needed)



0.2-mL microfuge tube containing 3 µL ddH_2O



2 0.2-mL microfuge tubes containing 12.5 µL PCR master mix (“MM”) (must be kept on ice)



2 0.2-mL microfuge tubes, one containing 11 µL TAS2R38 forward primer ("FWD") and one containing 11 µL TAS2R38 reverse primer (“REV”) (must be kept on ice)

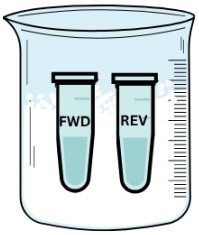
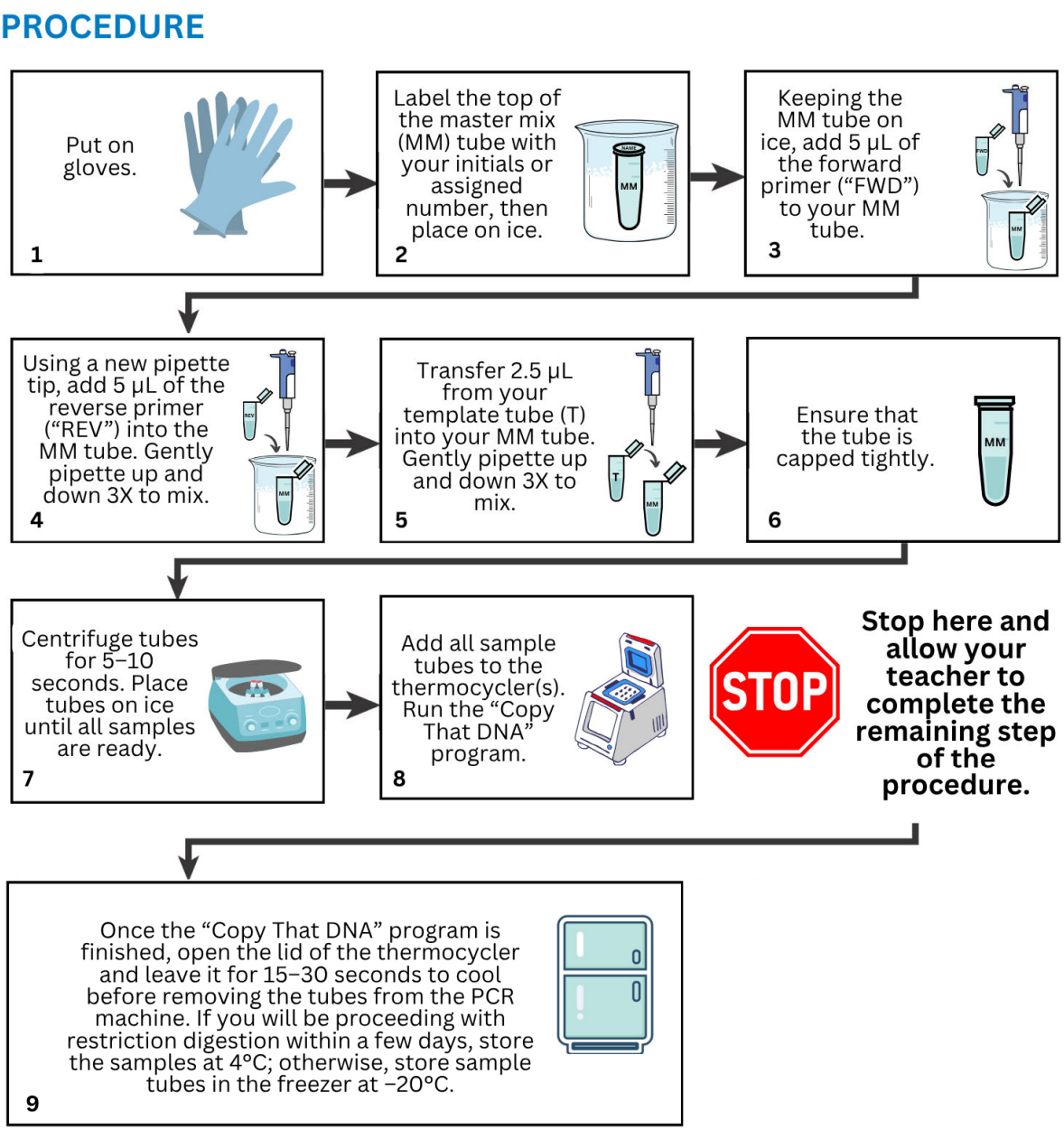


Figure 3.4: Flowchart for PCR



READING: How Is DNA Sequenced?

The human genome contains approximately 3 billion base pairs in our 23 pairs of chromosomes! How do we know this? Technology has allowed scientists to use ***sequencing*** (the process of determining the order of nucleotides in a DNA sample) to decode DNA from any organism. Although there are now multiple technologies used to sequence DNA, one of the early methods was ***Sanger sequencing*** (named after Frederick Sanger, who developed the method with his colleagues in 1977). The ***Human Genome Project*** (1990–2003)—a large, collaborative, international project that generated the first full sequence of the human genome—used Sanger sequencing.

SANGER SEQUENCING

Decoding DNA is essential because DNA serves as a blueprint for making RNAs, including mRNA, which our cellular machinery translates into the proteins we need. Since 2003, when the results of the Human Genome Project were published, DNA sequencing technology has become much faster, cheaper, and more readily accessible. However, scientists are still working to understand precisely which regions of the human genome do what and under what circumstances. By analyzing and comparing thousands of genomes with powerful software, researchers will continue to unlock more of DNA's secrets in the years to come.

Sanger sequencing permits the rapid identification of the order of nucleotides in a segment of DNA. Sanger sequencing consists of three major phases: chain-termination PCR, size separation by gel electrophoresis or capillary electrophoresis, and laser excitation and detection.

CHAIN TERMINATION PCR

Sanger sequencing begins by harnessing the power of PCR. In a standard polymerase chain reaction, DNA polymerase adds nucleotides in an order determined by a template DNA strand. Sanger sequencing introduces specially modified nucleotides into the reaction at random, in addition to normal nucleotides. These modified nucleotides have two special properties: they lack an oxygen group, and they are also able to fluoresce.

When one of these fluorescent modified nucleotides is added to a strand of template DNA, the chemical difference jams the polymerase and stops it from replicating the remainder of the strand, much like a stuck zipper. The fragment now has a glowing nucleotide at its ***terminus*** (end), which can be detected by a laser, then saved to analytic software. The process repeats, creating numerous fragments of varying lengths, each ending in a fluorescent nucleotide.

SIZE SEPARATION BY GEL ELECTROPHORESIS OR CAPILLARY ELECTROPHORESIS

In the next phase of Sanger sequencing, the new fragments are sorted by size using an electric field (electrophoresis) to help determine the order of the nucleotides. In the past, this was done by hand, using an agarose gel in traditional gel electrophoresis. Nowadays, automated sequencing machines typically use ***capillary electrophoresis sequencing***, in which nucleic acid fragments are loaded into a polymer-filled capillary tube to which an electric field is applied for size separation.

In both gel electrophoresis and capillary electrophoresis sequencing, an electrical charge is run through a polymer to separate DNA fragments. The smallest, most streamlined pieces travel the

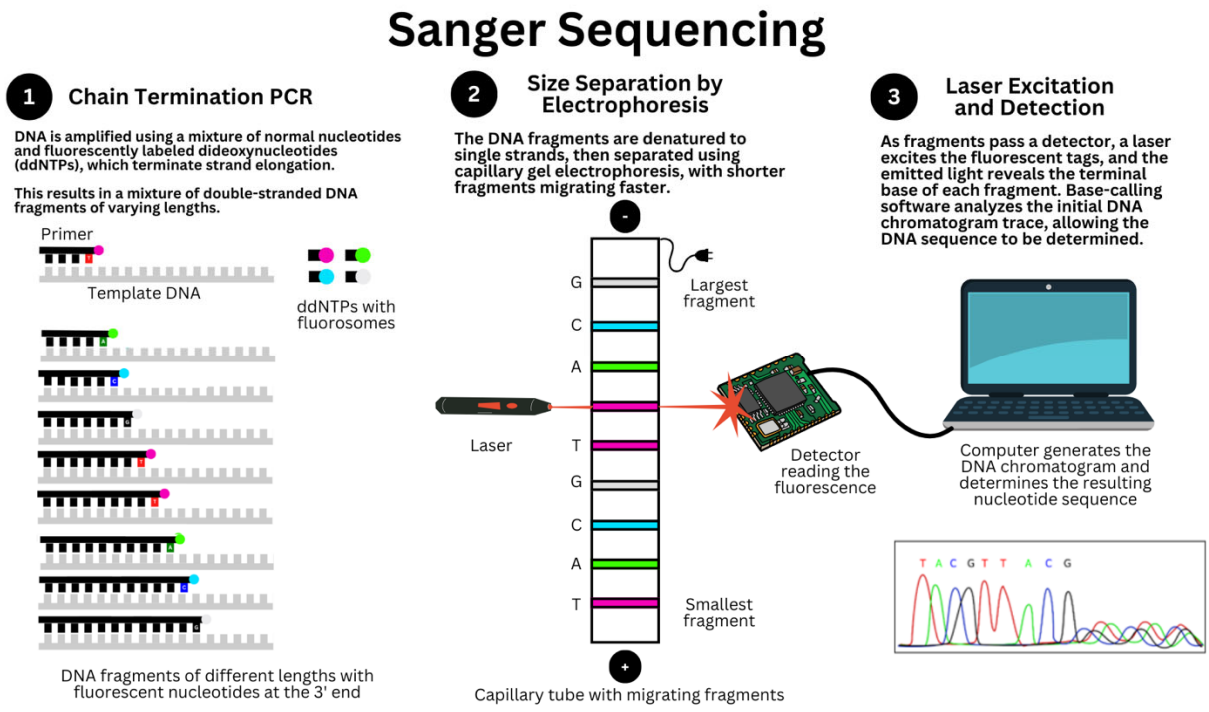
farthest through the polymer, whereas the largest, bulkiest pieces travel the shortest distance. Because DNA polymerase only synthesizes in the 5’ to 3’ direction, the smallest fragment corresponds with the first nucleotide at the 5’ end of the target strand. The next smallest fragment ends with the second nucleotide from the 5’ end, the third smallest with the third nucleotide, and so on.

LASER EXCITATION AND DETECTION

In the final phase, the gel results are analyzed within an automated sequencing machine. In the past, scientists read sequencing gels by eye, which was laborious and error prone.

