EDVOTEK®

THE **BIOTECHNOLOGY EDUCATION** COMPANY®

Includes EDVOTEK's All-NEW
EdvoQuick™ DNA Ladder

- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes:
2640, 1400, 1100, 700, 600, 400, 200

Edvo-Kit #
1125

Edvo-Kit #1125

Detecting Huntington's Disease

LyphoTemplate™

LyphoPrimer™

Experiment Objective:

In this experiment, students will conduct a DNA fingerprinting exercise on simulated patient samples to determine if family members are heterozygous or homozygous for Huntington's Disease. Students will then analyze the amplified DNA segments by agarose gel electrophoresis.

See page 3 for storage instructions.

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Table of Contents

Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

Experiment Components

	Component	Storage	Check (✓)
A	LyphoPrimer™ Mix	Freezer with desiccant	❑
B	EdvoQuick™ DNA Ladder	Freezer	❑
C	Positive DNA Control	Freezer with desiccant	❑
D	Father DNA	Freezer with desiccant	❑
E	Mother DNA	Freezer with desiccant	❑
F	Daughter DNA	Freezer with desiccant	❑
G	Son DNA	Freezer with desiccant	❑
H	TE Buffer	Freezer	❑
•	PCR EdvoBeads™	Room Temp. with desiccant	❑

Each PCR EdvoBead™ contains:
- *dNTP Mixture*
- *Taq DNA Polymerase*
- *$MgCl_2$*
- *Reaction Buffer*

Experiment #1125 contains enough reagents to amplify 25 DNA samples using the Polymerase Chain Reaction. This represents five complete sets of reactions.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

LyphoTemplate™

LyphoPrimer™

Reagents & Supplies *(Included with this experiment)*

Store all components below at room temperature.

Component	Check(✓)
• UltraSpec-Agarose™	❑
• Electrophoresis Buffer (50X)	❑
• SYBR® Safe Stain	❑
• FlashBlue™ Stain	❑
• Microcentrifuge Tubes	❑
• 0.2 mL PCR tubes	❑

Requirements *(NOT included with this experiment)*

- Thermal cycler (EDVOTEK Cat. #541 highly recommended)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Microcentrifuge
- UV Transilluminator or Blue Light visualization (EDVOTEK Cat. #558 or #557 highly recommended)
- White light visualization system (OPTIONAL - use if staining with FlashBlue™)
- UV safety goggles
- Automatic micropipettes (5-50 µL) with tips
- Microwave
- 250 mL flasks or beakers
- Hot gloves
- Disposable vinyl or latex laboratory gloves

Background Information

HUNTINGTON'S DISEASE

The nervous system is made of up many different cell types. Neurons are the cells responsible for processing information in the brain and are incredibly large and complex. In humans, a single neuron can be up to 1 meter long. Different areas of a neuron are responsible for different cellular processes. A neuronal dendrite receives information, and an axon sends information. Given a neuron's size and complexity, a mutation in a protein that is expressed in every cell oftentimes preferentially affects neurons. This is the case with Huntington's disease, a neurodegenerative disease that causes motor movement problems and impaired mental status.

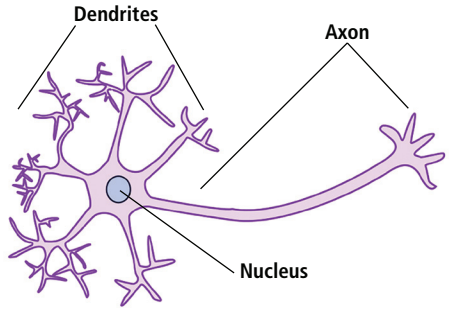


Figure 1

The brain is divided into regions based on location and responsibility. In Huntington's disease, the area of the brain that is affected is known as the basal ganglia. Neurons in the basal ganglia have a number of responsibilities, including inhibiting certain movements so that we can make very precise motions. In this way, we're able to sit still, accurately pick up a water bottle, or type on a keyboard. However, these neuron are affected during Huntington's Disease, and the inhibition becomes lifted. This results in erratic and uncontrolled movements known as chorea. Huntington's disease is caused by an autosomal dominant mutation in the *HTT* gene, resulting in a mutated huntingtin protein.

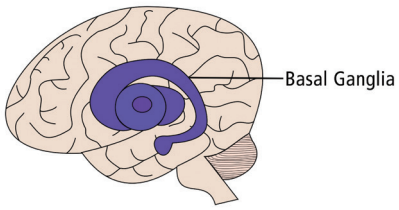


Figure 2: Basal Ganglia and Related Structures of the Brain

The Huntingtin Protein

The huntingtin protein is expressed in every cell of the human body. It is a large protein and is involved with almost every process within the cell. Indeed, it has been shown to interact with over 200 other proteins! Considering this complexity, research is still underway to describe all the different functions of the huntingtin protein in order to better understand how the mutation causes the disease.

