Hunting the Inheritance of Huntington's Disease MiniLab

Student's Guide

Cat# M3010

Version 081519

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Laboratory Safety

1. Do not eat, drink, or apply cosmetics in the lab. Do not eat food used in laboratory activities.
2. Wear lab coats, gloves, and eye protection as required by district protocol.
3. Use caution when using all electrical equipment such as electrophoresis units.
4. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
5. Wash your hands thoroughly after handling biological materials and chemicals.

Objectives

In this MiniLab you will use a family's pedigree to observe patterns and make predictions about the inheritance of the genetic disease Huntington's.

This will enable you to:

- Understand and use Mendelian inheritance to analyze data and to determine a probable conclusion.
- Develop an understanding of electrophoresis principles to confirm predictions and explain patterns observed using scientific data.

Introduction

Huntington's disease (HD) is an inherited progressive, neurodegenerative disorder that is caused when the neurons (nerve cells) in the brain die. Signs and symptoms of Huntington's disease include uncontrolled movement, memory loss, mood and behavior disturbances, and deteriorating cognitive (thinking) ability. Most people with Huntington's disease start to display symptoms typically between 30 and 50 years of age, although there is also a less common form of the disease known as the juvenile form that begins in childhood or adolescence. There is currently no cure for Huntington's disease but there are medications available to help manage the symptoms.

In 1872, George Huntington was the first person to provide a comprehensive description of this disease. The gene and mutation responsible for causing Huntington's disease, the huntingtin (HTT) gene, was first described by a team of scientists in 1983. It was discovered that the mutation was a result of a CAG trinucleotide repeat. While the normal allele of this gene usually carries between 10 and 35 copies of this CAG repeat, the disease allele usually carries between 40 and 120 repeats of this CAG sequence creating an expanded allele and thus an elongated mutant protein. This protein causes the symptoms of the disease by accumulating in the neurons and disrupting the normal function of these cells. It has also been found that the number of CAG repeats is inversely correlated with the age of onset, so the more repeats a person has, the earlier the onset of the symptoms of Huntington's disease.

The mutation in the HTT gene is inherited in an autosomal dominant pattern, which means that one copy of the mutant gene is enough to cause the disorder. Therefore, every child of a parent with Huntington's disease has a 50/50 chance of inheriting the disease allele and developing the disease. If a child does not inherit this gene, he or she will never develop the disease, and cannot pass it on to their children.

The CAG trinucleotide repeat has been observed to exhibit dramatic instability when transmitted to subsequent generations. This means that the number of CAG repeats may expand or contract from one generation to the next. As a result of this instability, a child who inherits the mutant HTT gene could exhibit a different phenotype from his or her parent, perhaps with a different age of onset of the disease.

Individuals who have a family history of Huntington's disease and are at risk of developing the disorder may undergo molecular genetic testing to determine if they carry the expanded version of the gene. The Huntington's Disease Society of America (HDSA) recommends that at-risk individuals considering genetic testing do so at an HDSA site where it is done under the close supervision of a healthcare practitioner, a genetic counselor, neurologists, and often a psychiatrist or a psychologist.

Scenario

Nathaniel and Jean were thrilled to find out they were pregnant with twins in 1992. When the ultra sound revealed they were fraternal twins they decided to name them Peter and Kim. Life was good when the twins were born in July of 1993. They were a happy family. In 1995, when Nathaniel began to show signs of Huntington's Disease, he and Jean wanted to learn more about his birth mother's family history. They contacted relatives to gather information to try to determine who had the disease, at what ages they started showing symptoms, the severity of the symptoms, and when those that inherited the disease as well as healthy members of the family died. Four relatives were forthcoming with information about the family. It is your job to examine the information Nathaniel and Jean received to construct a pedigree (family tree) to gain a better understanding of the mode of inheritance and age of onset of the disease.

In the second part of this MiniLab you will be given additional data about the molecular inheritance of the disease. You will need to add this information to your pedigree. This data is reported much like dominant (D) and recessive (d) alleles. A person who is homozygous dominant would be reported as (DD). HD alleles are reported as the number of trinucleotide repeats (CAG) associated with the disease. Someone with 6 repeats from dad and 12 repeats from mom would be reported as a genotype of (6, 12). Based on the data he received, Nathaniel knew there was a possibility of his passing the autosomal HD trait to his twins. He and Jean discussed the implications for the twins. Should they have them tested? Do treatments exist to mitigate symptoms of the disease so that Peter and Kim could prolong and led more fruitful lives if they are carriers?