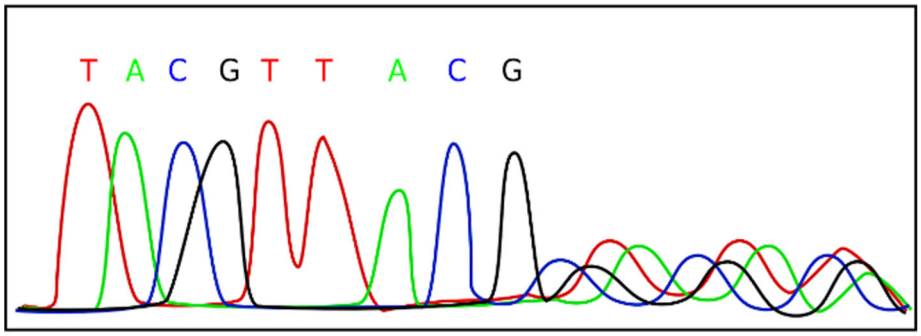
In automated machines, a laser excites the fluorescent nucleotides at the end of each fragment as they flow through the capillary. Each nucleotide—A, T, C, and G—emits a unique type of light, which a camera detects. The resulting fluorescence data are sent to automated ***base-calling software***, which compiles the information about the nucleotides at the end of each fragment, sorts the fragments into the correct order, and generates an output called a ***DNA chromatogram*** (see Figure 3.5).

Figure 3.5: The phases of Sanger sequencing by capillary electrophoresis



A DNA chromatogram (see Figure 3.6) displays the nucleotides at each position and indicates the level of fluorescence emitted by each nucleotide in the sequence. Higher peaks (on the left in the image) indicate stronger signals, whereas lower peaks (on the right) indicate weaker signals and less certainty.

Figure 3.6: A DNA chromatogram



CHAPTER 4:
How Is DNA Sequenced, and What Can We Learn?

INTRODUCTION

In this chapter, you will use bioinformatics software to analyze the nucleotide and amino acid sequences of alleles of TAS2R38 to determine how they differ. You will also explore how these genotypes relate to PTC taster phenotypes and discuss how small the differences are between the DNA sequences. Next, you will learn how to "read" the files created when DNA is sequenced. Lastly, you will learn about restriction enzymes in preparation for the activities in Chapter 5.

ACTIVITY: Finding TAS2R38 Differences

You are going to compare the DNA sequences of alleles of TAS2R38 to see how they differ and how those differences might relate to their phenotypes. Specifically, you will use DNA alignment software to compare the sequences of strong tasters and nontasters. How do the gene sequences of people with these PTC tasting phenotypes differ from one another?

NOTE: When you amplified your DNA in Chapter 3, you created many copies of a small fragment of the TAS2R38 gene. In this activity, however, you will be exploring the complete 1002-bp TAS2R38 gene.

MATERIALS

- Laptop or desktop computer with internet access
- 1 copy of **Finding TAS2R38 Differences (RM 4.1)**
- (Optional) Amino acid translation table (see **Table A.1** in the Appendix of this guide)

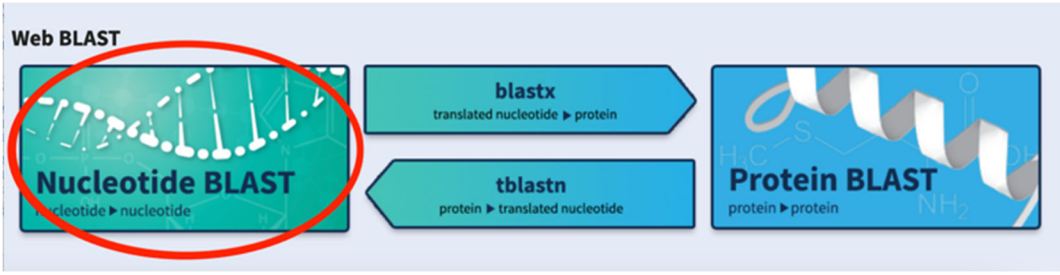
PROCEDURE

Before you begin, familiarize yourself with NCBI BLAST by watching the tutorial (4:03). Alternatively, read the Alignments Tab of the Web BLAST Quick Start Guide on the NCBI website.

Create an alignment between two nucleotide sequences: PTC taster and nontaster

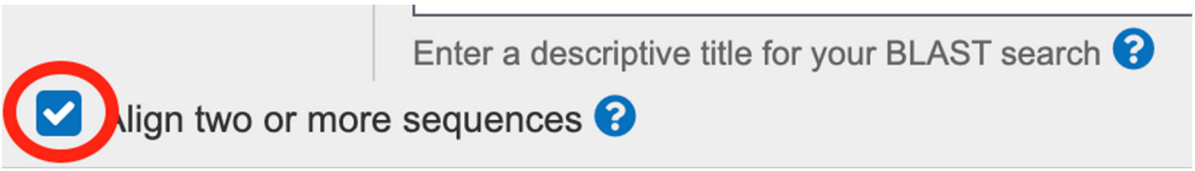
1. Navigate to the NCBI BLAST homepage.
2. Under the header "Web BLAST," click on the "Nucleotide BLAST" box (see **Figure 4.1**).

Figure 4.1: Nucleotide BLAST



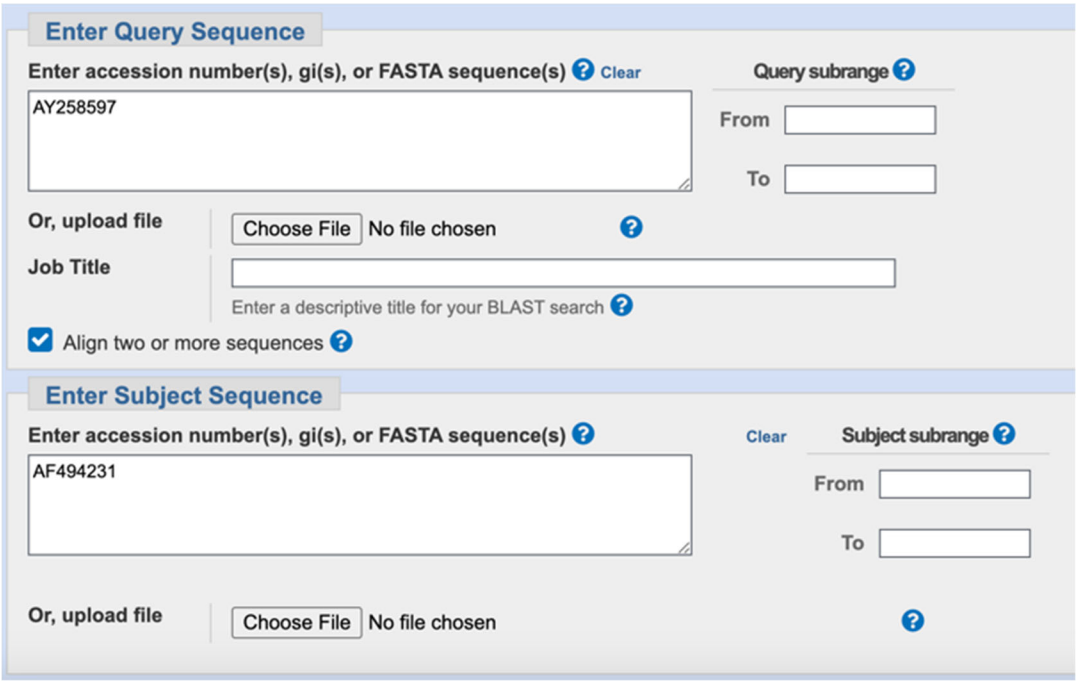
3. GenBank is a repository of nucleic acid and amino acid sequence files. You will be creating a DNA sequence alignment between two GenBank records: the nucleotide sequences of a PTC taster allele (AY258597) and a PTC nontaster allele (AF494231) of the TAS2R38 gene. Under "Enter Query Sequence," check the "Align 2 or more sequences" box (see **Figure 4.2**).

Figure 4.2: Align two or more sequences



4. Now, you should see two boxes, one called "Enter Query Sequence" and one called "Enter Subject Sequence" (see **Figure 4.3**). Enter the taster sequence accession number (AY258597) into the Enter Query Sequence box and the nontaster sequence accession number (AF494231) into the Enter Subject Sequence box.

Figure 4.3: Enter accession numbers for the query and subject DNA sequence

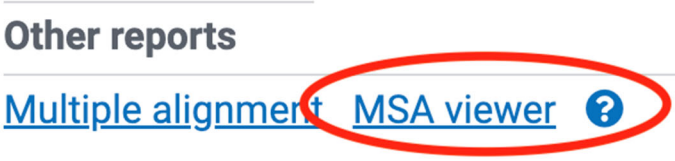


5. At the bottom of the page, select the "Show results in a new window" box and then click on the "BLAST" button (see **Figure 4.4**).

Figure 4.4: Show results and BLAST

6. An alignment analysis will open on a new page. Click "MSA viewer" next to "Other reports" (see **Figure 4.5**). A graphical representation of your nucleotide sequence alignment will open in a new window.

Figure 4.5: MSA viewer

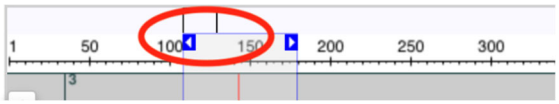


Analyze nucleotide differences between phenotypes

7. You should see three rows, labeled "consensus," "AY258597" (the taster sequence), and "AF494231" (the nontaster sequence). Above them you will notice a horizontal gray bar with several vertical red lines. These lines are ***SNVs*** (***single nucleotide variants)***, or differences in a gene's DNA sequence found within a population). Locate and count the red lines. How many SNVs do you see?

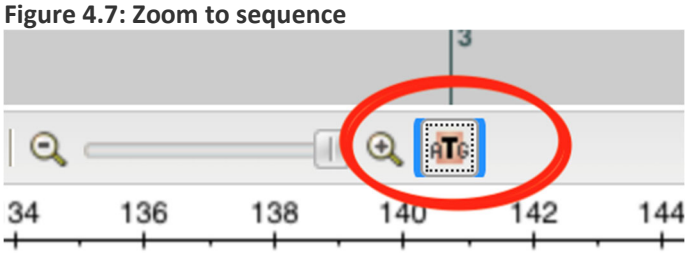
8. Move the uppermost rectangular slider until it is centered on the first single nucleotide variant (see **Figure 4.6**).

Figure 4.6: Move slider



9. Click the "Zoom to sequence" button (see **Figure 4.7**) to visualize both the consensus nucleotide sequence and the sequence of the taster and nontaster alleles at each SNV**.** You will notice that most of the taster and nontaster sequences are represented by dots, indicating that they are identical to the consensus sequence. Only the SNVs differ.

Figure 4.7: Zoom to sequence



10. What is the nucleotide position of the first SNV you find? Record the number as well as the difference in nucleotide sequences between the taster and nontaster alleles in the **Nucleotide Sequence Differences** table on **Finding TAS2R38 Differences (RM 4.1)**.