In Huntington's Disease, there is a nucleotide expansion in the gene that encodes the huntingtin protein, *HTT*. The N-terminus of the *HTT* gene contains CAG repeats. The CAG nucleotide order (cytosine-adenine-guanine) codes for the amino acid glutamine, which is represented as a Q. The normal huntingtin protein

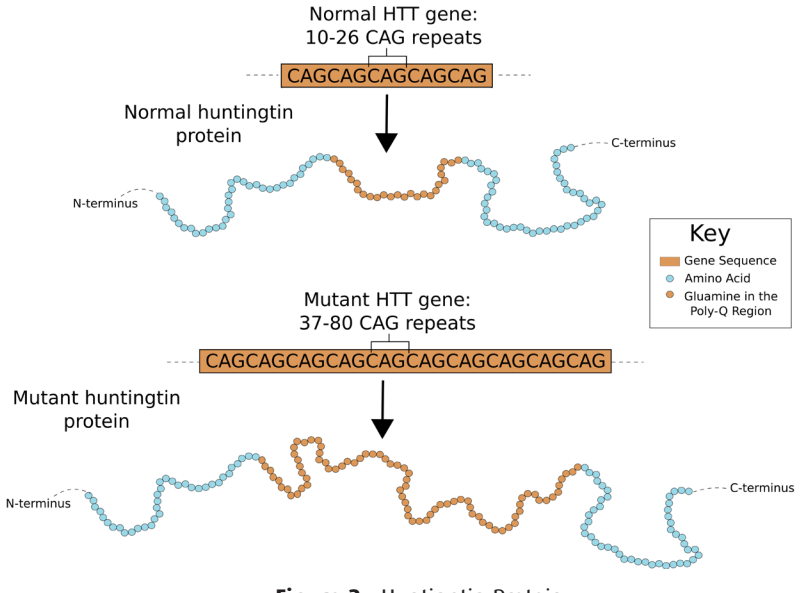


Figure 3: Huntingtin Protein

contains less than 26 Q's in a row. During Huntington's Disease, this is expanded to 40 or more Q's. These Q amino acids interfere with the ability of huntingtin to perform its normal functions and disrupt many of its binding partners. This increase in the amount of glutamines, or "poly-Q expansion" is characteristic of other neurodegenerative diseases as well including spinocerebellar ataxia and Machado-Joseph disease. In Huntington's disease, the poly-Q expansion follows Mendelian genetics and is autosomal dominant.

Mendelian Genetics

In the mid-1800's, Augustine monk Gregor Mendel established basic laws of genetics through careful experimentation using garden peas. Mendel's first law, the law of segregation, states that alternative forms of the same gene, called alleles, control the differences in the pea plants. Each offspring has two copies of the gene, one inherited from each parent. Next, he realized that alleles are dominant or recessive. When a dominant allele is inherited, it will mask the trait coded by the recessive allele. To impart the recessive trait, both alleles must be the recessive type. The second law of inheritance, the law of independent assortment, states that each pair of alleles will segregate separately from one another. Inheritance of a single gene can be illustrated with a two-by-two grid known as a Punnett Square (Figure 3). The alleles carried by one parent are placed across the top of the grid (columns), and the alleles contributed by the other parent are placed down the side of the grid (rows). By convention, the dominant allele is denoted by an upper-case letter P and the recessive allele by a lower-case letter p. Next, the parental alleles are used to fill in the grid. Each box in the grid is assigned the allele at the head of its column and row. For example, assuming each parent carries one dominant allele and one recessive allele, the Punnett Square predicts that one of the plants will receive two dominant alleles, two of the plants will receive one dominant and one recessive allele, and one will receive two recessive alleles. This represents the genetic makeup, or genotype, of the offspring.

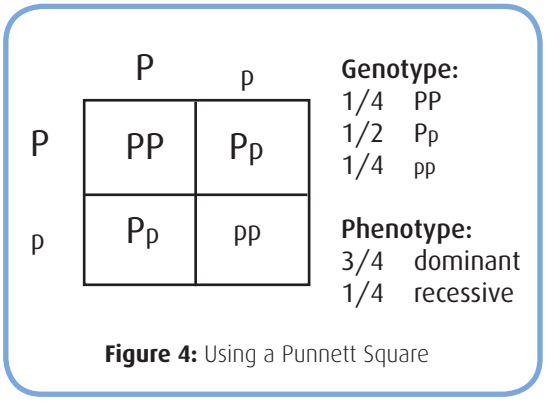


Figure 4: Using a Punnett Square

The *HTT* gene follows this rule, and the poly-Q expansion is dominant. Therefore, if a parent carries just one mutated allele, there is a 50% chance that it will be transmitted to their child. The symptoms of Huntington's disease often don't start until ages 30-50, therefore many people have already had children when they are diagnosed. That brings up the potential for the children in the family to be tested for the mutated allele.

Detecting Huntington's Disease

Given that Huntington's Disease results from a mutation within the DNA sequence, it can be detected using DNA sequencing methods. In this lab, a family that may have Huntington's Disease will be diagnosed using the polymerase chain reaction (PCR) and gel electrophoresis to compare the size of their *HTT* alleles.