They decided they want to test the children. You need to interpret the results and make recommendations.

Day 1 - Building Nathaniel's Family Tree

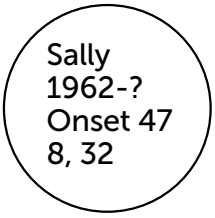
1. You will work with your group to complete Nathaniel's family tree using the data provided to you by your teacher.
2. Working with members from other groups communicate your data to further build your pedigree.

Include as much information as possible such as birth year, whether or not the person displayed symptoms of Huntington's disease, year of death, etc. Students may wish to construct a data chart listing family members on one side exhibiting the disease or symptoms of the disease on one side (including number of trinucleotide repeats, dates of onset or death) and unaffected members on the other. You may wish to construct a data chart listing family members on one side exhibiting the disease or symptoms of the disease on one side (including number of trinucleotide repeats, dates of onset or death) and unaffected members on the other.

Here is an example to help you determine what information should be included in the pedigree:

You gathered information on a family member named Sally. You know she was born in 1962. She started showing symptoms of HD when she was 47. Her genotype for the disease is 8, 32.

Her information on the family tree would look something like this:



For additional information on trinucleotide repeats you may wish to refer to the scholarly article found at:
https://ghr.nlm.nih.gov/condition/huntington-disease#inheritance

Analysis Questions

1. What is a trinucleotide repeat?

2. How is Huntington's Disease inherited? What is the mode of inheritance?

3. According to the article, how many repeats does an individual with adult onset Huntington's Disease have?

4. According to the article, how many repeats does an individual with juvenile onset Huntington's Disease have?

5. In general, children have to have a parent that has the disease to inherit it. However some children are still at risk of developing the disease. Why?

6. Does Nathaniel's family tree provide supporting evidence of a correlation between the number of repeats and the inheritance of the Huntington's gene? Explain.

7. Does Nathaniel's family tree provide supporting evidence of a correlation between the number of repeats and the age of onset? Explain.

8. What other patterns can you identify from the pedigree? What evidence can be used to support this answer?

Day 2 - Molecular Genetic Testing

Polymerase chain reaction, PCR, is a technique used to make millions of copies of a specific fragment of a gene in vitro. This method was first developed in the late 1970s by Frederick Sanger and revolutionized the field of biotechnology.

PCR works by mimicking the intricate steps of DNA replication that occurs in all our cells by using heat. Each PCR reaction is made up of several important ingredients, DNA to be copied, forward and reverse primers, free nucleotides (dNTPs), Taq polymerase, and a thermal cycler. There are three main steps to a PCR reaction, namely denaturation, annealing, and extension. A thermal cycler is used to control the temperature at which each of these three steps occurs.

In the first step, the double stranded DNA is heated up to a temperature from 90–98°C for 5–30 seconds in order to weaken the hydrogen bonds between the bases, causing the DNA strands to separate into single stranded DNA.

After the DNA strands separate, the temperature is then lowered to approximately 45–65°C for 5–30 seconds per cycle seconds in order for the annealing step to take place. During the annealing step, DNA primers bind to their complementary sequence on the target DNA. The temperature at which this step occurs depends on the sequence and length of the primers used. It should be low enough in order for hydrogen bonds to form between the primer and its specific complementary sequence, but not too low that random binding between primer and template occurs.

The third step in the PCR reaction is extension. In this step, Taq polymerase, a heat-stable DNA polymerase, binds to the primer-template complex and begins to add nucleotides (dNTPs) to the 3' end of the primer. This normally takes place at 72°C and the duration of this step depends on the length of the DNA segment being copied, usually between 30 seconds to 5 minutes.

This cycle is repeated between 20–40 times, and with each cycle, the number of DNA copies made increases exponentially. The final PCR product will number in the billions. The amplified PCR product can then be separated using gel electrophoresis which separates the DNA fragments according to the length of the products.

Below is the gene sequence for the instability region for the human huntingtin gene on chromosome 4, with the CAG trinucleotide repeat in the middle.