11. Move the slider to the next SNV and record its position and DNA sequence in **RM 4.1**. Continue with all the SNVs.

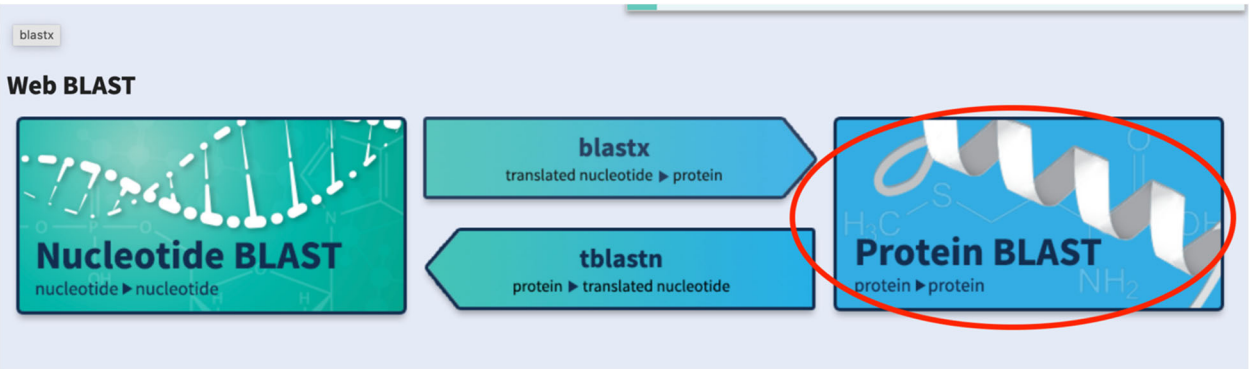
Analyze amino acid differences between phenotypes

You have identified SNVs in the taster and nontaster alleles. Now you will explore whether any of these nucleotide changes result in differences in the amino acid sequence of either allele. To do this, you will create another alignment in BLAST—this time aligning the amino acid translation of each of the same alleles.

12. Navigate to the NCBI BLAST homepage once more.

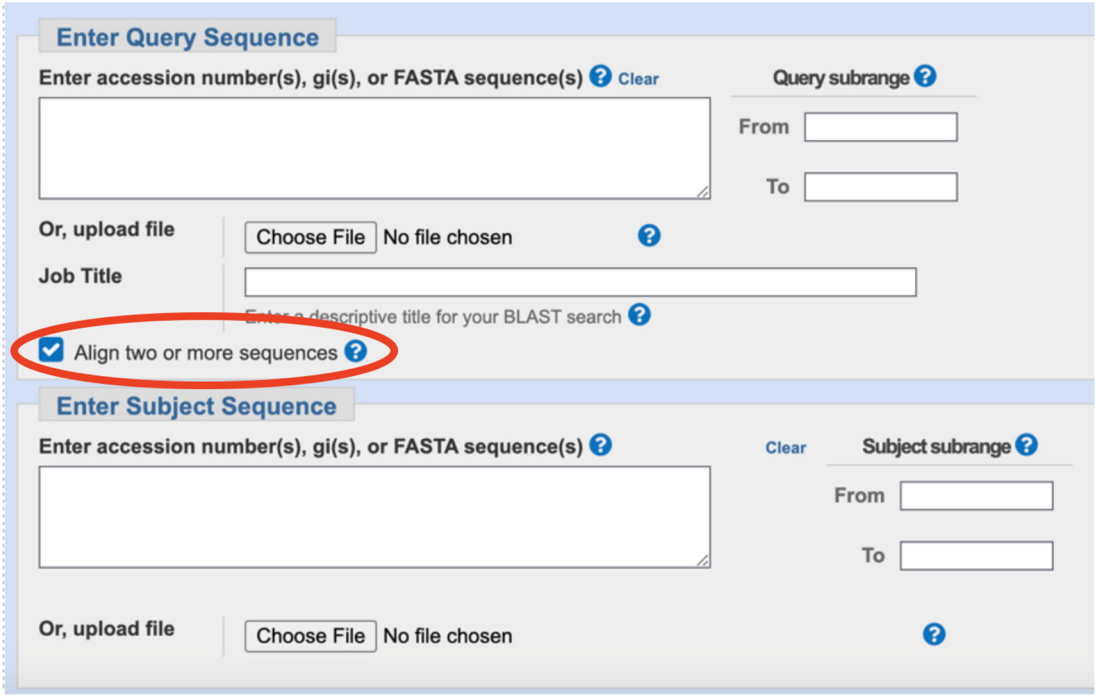
13. Under the header “Web BLAST,” click on the “Protein BLAST” box (**Figure 4.8**).

Figure 4.8: Protein BLAST



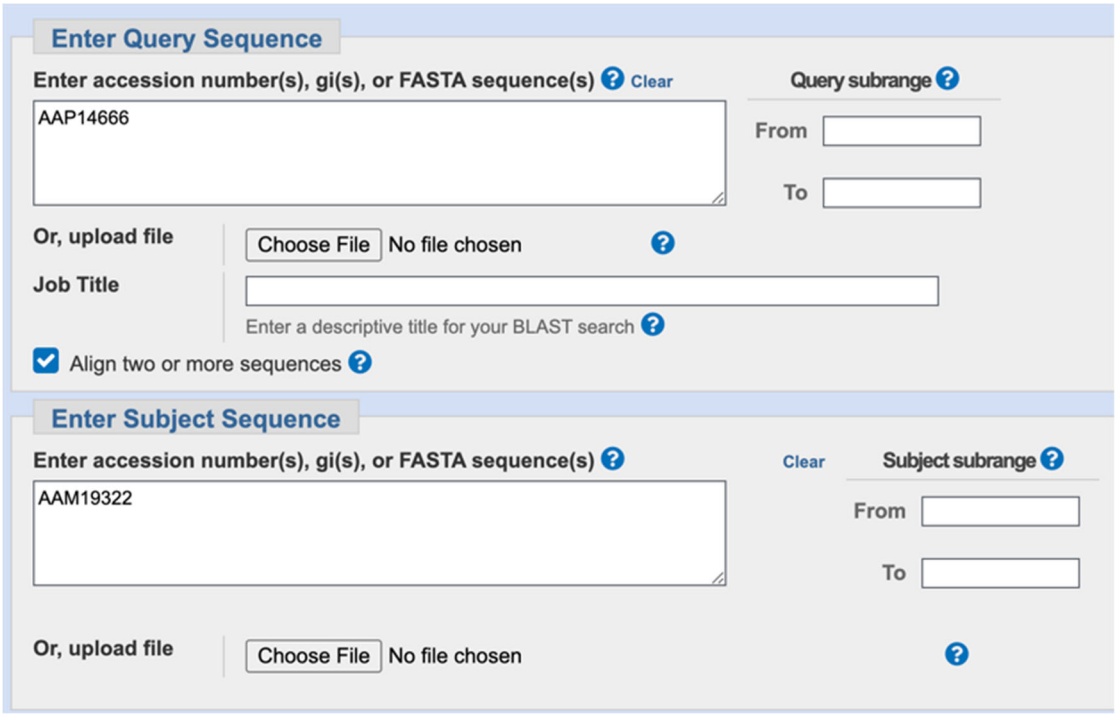
14. You will be creating a sequence alignment between two protein files from GenBank: the amino acid sequences of a PTC taster allele (AAP14666) and a PTC nontaster allele (AAM19322) of the TAS2R38 gene. Under “Enter Query Sequence,” check the “Align two or more sequences” box (see **Figure 4.9**).

Figure 4.9: Align two or more sequences



15. Now, you should see two boxes, one called “Enter Query Sequence,” and one called “Enter Subject Sequence” (see **Figure 4.10**). Enter the taster amino acid sequence accession number (AAP14666) into the Enter Query Sequence box and the nontaster amino acid sequence accession number (AAM19322) into the Enter Subject Sequence box.

Figure 4.10: Enter accession numbers for the query and subject amino acid sequences

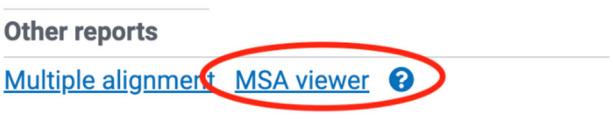


16. Select the "Show results in a new window" box and then click on the "BLAST" button at the bottom of the page (**Figure 4.11**).

Figure 4.11: Show results in a new window and BLAST

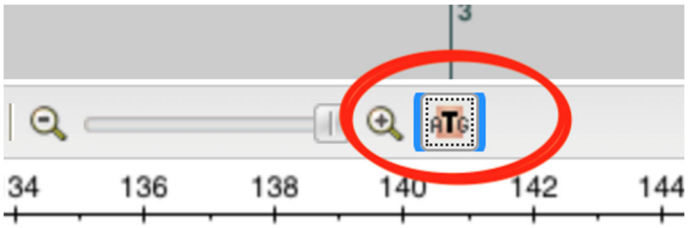
17. An alignment analysis will open on a new page. Click "MSA viewer" next to "Other reports" (see **Figure 4.12**). A graphical representation of your amino acid sequence alignment will open in a new window.

Figure 4.12: MSA viewer



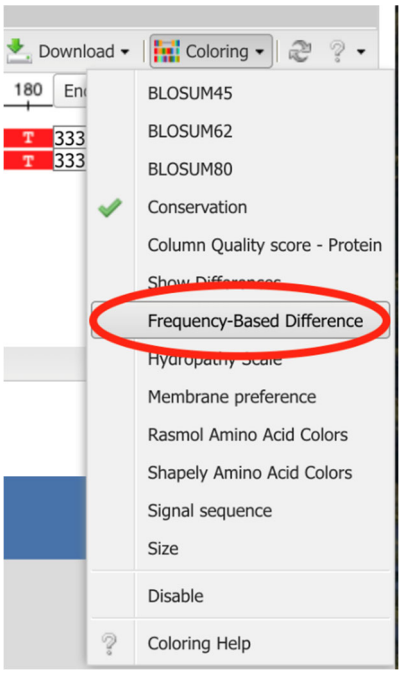
18. Click the "Zoom to sequence" button (see **Figure 4.13**) to visualize the amino acid sequences of the two TAS2R38 alleles in your alignment. You are going to scan the alignment in search of amino acid substitutions. As a reminder, AAP14666 is the taster sequence and AAM19322 is the nontaster sequence**.**

Figure 4.13: Zoom to sequence



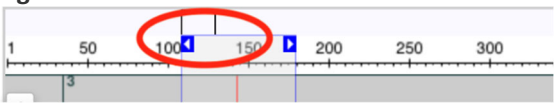
19. Under the "Coloring" pull-down menu, select "Frequency-Based Difference" (see **Figure 4.14**). Amino acids that differ between the two sequences will now be highlighted in red.

Figure 4.14: Frequency-based difference



20. Move the uppermost rectangular slider along the length of the sequence alignment to identify amino acid differences between the two sequences (see **Figure 4.15**). Record the amino acid positions and sequences in the **Amino Acid Sequence Differences** table on **Finding TAS2R38 Differences (RM 4.1)**.