In 1984, Dr. Kary Mullis revolutionized the field of molecular biology when he devised a simple and elegant method to copy specific pieces of DNA. Mullis recognized that he could replicate DNA in vitro using short, synthetic DNA oligonucleotides (known as primers) and DNA polymerase I in a process similar to DNA replication in a cell's nucleus. Because researchers can customize the primers to target a specific gene, this method allows for the rapid amplification of a selected DNA sequence. For the development of this technique, known today as the Polymerase Chain Reaction (or PCR), Mullis was awarded the Nobel Prize in Chemistry in 1993. Before performing PCR, template DNA is extracted from a biological sample. Two primers are designed to correspond to the 5′ and 3′ ends of the target sequence. The template DNA and primers are mixed with buffer, the four "free" deoxynucleotides (dATP, dCTP, dGTP, and dTTP), and a thermostable DNA polymerase (Taq). Next, the PCR mixture is subjected to sequential heating/cooling cycles at three different temperatures to amplify DNA.

In the first step, known as "denaturation", the mixture is heated to 94° C to disrupt the hydrogen bonds between the complementarity strands. This causes the target DNA to unzip into single strands (or melt). It is important to use a thermostable DNA polymerase for PCR because this enzyme remains stable at high temperatures. In the second step, known as "annealing", the reaction mixture is cooled to 45° C - 65° C. This allows the primers to base pair with the target DNA sequence. In the third step, known as "extension", the temperature is raised to 72° C. This temperature is optimal for Taq polymerase to add nucleotides to the 3' end of the primer, synthesizing a new strand of DNA.

Together, these three steps - denaturation, annealing, and extension – make up one PCR "cycle" (Figure 5). To simplify this process, a specialized machine, called a "thermal cycler" or a "PCR machine", was created to heat and cool the samples rapidly. Each PCR cycle doubles the amount of the target DNA in less than five minutes. This makes PCR a very sensitive technique, as only a few copies of the template DNA are required to produce a large amount of signal.

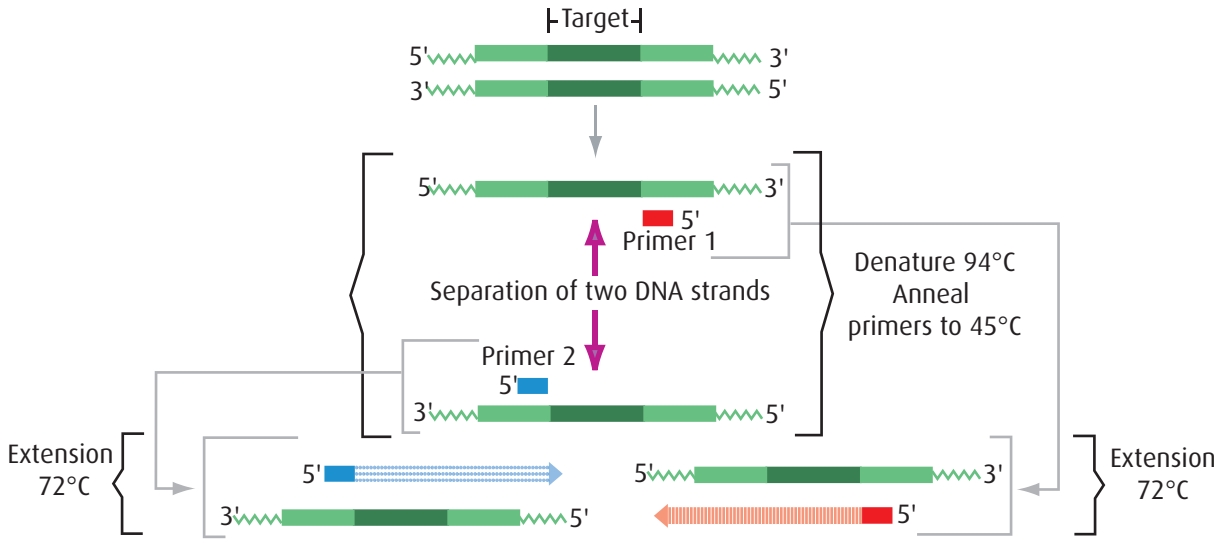


Figure 5: Polymerase Chain Reaction

A Family with Huntington's

Recently, a man age 55 came to the neurology clinic exhibiting chorea and impaired mental abilities. While taking his history, the doctor hears that his mother also exhibited these symptoms before she passed away. They suspect that he may have Huntington's Disease. After a discussion with him, his wife, his son, and his daughter, the family chooses to all be tested for Huntington's Disease. A blood sample is taken, and DNA is purified away from the rest of the cells. In order to test for Huntington's Disease, the DNA will have to be amplified by PCR and separated using agarose gel electrophoresis. The primers will be towards the *HTT* gene. Because larger DNA fragments run slower on a DNA gel, if anyone in the family has an expanded *HTT* gene due to CAG repeats, that DNA will be of a higher molecular weight. You will compare each family member's DNA against a positive control, which is the mutated *HTT* allele with 45 CAG repeats.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will conduct a DNA fingerprinting exercise on simulated patient samples to determine if family members are heterozygous or homozygous for Huntington's Disease. Polymorphic regions of DNA are amplified using PCR. Students will then analyze the amplified DNA segments by agarose gel electrophoresis.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Module I: 2 hours
Amplification of Extracted DNA by PCR