GAGTCGGCCCGAGGCCTCCGGGGACTGCCGTGCCGGGCGGGAGACCGCCATGGCG
ACCCTGGAAAAGCTGATGA**AGGCCTTCGAGTCCCTCA**AGTCCTTCCAGCAGCAGC
AGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAACAGC
CGCCACCGCCGCCGCCGCCGCCGCCTCCTCAGCTTCCTCAGCCGCCGCCGCAG
GCACAGCCGC**TGCTGCCTCAGCCGC**AGCCGCCCCCGCCGCCGCC

1. The positions of the forward and reverse primers are **bolded** in the sequence above.

2. Using a highlighter, highlight the region of instability of CAG repeats.

3. In order to calculate the length of the gene fragment amplified by PCR, you will need two pieces of information:

   a. the number of bases in the gene sequence that is amplified that **does not include** the region of instability (CAG repeats)

   b. and the number of trinucleotide repeats

4. Calculate the number of bases in the gene sequence above. This number will remain the same for all chromosomes.

   a. Count the number of bases starting with the forward primer and ending with the reverse primer. Do not count the region that is highlighted.

   Number of bases _____

5. Using your pencil mark every CAG repeat. Count the number of repeats that are marked; this is the region of instability and varies among individuals. To figure out the number of bases; multiply the number of repeats by 3.

   a. **Number of repeats** _____

   b. **Number of bases in the region of instability** _____

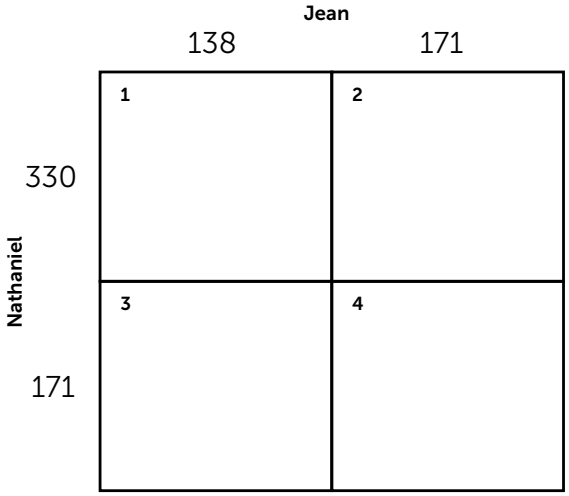
6. Calculate the target fragment size of a PCR product by adding the number of bases in the gene sequence and the number of bases from the trinucleotide repeats.

Number of bases in the gene sequence	Number of CAG repeats	Number of bases on CAG repeat area	Total fragment size of PCR product

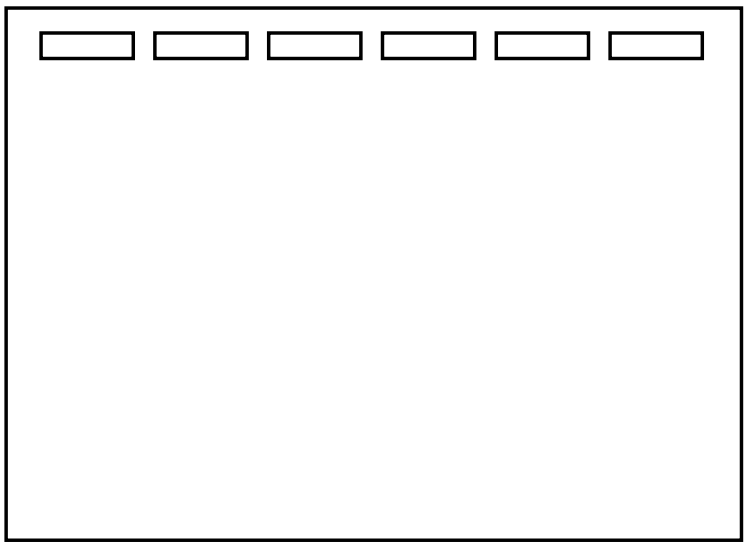
7. Using this information, calculate the expected fragment size to identify Nathaniel and Jeans trinucleotide repeat count. Recall that Jean's genotype was (8, 19) and Nathaniel's genotype was (72, 19).

Person	Number of bases in the gene sequence	Number of CAG repeats	Number of bases on CAG repeat area	Total fragment size of PCR product
Jean		8		
Jean		19		
Nathaniel		72		
Nathaniel		19		

8. Using the data from the chart in question 7 complete a punnet square for possible genotypes for Jean and Nathaniel's offspring:



9. What would the bands look like in each of the four outcomes you predicted for Jean and Nathaniel's offspring? Draw band patterns for the possible outcomes on the gel below.



Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____

Analysis Questions

1. What does PCR stand for and what is the purpose of this method?

2. What can you conclude if one of Jean and Nathaniel's offspring had a PCR fragment of the huntingtin instability region that was 455bp in size? How many repeats would that child have in that gene? Can you predict his/her age of onset of Huntington's disease? Use data from the pedigree to support your claim.