Figure 4.15: Move slider



21. Before your class discussion, read the passage that follows to learn about the distinction between SNVs and SNPs.

READING: SNVs and SNPs

SINGLE NUCLEOTIDE VARIANTS VS. SINGLE NUCLEOTIDE POLYMORPHISMS

Between any two individuals, there are different nucleotides in their DNA on average once every 1,300 bases. These are single nucleotide variants (SNVs) like the ones you identified in alleles of the TAS2R38 gene. Some SNVs occur frequently in a population—at a rate of 1% or higher. These variants are known as ***single nucleotide polymorphisms***, or ***SNPs*** (pronounced "snips"). In both SNVs and SNPs, any nucleotide can be substituted—in other words, an A might become a C, G, or T. The substitutions are caused by errors during DNA replication or by ***mutagenesis*** (the process of permanent changes to DNA due to external agents). Single nucleotide substitutions can also occur in dividing somatic cells (for example, in cancer), but SNVs and SNPs must occur in ***germline DNA*** (the genome of reproductive cells) to be inherited.

While most SNVs occur in the non-coding portions of our DNA, some do produce phenotypic differences—like the ability to taste bitterness. Scientists use large human genomic datasets to map our genes and identify the location of these variants, just like you did with the TAS2R38 gene using BLAST.

How, then, do researchers determine that a SNV occurs with sufficient frequency to be called a SNP? They sequence DNA from databases of populations around the world, often examining thousands of individual sequences to determine the frequency of particular combinations of genetic variants.

ARE THERE ANY SNPS IN TAS2R38?

Three single nucleotide variants in TAS2R38 occur at sufficiently high frequencies to be classified as SNPs. These are located at nucleotide positions 145, 785, and 886. In Chapter 5, you will focus on the SNP at 145 in greater detail. If you wish to learn more about the biomedical research behind these SNPs, visit SNPedia and search for TAS2R38.

ACTIVITY: Reading DNA Chromatograms

MATERIALS

- Laptop or desktop computer with internet access
- 1 copy of **Exploring DNA Sequences (RM 4.2)**

NOTE FOR COLORBLIND STUDENTS: Being able to tell apart different colors is crucial to this activity. If you have red–green colorblindness, both macOS and Windows operating systems offer accessibility options that help users distinguish between colors that others experience as red or green. Make sure to ask your teacher for access to digital versions of the DNA chromatograms featured below.

BACKGROUND

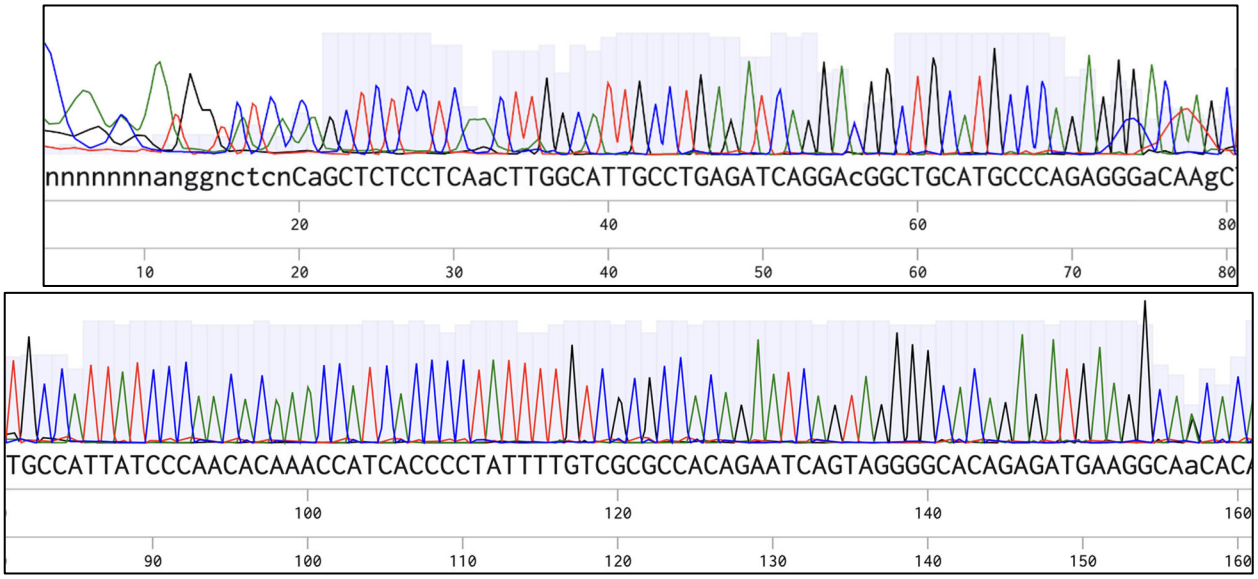
As you learned in Chapter 3, a DNA chromatogram is a visual representation of the nucleotide sequence of a DNA sample produced by an automated sequencing machine. In the DNA chromatogram, each color represents a specific nucleotide (see **Table 4.1**). The height of each color peak in the DNA chromatogram represents the relative fluorescent intensity of a nucleotide whose identity is determined by base-calling software. Should the software be unable to assign a nucleotide base with confidence, an "N" (for nucleotide) will appear as a placeholder.

Table 4.1: Key to the DNA chromatogram colors

Key to the DNA chromatogram Colors	
Black = Guanine (G)	**Red = Thymine (T)**
Blue = Cytosine (C)	**Green = Adenine (A)**

A DNA chromatogram looks like the image below—four different colored strands ("traces") representing the four nucleotides (see **Figure 4.16)**. The gray blocks behind the traces represent the ***confidence interval***—a measure of the probability that the software correctly identified the nucleotide in the DNA sequence. Basically, the taller the gray area, the more confident the program is in assigning the nucleotide.

Figure 4.16: Example DNA chromatogram



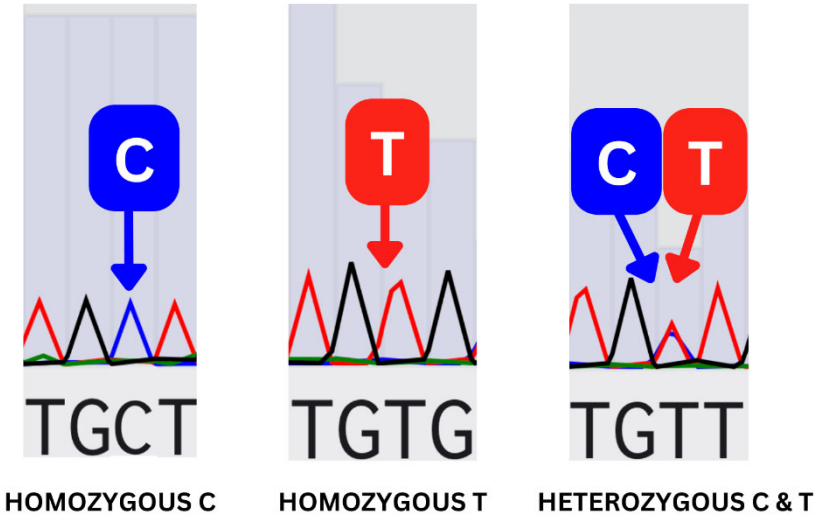
In a "good" DNA chromatogram, the peaks are spaced evenly (to the right of nucleotide position 20 in **Figure 4.16** above, for the most part). Peak heights may vary, which is normal. Often, the first 20–40 base pairs of a sequence are unclear (as shown in the lower confidence intervals above), and you can see many "N" (for "nucleotide") placeholders at positions where the computer cannot assign a nucleotide base with confidence.

Occasionally, broad peaks are visible at locations where the sequence repeats the same nucleotide multiple times. However, the trace will still show defined peaks at each nucleotide position; the software reads these as distinct signals and assigns the correct nucleotide bases (see **Figure 4.17)**.

Figure 4.17: Broad peaks in a DNA chromatogram

If a person is heterozygous for a particular base pair, you should see two peaks at that location. The images below represent sequences that are homozygous C, homozygous T, and heterozygous C and T (see **Figure 4.18)**. Note that in the heterozygous individual shown, the height of the peaks for C and T, respectively, are approximately half the height of the peaks for that position in the homozygous examples.

Figure 4.18: Examples of heterozygous vs. homozygous nucleotide peaks in DNA chromatograms

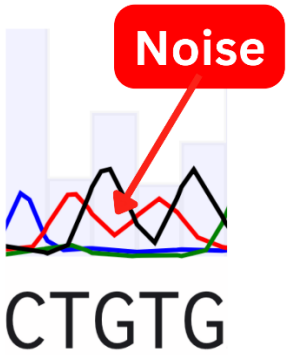


Sometimes, portions of a DNA chromatogram are illegible. There are numerous potential reasons for "noisy" traces, including poor DNA quality, low concentration of template DNA, suboptimal PCR conditions, and degradation of sequencing reagents.

How can you distinguish between a clear-cut, heterozygous position and a "noisy" one? In any DNA chromatogram, there is always some background "noise" from the other traces, but the base-calling software can usually tell the difference between "noise" and a heterozygous base pair at a position.

For this DNA chromatogram analysis, peaks that are less than half the height of the main peak at a nucleotide position can be considered “noise” (see **Figure 4.18**). Sometimes there are single broad peaks that span several nucleotide positions. These can also be considered “noise.”

Figure 4.18: “Noise” in a DNA chromatogram



PROCEDURE

Now try it yourself. Explore the DNA chromatogram on **Exploring DNA Sequences (RM 4.2)** and then answer the questions.