↓

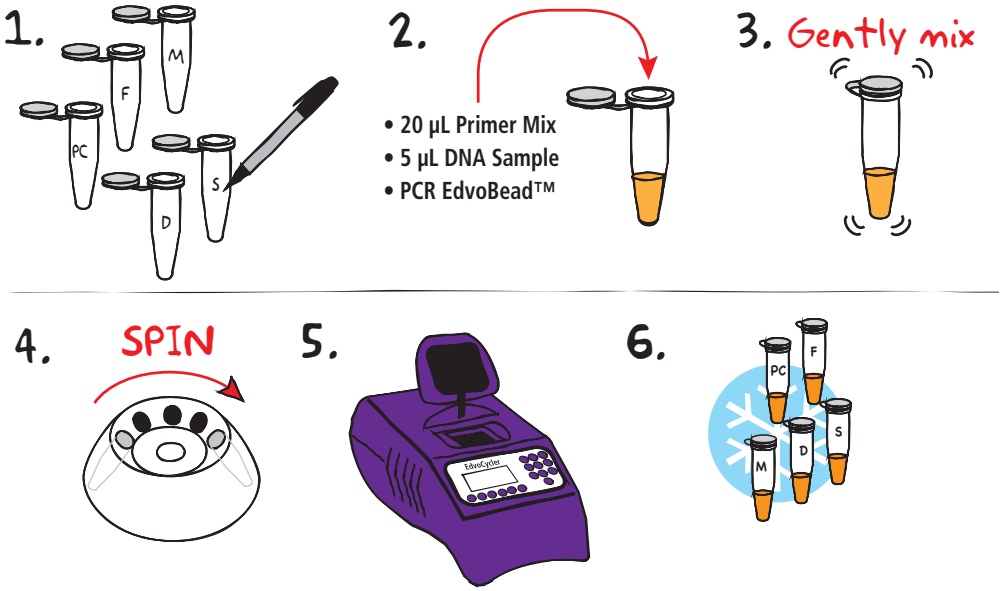
Module II: 50-70 min.
Separation of PCR Product by Electrophoresis

↓

Module III : 10-30 min.
(OPTIONAL) Staining Agarose Gels with FlashBlue™

NOTE: Experimental times are approximate.

Module I: PCR Amplification of a Family's DNA



1. **LABEL** 5 PCR tubes for: Positive DNA Control (PC), Father DNA (F), Mother DNA (M), Daughter DNA (D), and Son DNA (S). Put your initials or group number on the tubes.
2. All PCR reactions should be prepared as follows: **ADD** 20 µL primer mix (yellow), 5 µL DNA sample (red) and one PCR EdvoBead to the appropriately labeled 0.2 mL PCR tube.
3. **MIX** each PCR sample. Make sure the PCR EdvoBeads™ are completely dissolved. ***NOTE: Double-check that both the primer and DNA have been added by looking at the color of the mixture in the PCR tube. The mixture should be orange with the primer and DNA mixed together.***
4. **CENTRIFUGE** the samples for a few seconds to collect the sample at the bottom of the tubes.
5. **AMPLIFY** the DNA using PCR:

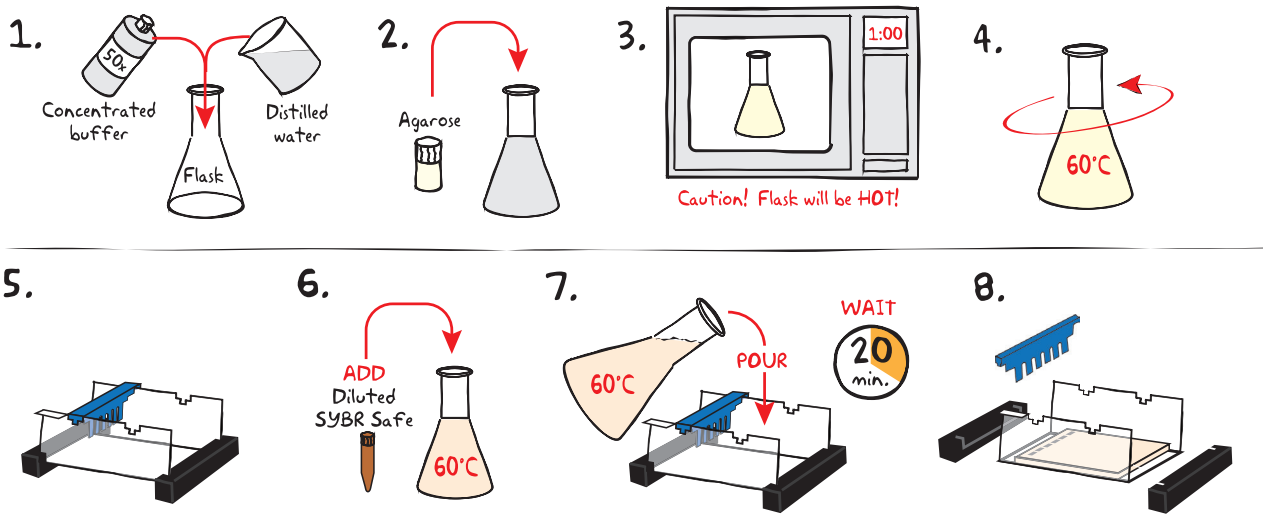
   PCR cycling conditions:
   Initial denaturation 94°C for 3 minutes
   94° C for 30 seconds
   55° C for 65 seconds } 30 cycles
   72° C for 30 seconds
   Final Extension 72° C for 4 minutes