3. What can you conclude if one of Jean and Nathaniel's offspring had a PCR fragment of the huntingtin instability region that was 272bp in size? How many repeats would that child have in that gene? Can you predict his/her age of onset of Huntington's disease? Use data from the pedigree to support your claim.

Day 3 - Gel Electrophoresis and Data Analysis

Gel Electrophoresis

Gel electrophoresis is a technique used in many areas of science to separate and analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after the agarose gel is solidified. This creates wells into which the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive (⊕ anode) and negative (⊖ cathode) electrodes. A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the ⊖ cathode and negatively charged molecules migrate toward the ⊕ anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its size. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

Pre-Lab Questions

1. What is DNA and what does it do?

2. What are chromosomes and how many copies do you have in each of your cells? Who did they come from?

3. How much DNA do you share with each of your parents? How much DNA do you share with your siblings?

4. What are some characteristics or properties of DNA?

Part I: Electrophoresis

Materials

MiniOne® Casting System

1 MiniOne® Electrophoresis System

1 agarose GreenGel™ cup (2%)

5 samples (4 DNA and (1) 100 bp marker)

TBE running buffer (135 mL)

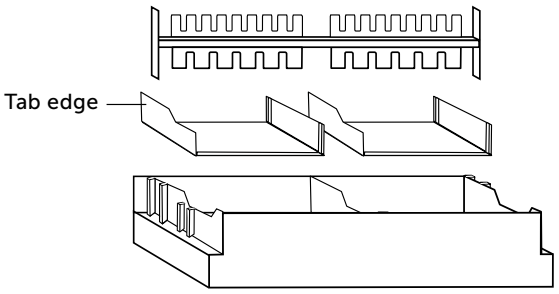
1 micropipette (2–20 µL) and 6 pipette tips

SAMPLE LOADING CHART:

Well	Sample	Volume in µL
1	100 bp marker	10 µL
2	Nathaniel (N)	10 µL
3	Jean (J)	10 µL
4	Peter (P)	10 µL
5	Kim (K)	10 µL

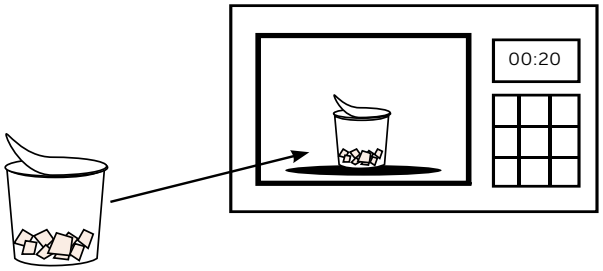
How to Cast a Gel

1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down.

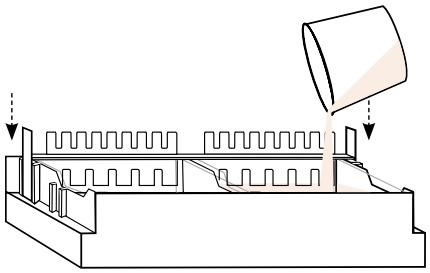


2. Partially peel the film off a GreenGel™ cup and microwave for 20 seconds. Allow to cool for 15 seconds. **DO NOT microwave more than 5 gel cups at a time.**

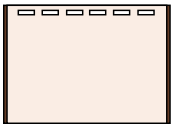
   ⚠**Safety requirement:** Adult supervision required if students are handling gel cups!

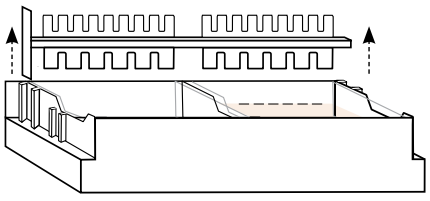


3. **One gel cup is for making one agarose gel!** Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque. **DO NOT disturb the gel until time is up.**



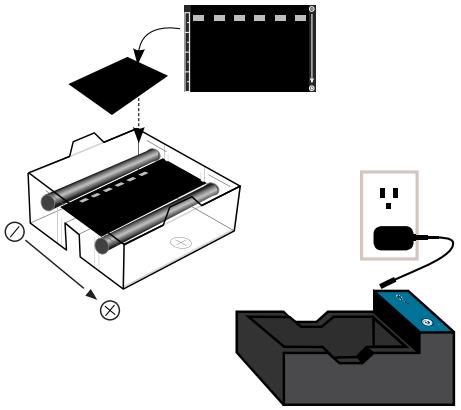
4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.