ACTIVITY: Explore the DNA Chromatogram of a Weak PTC Taster

MATERIALS

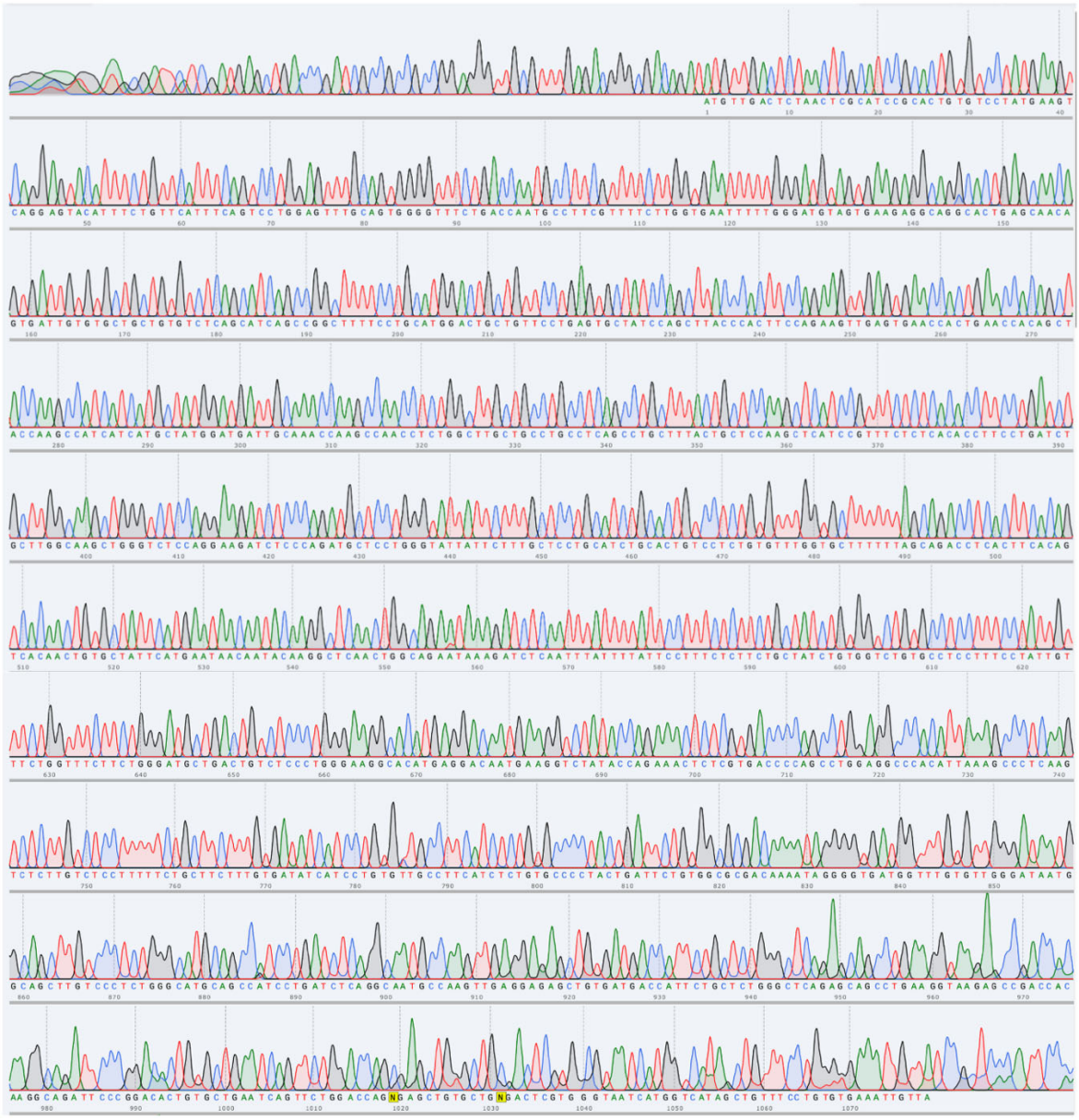
- 1 copy of **Finding TAS2R38 Differences (RM 4.1)**
- 1 copy of **Interpreting a Heterozygous DNA Sequence (RM 4.3)**

PROCEDURE

1. Now you have learned how to read a DNA chromatogram and you have identified SNPs which differentiate the taster from the nontaster allele. Review your answers on **Finding TAS2R38 Differences (RM 4.1)**. What do you predict you will find at those same nucleotide positions in the DNA sequence of a weak taster? Keep in mind they would have inherited one taster allele from one parent, and one nontaster allele from the other parent. Write your predictions in the table on **Interpreting a Heterozygous DNA Sequence (RM 4.3)**.

2. Next, examine the DNA chromatogram below (see **Figure 4.19),** which shows the DNA sequence of a heterozygous individual who must have one copy of each allele. Please note that this DNA chromatogram has been trimmed to reduce “noise” and remove "N" positions, and the confidence interval is not displayed.

Figure 4.19: DNA chromatogram of TAS2R38 from a heterozygous, weak PTC taster



3. Keeping the diagnostic SNP positions from **RM 4.1** in mind, examine those same nucleotide locations in the weak taster DNA chromatogram in search of heterozygous peaks. As a reminder, a heterozygous position will have two overlapping peaks with different colors. Note any heterozygous positions you find in the table on **RM 4.3**.

READING: Exploring the Differences in Our DNA

As you read the passage below, which will prepare you for the lab activities of Chapter 5, write down definitions in your science notebook.

WHY LOOK FOR SNPS?

SNPs are molecular signposts that help researchers study molecular evolution. These single nucleotide substitutions represent a historical record of minor, heritable changes accumulating in the human genome and being passed down from one generation to the next. Scientists can use mathematical formulas to determine how much time has passed since a particular nucleotide variant has emerged in a DNA sequence. With collections of DNA sequences from geographical populations worldwide, they can also map the movement of specific haplotypes from one place to another. This information provides insight into selective forces that have acted on human populations over time.

Some SNPs, such as synonymous nucleotide substitutions within ***exons*** (portions of coding sequence that are spliced together to make mRNA), do not affect the structure or expression of proteins. The same is true for most nucleotide substitutions in noncoding DNA, unless mutations occur in important upstream regulatory regions, such as promoter sites. (A ***promoter*** is a region of DNA upstream from a gene where proteins bind to initiate transcription of the gene). Nonsynonymous SNPs, such as those that cause amino acid substitutions, are noteworthy from both an evolutionary biology and biomedical standpoint. Mutations that cause physiological changes are valuable targets for drug development and genetic engineering.

SNPs are often detected when researchers compare the DNA sequences of many individuals at once. This process typically requires large-scale lab equipment and multiple personnel.

DETERMINING DIFFERENCES IN OUR DNA WITHOUT SEQUENCING

Large DNA sequencing projects can be expensive and time-consuming. However, sometimes simply knowing which nucleotide is present at a specific position in a gene's coding sequence can reveal which allele you have. In the activity **Finding TAS2R38 Differences**, you found places in which the sequence of nucleotides in the TAS2R38 gene was different between people who are PTC tasters and nontasters.

Knowing the locations of diagnostic SNPs like these allows you to use a less expensive and more widely available technology to determine your particular genotype. To do this, you can use restriction enzyme digestion followed by gel electrophoresis.

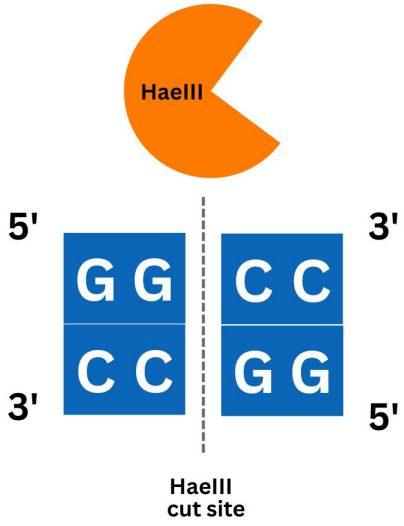
WHAT ARE RESTRICTION ENZYMES?

Restriction enzymes are specialized bacterial proteins that cut DNA into fragments at or near specific sequences of bases. When these proteins are used in the lab to cut double-stranded DNA at particular sites for diagnostic or gene cloning purposes, the technique is known as a ***restriction enzyme digest***.

In the early 1950s, scientists observed that certain strains of *E. coli*, a common bacterium found in the human gut, were resistant to infection by ***bacteriophages***—viruses that infect bacteria by injecting their DNA into the cell and commandeering the host cell's molecular processes to make more bacteriophages. Investigation of this primitive bacterial "immune system" led to the discovery of restriction enzymes, which restrict bacteriophage growth by recognizing and destroying phage DNA without damaging the host DNA. Subsequent studies demonstrated that restriction enzymes from different species of bacteria cut DNA at specific sequences, which are called ***restriction sites***. There are currently at least 3,000 different restriction enzymes commercially available!

One class of restriction enzymes recognizes specific 4–8-bp-long DNA sequences and typically cuts the strands at a particular position within or outside of the recognition site (See **Figure 4.20**). During a restriction enzyme digest *in vitro*, cuts at each of these recognition sites generate DNA fragments of different sizes. Then these DNA fragments can be separated by size using gel electrophoresis. You will use restriction enzyme digestion and gel electrophoresis to learn more about your own taster genotype in the next chapter.

Figure 4.20: An illustration of the restriction enzyme *HaeIII* and its recognition site



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:

```
  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
```

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.

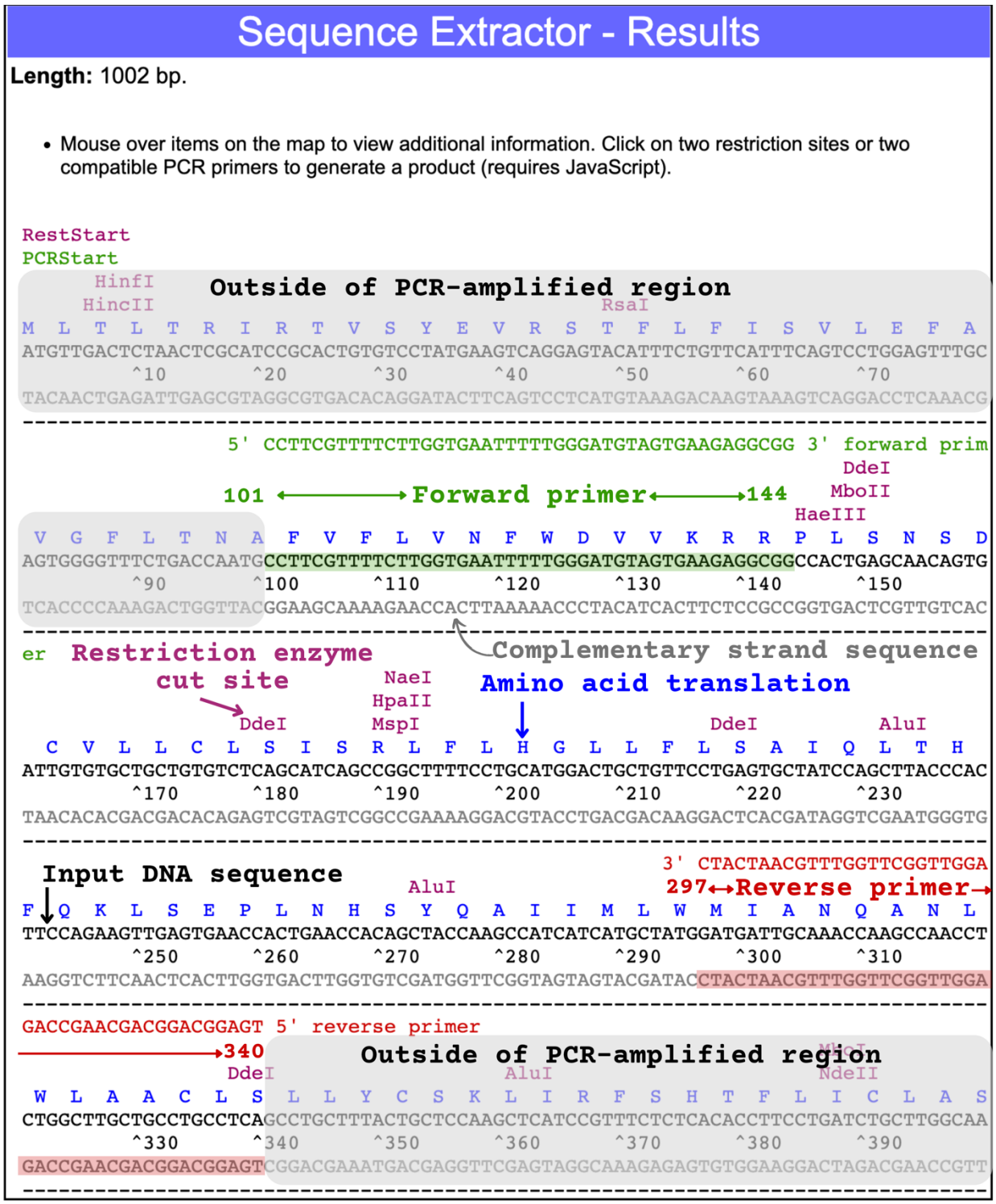
   Important: Be sure to separate the forward and reverse primers with a comma!