6. After PCR, **PLACE** the tubes on ice. **PROCEED** to Module II: Separation of PCR Products by Electrophoresis.

OPTIONAL STOPPING POINT

The PCR samples may be stored at -20° C for electrophoresis at a later time.

Module II: Separation of PCR Products by Electrophoresis



PREPARING THE AGAROSE GEL WITH SYBR® SAFE STAIN

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** the agarose to 60° C by carefully swirling the flask to promote even dissipation of heat.
5. While the agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the comb in the appropriate notch.
6. Before casting the gel, **ADD** diluted SYBR® Safe stain to the cooled molten agarose and swirl to mix (see Table A).
7. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
8. **REMOVE** the end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Wear gloves and safety goggles

IMPORTANT:

7 x 7 cm gels are recommended. Place the comb in the first set of notches.

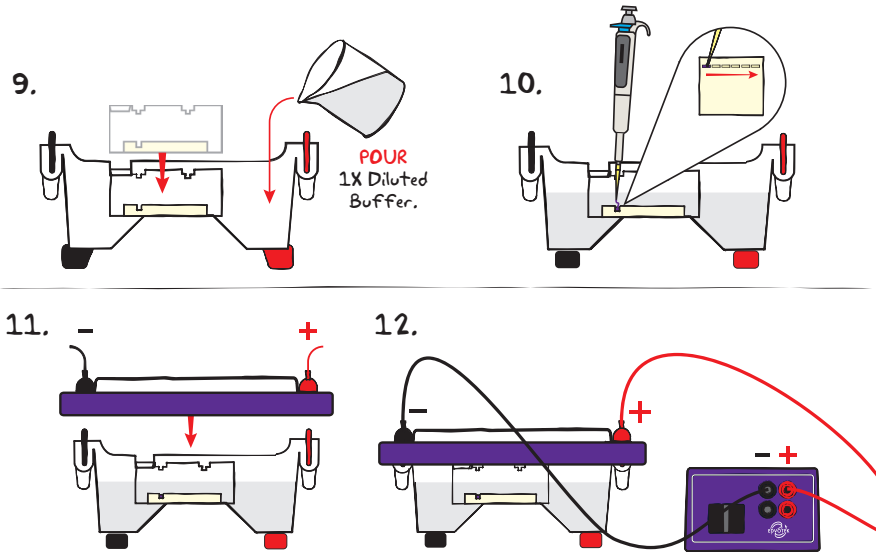
If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

Table A

Individual 1.0% UltraSpec-Agarose™ Gel with SYBR® Safe Stain

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume	Diluted SYBR® (Step 6)
7 x 7 cm	0.5 mL	24.5 mL	0.25g	25 mL	25 µL
7 x 14 cm	1.0 mL	49.0 mL	0.50 g	50 mL	50 µL

Module II: Separation of PCR Products by Electrophoresis, continued



Reminder:

Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

Wear gloves and safety goggles

RUNNING THE GEL

9. **PLACE** the gel (on the tray) into the electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
10. **LOAD** the entire volume (25 µL) into the well in the order indicated by Table 1, right.
11. **CHECK** that the gel is properly oriented, then **PLACE** the safety cover onto the chamber. Remember, the DNA samples will migrate toward the positive (red) electrode.
12. **CONNECT** the leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
13. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

Table 1: Gel Loading

Lane 1	EdvoQuick™ DNA Ladder
2	Positive Control
3	Father DNA
4	Mother DNA
5	Daughter DNA
6	Son DNA

OPTIONAL STOPPING POINT:
Gels can be stored for several days. Place gel in a watertight plastic bag with 2 mL of electrophoresis buffer and store in the refrigerator.