How to Load a Gel

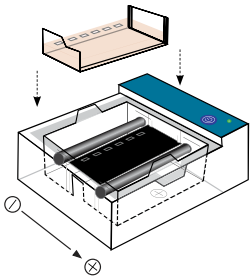
1. Ensure the black viewing platform is in the tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.

3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

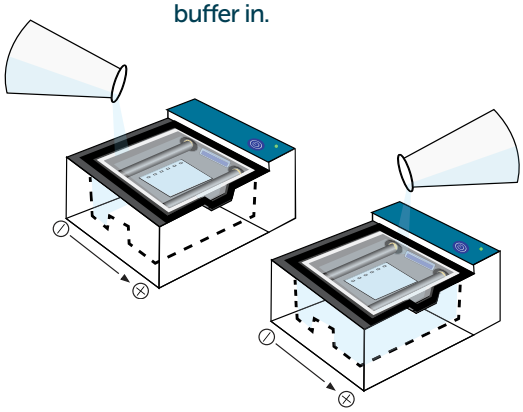
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.



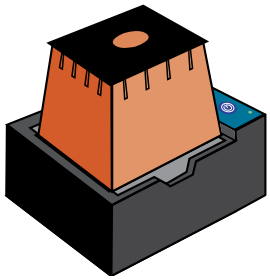
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

5. Turn the low intensity blue LED on by pressing the button on the carriage.

6. Measure 135 mL of TBE running buffer and pour into **one side** of the tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the other side of the gel tank.

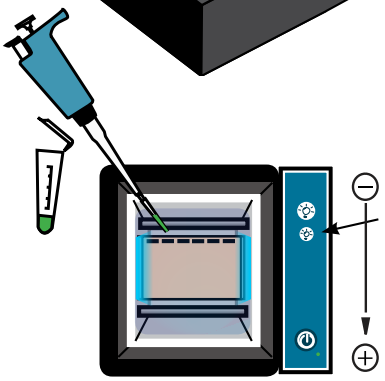


7. Place photo hood on the carriage.



8. Press the power button which should now be a solid green light. If **green light is solid,** turn off the unit and proceed to loading gels.

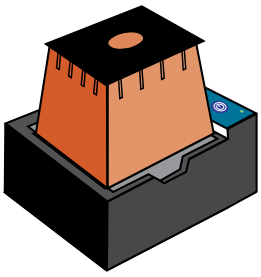
9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading.



10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

Run, Visualize and Capture Image

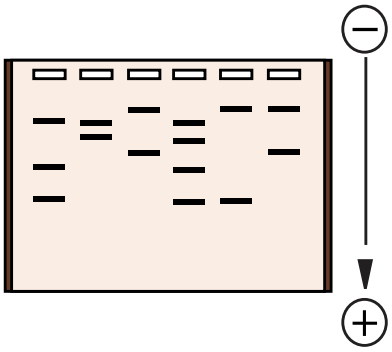
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.



The green power LED will not turn on if:

- The tank is not properly placed inside the carriage
- There is no buffer in the tank
- The buffer is too concentrated or too diluted
- The photo hood is not on the carriage
- There is too much or too little running buffer
- The power supply is not plugged in. Check by turning on the blue LEDs

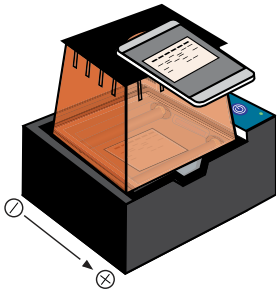
2. Check the migration of the bands (~every five minutes).



3. Allow the gel to run for approximately 25 minutes or until DNA separation is sufficient. After your run is complete, turn off the power by pressing the button. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.

4. **Document your results.**

Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.)