   `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

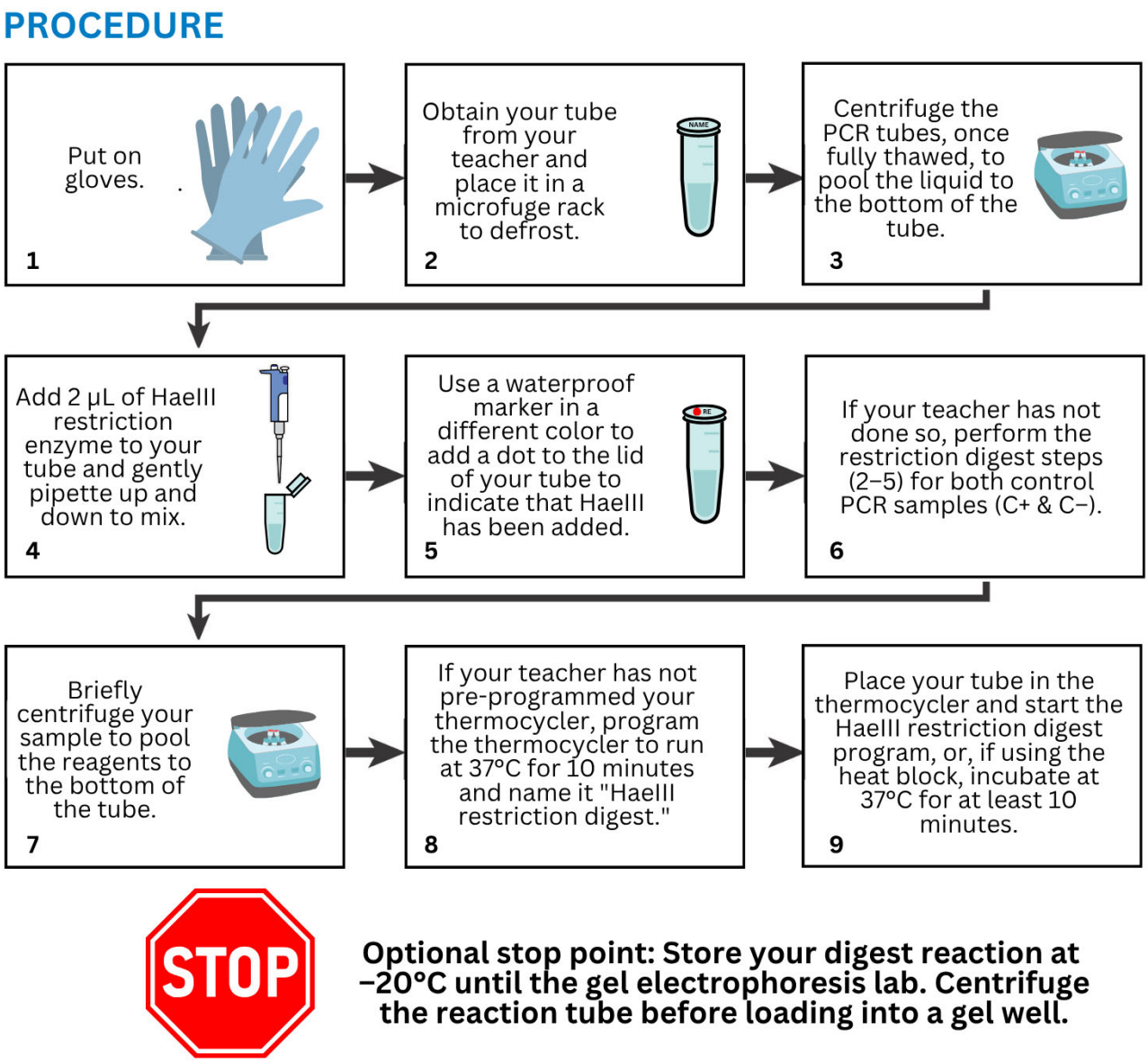
PROCEDURE (see **Figure 5.3** for flowchart)

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

Figure 5.3: Procedure flowchart for restriction digest



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?	

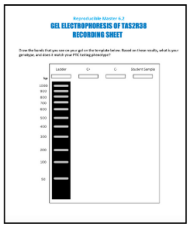
Step	Notes
2. One scientist speculated that iodine might play a role in natural selection of this gene. What did they suggest? 3. What is another reason that Africans have more genetic diversity than Europeans?	
Once the gel is complete . . .	
7. Place your gel on the transilluminator and observe the 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

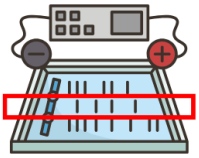
For each student:

A pair of gloves

RM 6.2: Gel Electrophoresis of TAS2R38 Recording Sheet



1 lane of an agarose gel



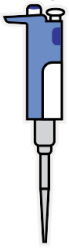
Student restriction enzyme digest

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

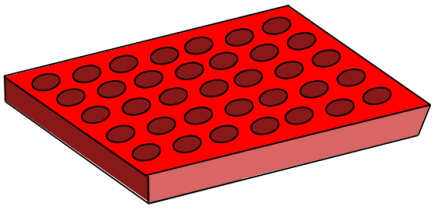


Figure 6.1: Materials for gel electrophoresis (continued)

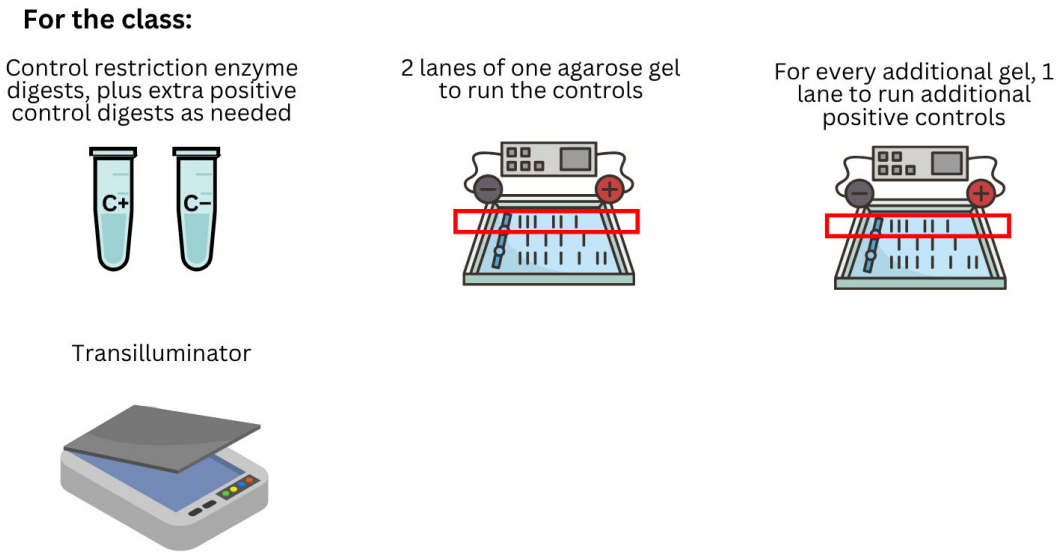
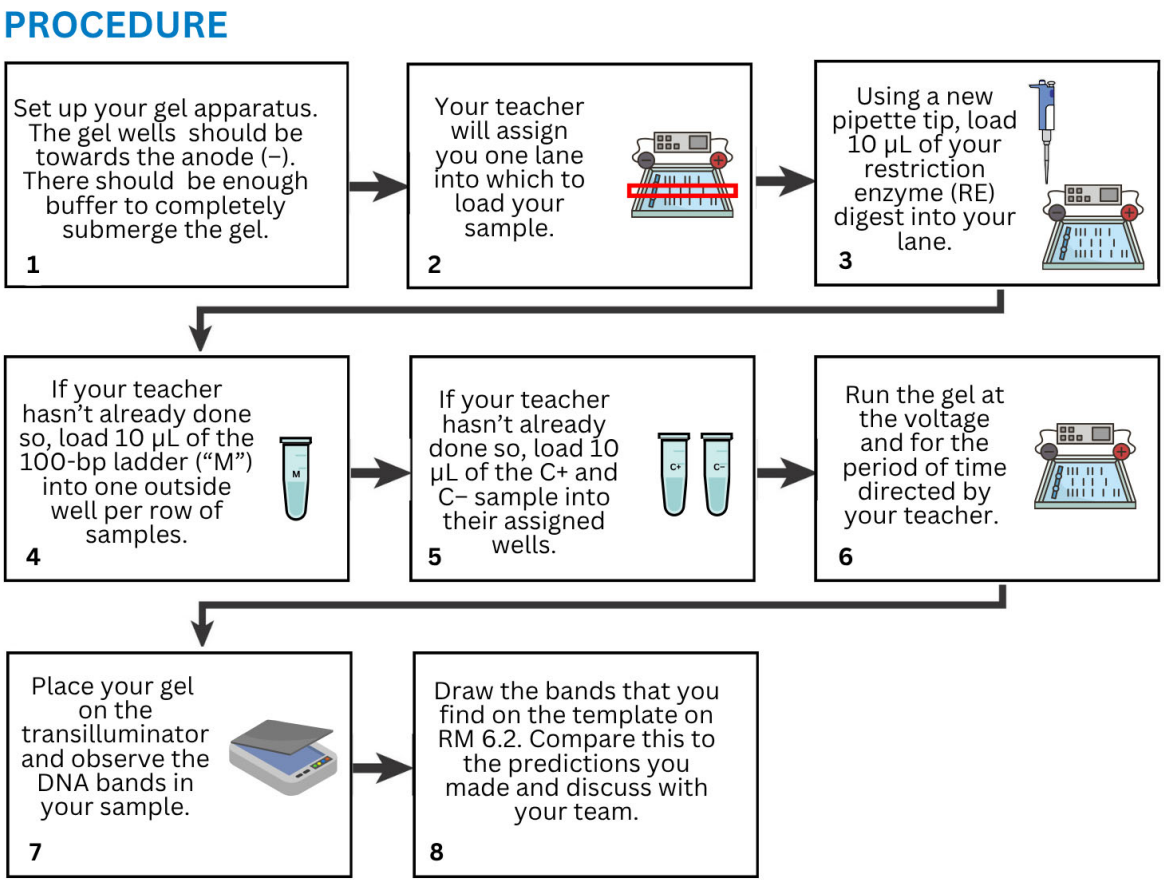