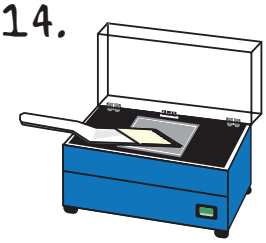
Table B: 1x Electrophoresis Buffer (Chamber Buffer)

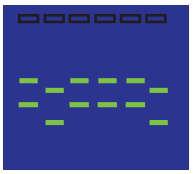
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 mL	6 mL	294 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

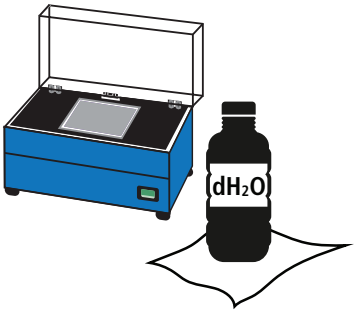
Table C: Time and Voltage Guidelines (1.0% – 7 x 7 cm Agarose Gel)

Volts	Recommended Time: Minimum	Maximum
150	15 min.	20 min.
125	20 min.	35 min.
70	35 min.	1 hour

Module II: Separation of PCR Products by Electrophoresis, continued

14. 

15. 

16. 

VISUALIZING THE SYBR® GEL

14. **SLIDE** the gel off the casting tray onto the viewing surface of the transilluminator and turn the unit on. **ADJUST** the brightness to the desired level to maximize band visualization. DNA should appear as bright green bands on a dark background.

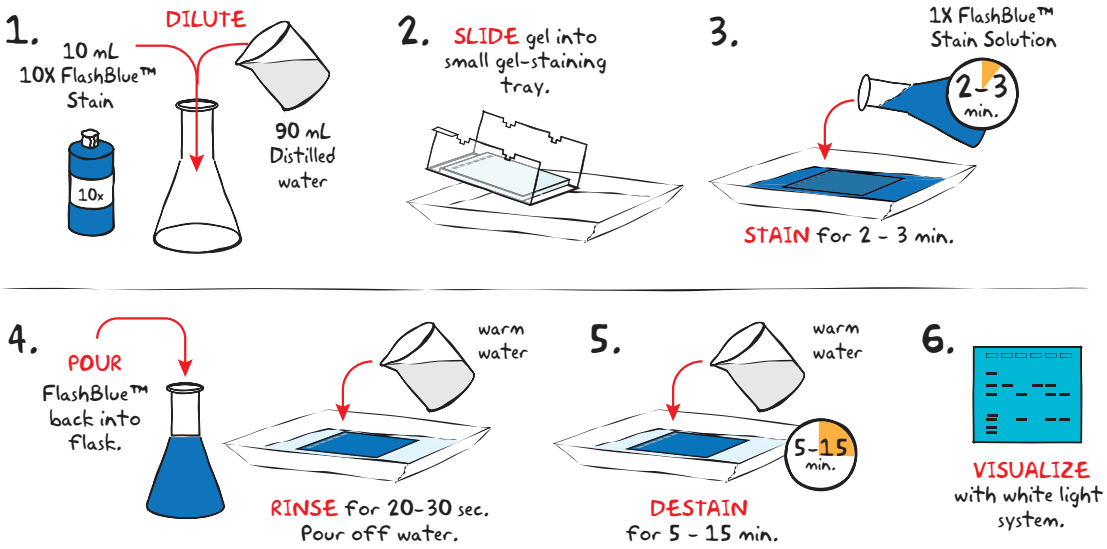
15. **PHOTOGRAPH** the results.

16. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.

Be sure to wear UV goggles if using a UV transilluminator.

Module III: Staining with FlashBlue™ Stain (OPTIONAL)

FlashBlue™ Stain is a simple and effective visible DNA stain that can be used as an alternative, or in addition to, UV-reactive DNA stains like SYBR® Safe. ***IF staining with both SYBR® Safe and Flash Blue, you must examine and record the SYBR® Safe bands before beginning the FlashBlue™ Staining.***



1. **DILUTE** 10 mL of 10X concentrated FlashBlue™ with 90 mL of distilled water in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1X FlashBlue™ stain solution. **STAIN** the gel for 2-3 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 3 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **POUR** the 1X FlashBlue™ back into the flask (the stain can be reused). **COVER** the gel with warm water (40-45° C). Gently **RINSE** the gel for 20-30 seconds. **POUR** off the water.
5. **COVER** the gel with clean, warm water (40-45° C). **DESTAIN** for 5-15 minutes with gentle shaking (longer periods will yield better results). DNA bands will start to appear after 5 minutes of destaining. Changing the water frequently will accelerate destaining.
6. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Wear gloves and safety goggles

ALTERNATIVE FLASHBLUE™ STAINING PROTOCOL:

1. **DILUTE** 1 mL of 10X FlashBlue™ stain with 499 mL distilled water.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
4. Carefully **REMOVE** the gel from the staining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Study Questions

1. What causes Huntington's Disease?
2. What are the three steps of PCR and what do each accomplish?
3. Who in this family had Huntington's Disease? Are they heterozygous or homozygous?
4. Draw a Punnett square of this family's *HTT* gene.