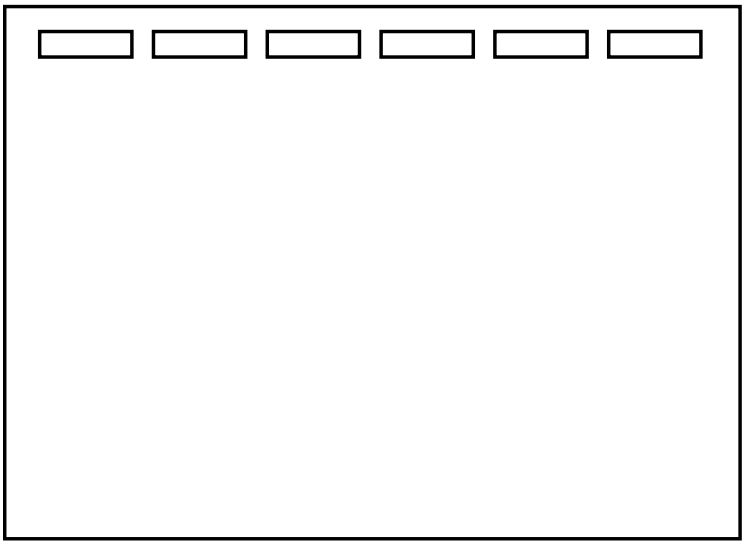


Clean Up

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage. Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or kimwipe to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for cleanup and storage.

Part II: Results

What does your gel look like? Record images of the gel.



Lane 1: _____

Lane 2: _____

Lane 3: _____

Lane 4: _____

Lane 5: _____

The marker has the following number of base pairs:

100, 200, 300, 400, 500, 600, 700, 800, 900, and 1,000

Part III: Analyze Your Data

1. In your own words, explain how gel electrophoresis works.

2. What electrical charge did your samples carry? How do you know?

3. What can you say about the number of repeats each child has for each of their chromosomes? Use the gel to provide evidence of your answer.

4. What can you conclude about the allele that Peter inherited from his father, Nathaniel? What can you deduce about Peter's age of onset of symptoms of Huntington's disease? Use data, add from the pedigree to support your answer.

Appendix A - Glossary

Term	Definition
Allele	One of two or more distinct forms of a gene located at the same position on homologous chromosomes.
Annealing	As the temperature of a PCR reaction is lowered, short pieces of DNA, called primers, bind to specific sequences within the genome targeting this region to be copied. Annealing temperature is specific to the primers used in your reaction—typical settings are 45–65°C for 5–30 seconds per cycle.
Cycle	A cycle refers to one round of denaturation, annealing, and extension steps of the PCR reaction. The number of cycles needed for a particular reaction will depend on how much DNA you are starting out with and how much DNA you are trying to produce. With high starting concentration, 20–25 cycles is sufficient to produce enough DNA to visualize on a gel. Where the starting concentration is low or large quantities of product are needed, 35–40 cycles can be used.
Denaturation	Denaturation uses high temperature to break the bonds between bases on opposing strands. Double-stranded DNA is split into single-stranded DNA exposing the bases so they can be copied. Typical settings are 90–98°C for 5–30 seconds per cycle.
dNTPs	Nucleotides, the molecular building blocks of DNA.
Extension	At around 70°C the polymerase gets to work and starts adding nucleotides (dNTPs) to the 3' end of the annealed primers, copying the complementary strand. Typical settings are 72°C for 5 seconds– 5 minutes per cycle.
Genotype	The genetic makeup of an individual organism.
Phenotype	The set of observable characteristics of an individual organism arising from the genotype and the environment.
Primers	Short pieces of DNA with sequences complementary to the sequences flanking the region to be copied. Primers are designed specifically for every PCR reaction taking many variables into account, including length, nucleotide content, and structural features. Many computer tools are available to assist in primer design.
Template	DNA containing the sequence that will be copied in a PCR reaction. Can be a short fragment or a whole genome.
Thermal Cycler	Also called a PCR machine, a thermal cycler is an instrument that automatically changes the temperature of the PCR reaction according to a program set up by the user. It heats and cools the reaction between denaturation, annealing, and extension temperatures over a specified number of cycles.

Appendix B - References and Recommended Reading

Chial, Heidi. "Huntington's disease: The discovery of the Huntingtin gene." *Nature Education*, vol. 1, no. 1, 2008, pp. 71

"Huntington's Disease and Predictive Testing." *National Society of Genetic Counselors*, www.aboutgeneticcounselors.com/Genetic-Conditions/Huntingtons-Disease-and-Predictive-Genetic-Testing

"Huntington's Disease." *Mayo Clinic,* Mayo Foundation for Medical Education and Research (MFMER), 16 May 2018, www.mayoclinic.org/diseases-conditions/huntingtons-disease/symptoms-causes/syc-20356117

"Huntington Disease." *Genetics Home Reference,* U.S. National Library of Medicine, National Institutes of Health,15 January 2019, https://ghr.nlm.nih.gov/condition/huntington-disease

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