Figure 6.2: Flowchart procedure for gel electrophoresis



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

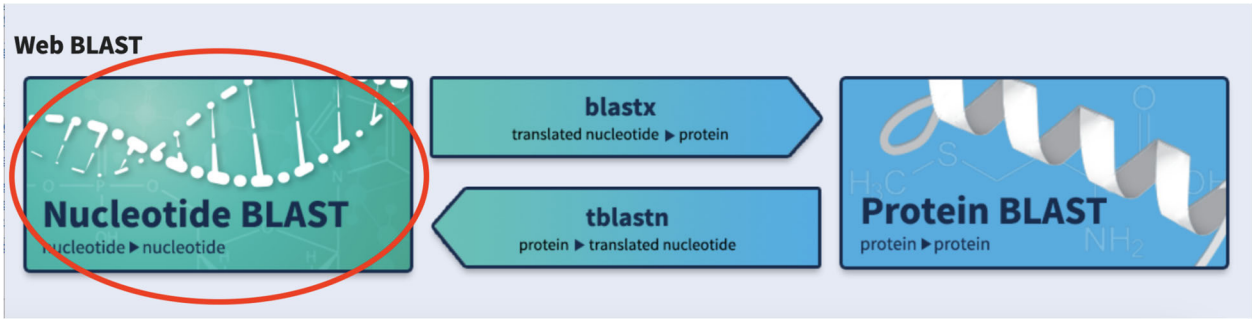
Research SNPs of CYP2C19

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"



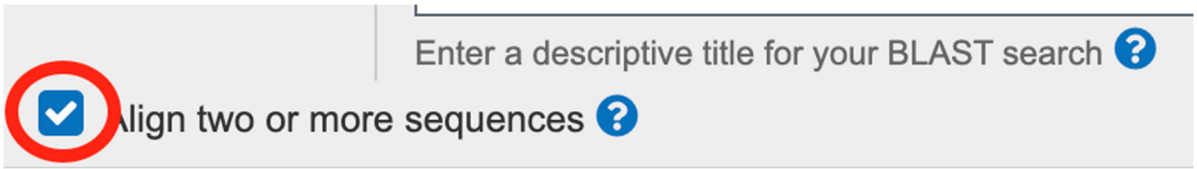
This time, you will create an alignment of a **selected range** of nucleotides from Exon 5 of CYP2C19. Please note that this is not the sequence of the entire exon—it is the sequence of a small portion of the DNA that contains a SNP of interest.

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

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

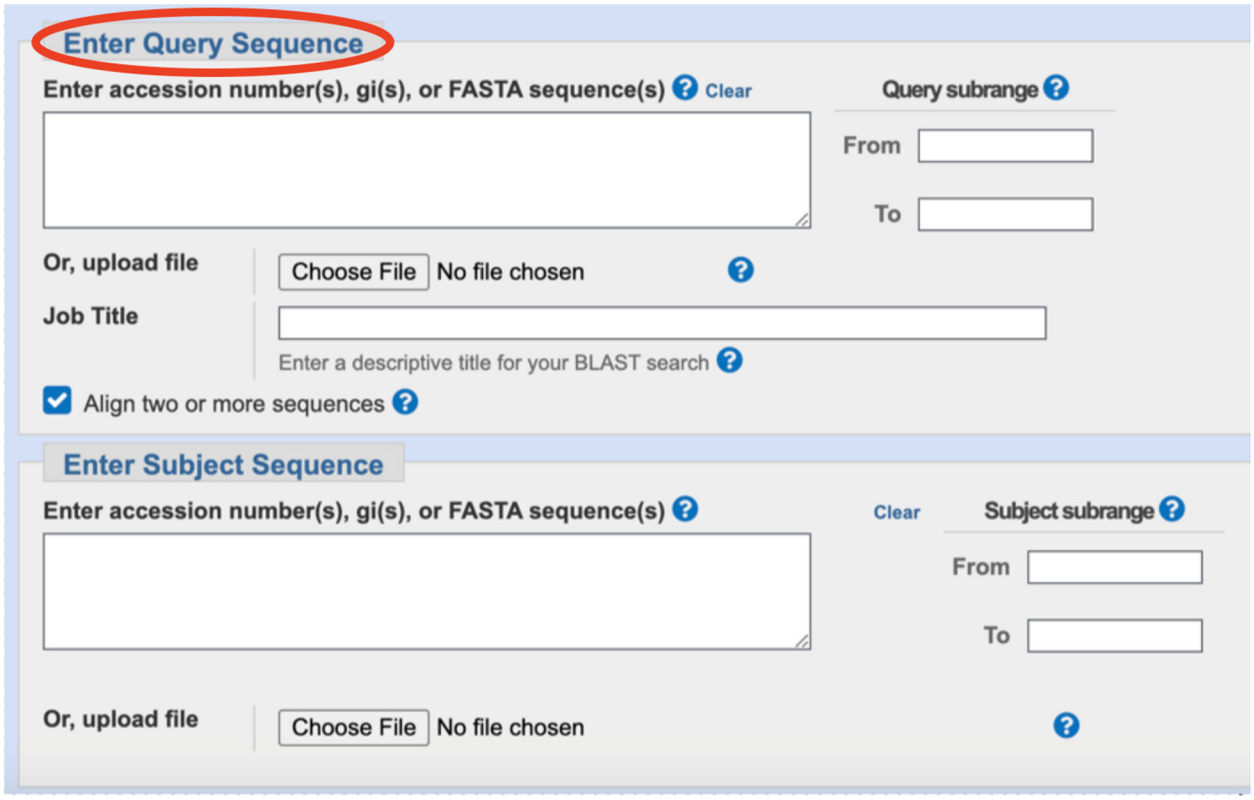
6. Under "Enter Query Sequence," check the "Align 2 or more sequences" box (see **Figure 6.4**).

Figure 6.4: Align two or more sequences



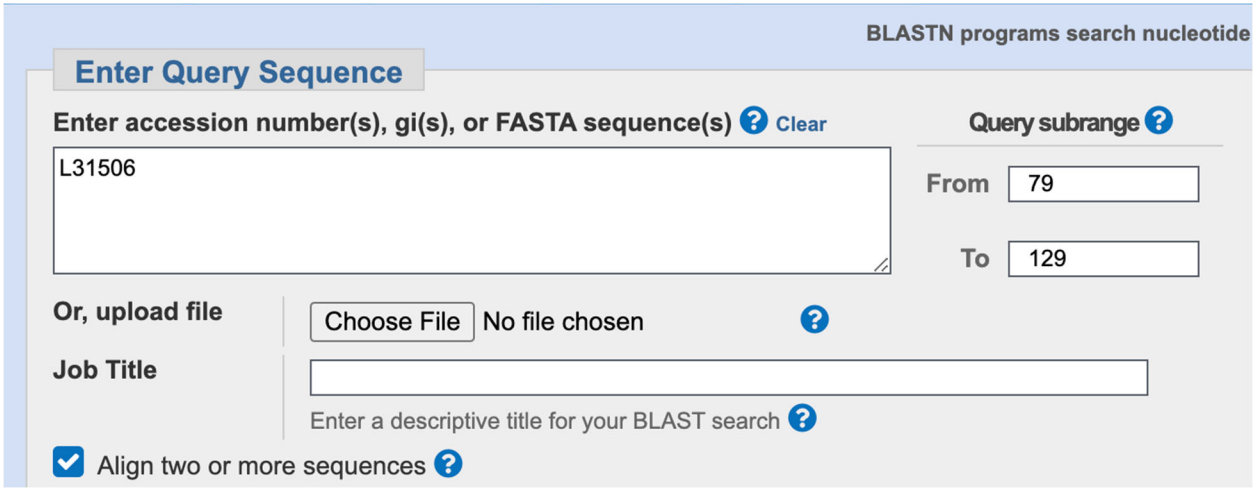
7. Now, you should see two boxes, one called “Enter Query Sequence” and one called “Enter Subject Sequence” (see **Figure 6.5**). Enter the wild-type CYP2C19 Exon 5 sequence reference number (L31506) into the Enter Query Sequence box.

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**).

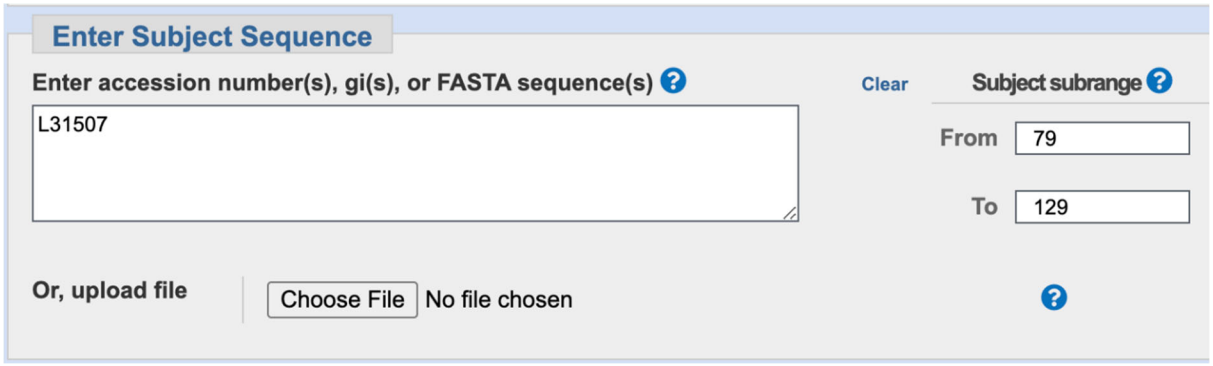
Figure 6.6: Enter the range of nucleotides in the Query Subrange box



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

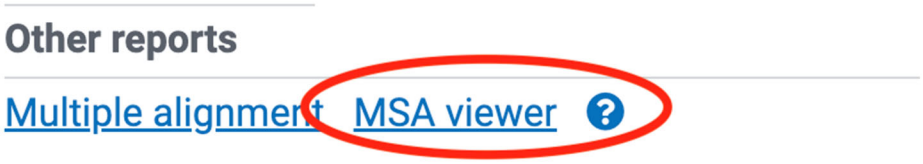


11. Select "Show results in a new window," then click on the "BLAST" button at the bottom of the page (see **Figure 6.8**). An alignment analysis will open on a new page.

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.

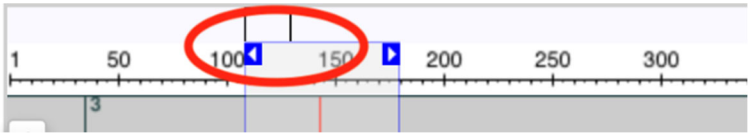
Figure 6.9: MSA viewer



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**.

CHAPTER 7:
SNPS and Drug Metabolism

INTRODUCTION

By working through the activities in this module, you have learned why genetic variation is important in medicine, how this variation is detected, and options healthcare practitioners have for treating patients whose genetic variations affect how they metabolize drugs.