Instructor's Guide

ADVANCE PREPARATION:

Preparation For:	What to do:	When:	Time Required:
Module I: PCR Amplification of a Family's DNA	Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.)	Up to 2 hours before the experiment.	30 min.
	Program Thermal Cycler	Any time before performing the experiment.	15 min.
Module II: Separation of PCR Product by Electrophoresis	Prepare 1X Electrophoresis Buffer and dilute SYBR® Safe Stain	Up to one day before performing the experiment.	45 min.
	Prepare molten agarose and pour gel (optional)		
Module III: Staining with FlashBlue™ (OPTIONAL)	Prepare staining components	Any time before the class period.	10 min.

Yellow = Prepare shortly before module. Green = Flexible / prepare up to a week before the module.

Pre-Lab Preparations

MODULE I

There are four different DNA samples provided and one positive control. This kit features the NEW EDVOTEK® LyphoPrimer™ and LyphoTemplate™. The reagents are color coded so that a correctly assembled PCR reaction should appear orange in color. These innovations will help ensure experimental success.

NOTE:
There is enough material to perform 25 PCR reactions and 5 gels. Students can be divided into groups of five students per group and samples from each group can be run on a gel.

FOR MODULE I
Each Group should receive:
- 5 PCR tubes
- 5 PCR EdvoBeads™
- 7 µL Positive Control
- 7 µL Father DNA
- 7 µL Mother DNA
- 7 µL Daughter DNA
- 7 µL Son DNA
- 120 µL Diluted Primer Mix

Preparation of the DNA Template

1. Add 50 µL TE Buffer (H) to each LyphoTemplate™ and mix to dissolve.

2. Label twenty-five 1.5 mL snap-top microcentrifuge tubes as follows:
   5 - Positive DNA Control (Label "PC").
   5 - Father DNA (Label "F").
   5 - Mother DNA (Label "M").
   5 - Daughter DNA (Label "D").
   5 - Son DNA (Label "S").

3. Dispense 7 µL of each of the samples into the appropriately labeled tubes.

Preparation of the Primer

1. Add 1 mL of TE Buffer (H) to the tube of LyphoPrimer™ Mix (A). Cap tube and mix. The solution should be light yellow and no solid pieces should remain.

2. Pipette 120 µL of the diluted Primer Mix into five labeled snap-top microcentrifuge tubes.

3. Distribute one tube of diluted Primer Mix to each student group.

Programming the Thermal Cycler

The Thermal cycler should be programmed as outlined in Module I in the Student's Experimental Procedure.

- Accurate temperatures and cycle times are critical. A pre-run for one cycle (takes approximately 3 to 5 min.) is recommended to check that the thermal cycler is properly programmed.

- For thermal cyclers that do not have a heated lid, it is necessary to place a layer of wax or mineral oil above the PCR reactions in the microcentrifuge tubes to prevent evaporation. Visit **www.edvotek.com** for more information.

Pre-Lab Preparations

MODULE II: SEPARATION OF PCR PRODUCTS BY ELECTROPHORESIS

Preparation of Agarose Gels:

This experiment requires one 1.0% agarose gel per student group. For best results, we recommend using 7 x 7 cm gels. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Individual Gel Preparation
Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module II in the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

Batch Gel Preparation
To save time, a larger quantity of agarose solution can be prepared for sharing by the class (see Appendix B).

SYBR® Safe Stain Preparation
Prepare diluted SYBR® Safe by adding 250 µL of 1X TAE electrophoresis buffer to the tube of concentrated SYBR® Safe and tapping the tube several times to mix. For individual gel preparation, each group will need 25 µL of the diluted SYBR® Safe for a 7 x 7 cm gel. For Batch Gel Preparation, you will use the entire tube of SYBR® Safe (see Appendix B).

Preparing Gels in Advance
Gels may be prepared ahead and stored for later use. Solidified gels can be stored for up to a week in the refrigerator in plastic bags containing a small amount of buffer to prevent drying. We recommend adding only 2 mL of buffer to the bag to prevent SYBR® Safe Stain from diffusing out of the gel.

Do not store gels at -20° C because freezing will destroy them.

Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

Additional Materials
Each 1.0% gel should be loaded with the EdvoQuick™ DNA ladder and PCR reactions from one student group.

- Pipette 30 µL of the EdvoQuick™ DNA ladder (B) into labeled microcentrifuge tubes and distribute one tube of EdvoQuick™ DNA ladder per gel.

NOTE:
Accurate pipetting is critical for good experiment results. This experiment is designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students do not know how to use micropipettes, we recommended performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this advanced level experiment.

FOR MODULE II
Each Group should receive:
- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™ Powder
- 25 µL diluted SYBR® Safe Stain
- 30 µL EdvoQuick DNA ladder
- PCR Samples from Module I

NOTE:
QuickGuide instructions and guidelines for casting various agarose gels can be found our website. **www.edvotek.com/quick-guides**

Pre-Lab Preparations

MODULE III: (OPTIONAL) STAINING WITH FLASHBLUE™ STAIN

FlashBlue™ can be used as an alternative or in addition to SYBR® Safe in this experiment. If only staining with FlashBlue™, you can omit SYBR® Safe from the gel preparation. However, FlashBlue™ is less sensitive than SYBR® Safe and will take a longer time to obtain results. Alternatively, gels can be visualized first with SYBR® Safe and then with FlashBlue™.

Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to develop in the destaining solution, resulting in dark blue DNA bands that contrast with a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks if they are refrigerated, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels should be discarded in the garbage and destaining solutions should be disposed of down the drain.

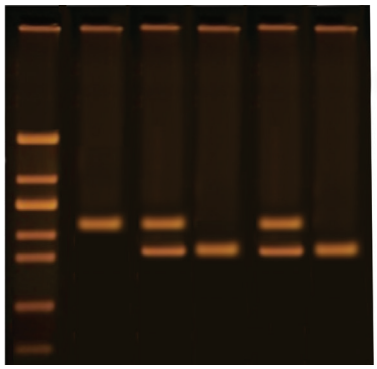
Wear gloves and safety goggles

FOR MODULE III
Each Group should receive:
- 10 mL 10x concentrated FlashBlue OR 100 mL 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

Photodocumentation of DNA (Optional)

Once the gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that interface directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

Experiment Results and Analysis



Includes EDVOTEK's All-NEW
EdvoQuick™ DNA Ladder

- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes:
2640, 1400, 1100, 700, 600, 400, 200

Lane	Sample	Genotype
1	EdvoQuick™ DNA Ladder	---------
2	Positive DNA Control	45 CAG repeats: homozygous dominant
3	Father DNA	Heterozygote
4	Mother DNA	WT (Wild-Type): homozygous recessive
5	Daughter DNA	Heterozygote
6	Son DNA	WT (Wild-Type): homozygous recessive

Please refer to the kit insert for the Answers to Study Questions

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Electrophoresis Buffer and Agarose Gels

Safety Data Sheets can be found on our website:
www.edvotek.com/safety-data-sheets

Appendix A
EDVOTEK® Troubleshooting Guides

PCR AND ELECTROPHORESIS

PROBLEM:	CAUSE:	ANSWER:
There is very little liquid left in tube after PCR.	Sample has evaporated.	Make sure the heated lid reaches the appropriate temperature.
		If your thermal cycler does not have a heated lid, overlay the PCR reaction with wax (see www.edvotek.com for more info).
		Make sure students close the lid of the PCR tube properly.
	Pipetting error.	Make sure students pipet 20 µL primer mix and 5 µL DNA into the 0.2 mL tubes.
The ladder, control DNA, and PCR products are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
		Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels.
	The gel was not stained properly.	Repeat staining.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
After staining, the ladder is visible but no PCR products are present.	PCR amplification was unsuccessful.	Repeat PCR with fresh PCR EdvoBeads™ and primers.
		Ensure that the thermal cycler has been properly programmed. See Module I for guidelines.
DNA bands were not resolved.	Blue tracking dye **in ladder** should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 3.5 cm (7x7 cm tray) or 6 cm (7x14 cm tray) before staining and visualizing the DNA. (See Table C for time and voltage guidelines.)
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time.	Re-stain the gel with FlashBlue™.

Appendix B
Bulk Preparation of Electrophoresis Buffer and Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities that the whole class can share. Leftover diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in **Table D**.

Table D — Bulk Preparation of 1X Electrophoresis Buffer

50x Conc. Buffer	+	Distilled Water	Total Volume 1X Buffer
60 ml		2,940 ml	3000 ml (3 L)

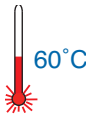
Batch Agarose Gels (1.0%)

For quantity (batch) preparation of 1.0% agarose gels, reference **Table E**.

Table E — Batch Preparation of 1.0% UltraSpec-Agarose™

50x Conc. Buffer	+	Distilled Water	+	Amt of Agarose	=	Total Volume
6.0 ml		294 ml		3.0 g		300 ml
8.0 ml		392 ml		4.0 g		400 ml

1. Use a 500 mL flask to prepare the 1X Diluted gel buffer.
2. Pour the measured UltraSpec-Agarose™ into the prepared buffer. Refer to **Table E** for the mass. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60° C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. If staining with SYBR® Safe, add the entire volume of diluted SYBR® Safe, prepared on page 17, to the cooled agarose. This concentration of SYBR® Safe will work for either 300 mL or 400 mL preparations of agarose gels.
7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 25 mL for each 7 x 7 cm gel, 50 mL for each 7 x 14 cm gel. ***For this experiment, 7 x 7 cm gels are recommended.***
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis. Alternatively, gels can be stored in water-tight plastic bags with 2 mL of 1X electrophoresis buffer for up to 1 week in the refrigerator.

NOTE:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

NOTE:
QuickGuide instructions and guidelines for casting various agarose gels can be found our website. **www.edvotek.com/quick-guides**