In this chapter, you will return to the case of Renee Jackson, whose medical history you reviewed in Chapter 1. You will learn her genotype for the CYP2C19 gene and make a recommendation regarding her treatment. You will also explore other genes relevant to pharmacogenomics and begin to consider other diagnostic methods used in genotyping.

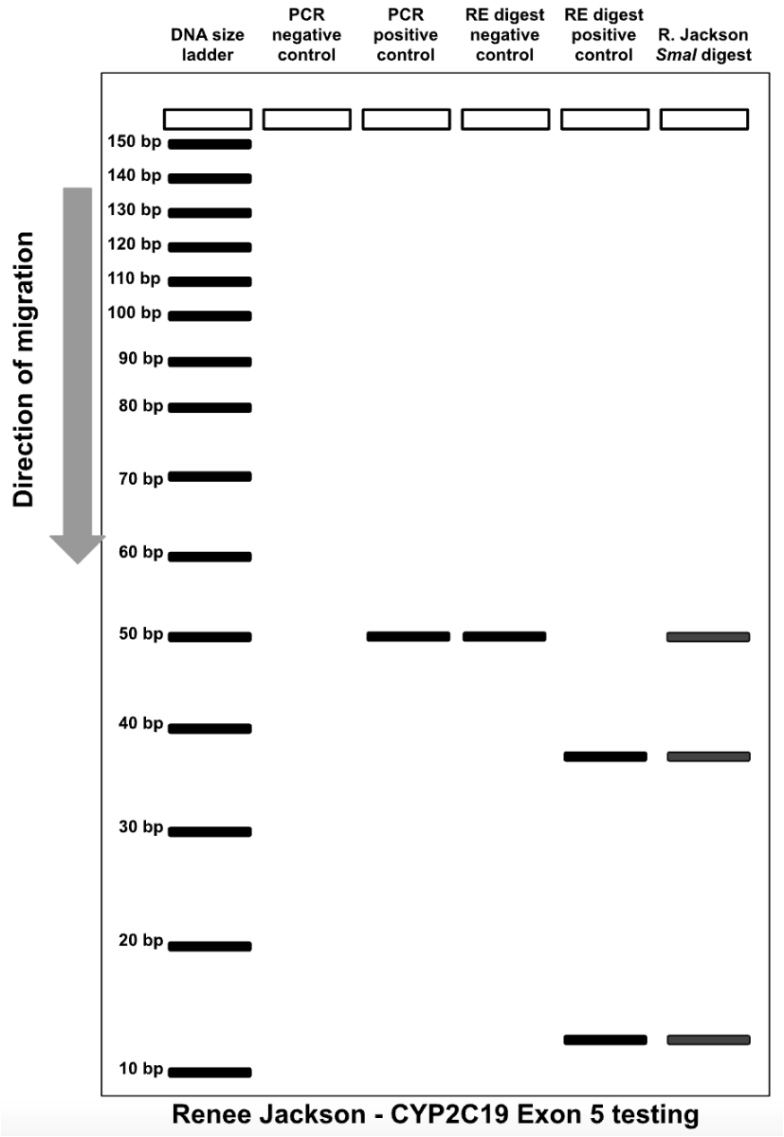
Finally, you and your classmates will discuss the pros and cons of using genetic information to improve patient care.

ACTIVITY: Pharmacogenomics of Clopidogrel

1. Review the diagnostic tests you explored in Chapter 6. As a review, recall that you could use PCR to amplify a portion of Exon 5 of the cytochrome P450 gene CYP2C19, and restriction enzyme digestion, followed by gel electrophoresis, to distinguish between normal (CYP2C19*1) and mutant (CYP2C19*2) alleles at the SNP rs4244285.

2. Examine the gel image (**Figure 7.1** on the following page), which shows the results of Renee Jackson's genotyping test. Then answer the questions on **Pharmacogenomics and Patient Care (RM 7.1)**.

Figure 7.1: Results of Renee Jackson's genotyping test



GLOSSARY

allele: One of two or more alternative forms of a gene.

amplify: To make many copies of a specific region of DNA.

angina: Chest pain caused by reduced blood flow to the heart, often from narrowed, nearby arteries due to the buildup of plaque. See also: unstable angina.

anneal: To attach, for example, as when primers form hydrogen bonds with single-stranded template DNA in PCR.

antiplatelet therapy: Medication that reduces the blood's ability to clot in order to prevent dangerous clots after a medical or surgical procedure.

bacteriophage: A virus that infects bacteria by injecting its DNA into the cell and commandeering the host cell's molecular processes to make more bacteriophages.

base-calling software: Computer code used to analyze the output of automated DNA sequencing. It compiles laser excitation output from fluorescent nucleotides in DNA fragments, sorts the fragments into the correct order, and generates an output called a DNA chromatogram. See also: DNA chromatogram, Sanger sequencing.

capillary electrophoresis sequencing: A form of Sanger sequencing; nucleic acid fragments are loaded into a polymer-filled capillary tube to which an electric field is applied for size separation. See also: Sanger sequencing.

chelate: To bind chemically.

cofactors: Metallic ions required for the catalytic activity of enzymes such as DNases.

confidence interval: A measurement of confidence in statistics. In the case of DNA sequencing, it is an estimate of the probability that a particular computer-assigned nucleotide is actually the nucleotide in the DNA sequence.

cytochrome P450: A member of a class of enzymes active in the endoplasmic reticulum, where they metabolize substances such as medications.

DNA chromatogram: A computer-generated chart of a DNA sequence that shows the nucleotides in each position and the level of fluorescence emitted by each nucleotide in the sequence.

DNA polymerase: The enzyme that builds new DNA molecules by connecting nucleotides.

DNA replication: The process by which organisms assemble new strands of DNA from existing templates using free nucleotides and the enzyme DNA polymerase.

DNases: Enzymes that break down DNA.

enzyme: A protein that catalyzes a chemical reaction without being altered itself.

exon: A segment of DNA that encodes a protein. Exons are spliced together to make mRNA.

gel electrophoresis: A laboratory technique for separating nucleic acids or proteins based on their relative size by applying an electric current that pulls materials through a gel in a buffer solution.

gene cloning: Using reagents derived from living organisms, such as restriction enzymes and DNA polymerase, to isolate and make copies of specific genes.

gene product: The protein that results from the expression of a gene, such as a human therapeutic protein like insulin.

genotype: The genetic makeup of an individual.

germline DNA: The haploid genome of gametes (sperm or eggs) generated by meiosis.

Human Genome Project: A large, collaborative, international project that generated the first full sequence of the human genome. This used Sanger sequencing; see also Sanger sequencing.

in vitro: A laboratory technique which occurs outside a living organism.

in vivo: A laboratory technique which occurs inside a living organism.

lysis: Breaking down a cell's plasma and nuclear membranes using heat or chemical means.

mutagenesis: The process of permanent changes to DNA due to external agents.

nucleotide: The basic building block of DNA.

percutaneous coronary intervention: (PCI): Also known as "angioplasty," a procedure to remove blockages in coronary arteries using X-ray-guided flexible catheters. May involve the placement of stents.

pharmacogenomics: The science of understanding how individuals' genotypes influence their response to medications.

phenotype: The set of observable characteristics of an individual based on how their genotype is expressed; see genotype.

phenylthiocarbamide (PTC): A chemical compound which some individuals perceive as bitter, while others do not taste it at all, depending on their genetics. It is commonly used to study inherited taste traits.

plasmids: Circles of DNA found in bacteria and other microorganisms that are separate from chromosomal DNA and can replicate independently.

polymerase chain reaction (PCR): A technique to make many copies of a portion of DNA ranging from as small as 200 base pairs to as large as 40 kilobases!

precision medicine: An approach to the prevention and treatment of disease that considers individual variability in genes, environment, and lifestyle.

promoter: A region of DNA upstream from a gene where proteins bind to initiate transcription of the gene.

restriction enzyme: A species-specific bacterial protein that restricts the growth of the harmful viruses known as bacteriophages by recognizing and destroying the phage DNA without damaging the host (bacterial) DNA. Each bacterial species cuts DNA at a different sequence of nucleotides (see also restriction sites).

restriction enzyme digest: A laboratory technique using specialized bacterial proteins which cut double-stranded DNA at particular sites for diagnostic and gene cloning purposes (see also restriction enzymes and restriction sites).

restriction sites: Short sequences of DNA that bind specific bacterial restriction enzymes, allowing the enzymes to cut the DNA.

running buffer: A solution of electrolytes capable of forming ions and maintaining a stable pH; used to facilitate gel electrophoresis.

Sanger sequencing: An early method of DNA sequencing developed by Frederick Sanger and his colleagues.

sequencing: The process of determining the order of nucleotides in a nucleic acid sample.

single nucleotide polymorphism (SNP): A variation at a single position in individuals' DNA sequences. This can occur either in coding or non-coding DNA. If it occurs within coding DNA in a gene, each SNP represents a different allele. SNPs occur with greater than 1% frequency in populations.

single nucleotide variant (SNV): Single nucleotide variation in individuals' DNA sequences (see also SNP), but that occurs with less than 1% frequency in populations.

somatic cells: Body cells, excluding reproductive cells.

stent: A miniature, expandable flexible wire "cage" used to hold open a major blood vessel.

***Taq* polymerase:** A DNA-replicating enzyme from the thermophilic bacterium *Thermus aquaticus* that can copy its DNA at high temperatures. This enzyme is used in PCR, where DNA must be denatured at high temperatures.

template DNA: The sample DNA that contains the target sequence.

terminus: The end of a chain of nucleotides.

thermocycler: A laboratory instrument that controls the temperature and length of time for different phases of a reaction.

thermophile: A heat-loving microorganism.

trait: A characteristic of living organisms that can be described, quantified, or measured, and that is due to the influence of genes, the environment, or both.

transformation: The process of introducing a plasmid to bacteria.

unstable angina: Sudden, severe chest pain brought on by a temporary blockage of a coronary artery which limits blood flow to the heart.

wild-type: The most prevalent form of a gene in a species, as found in nature.

APPENDIX

Table A.1: Amino acid translation table

First Base	Second Base: U		Second Base: C		Second Base: A		Second Base: G		Third Base
U	UUU	phe	UCU	ser	UAU	tyr	UGU	cys	U
	UUC		UCC		UAC		UGC		C
	UUA	leu	UCA		UAA	STOP	UGA	STOP	A
	UUG		UCG		UAG		UGG	trp	G
C	CUU	leu	CCU	pro	CAU	his	CGU	arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	ile	ACU	thr	AAU	asn	AGU	ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	lys	AGA	arg	A
	AUG		ACG		AAG		AGG		G
G	GUU	val	GCU	ala	GAU	asp	GGU	gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	glu	GGA		A
	GUG		GCG		GAG		GGG		G

Credit: Amino acid table: Scott Henry Maxwell, CC BY-SA 4.0,