EDVOTEK & PLTW Experiment #418

A Family Affair

(PBS 2.2.6)

Experiment Objective:

In this experiment, students will use agarose gel electrophoresis to explore the genetics of familial hypercholesterolemia and the molecular methods used to identify this disease.

See page 3 for storage instructions.

PLTW-PBS 418.200416

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Experiment Components

Store QuickStrip™ samples (A-H) and SYBR® Safe Stain in the refrigerator upon receipt.

Components		Check (√)
A	DNA Standard Marker	❑
B	Normal DNA Sample	❑
C	FH Control (FF)	❑
D	Patient #1 - Changman, Aki's Father	❑
E	Patient #2 - Jia, Aki's Sister	❑
F	Patient #3 - Aki	❑
G	Patient #4 - Justin, Aki's Brother-in-law	❑
H	Patient #5 - Amy, Aki's Niece	❑
•	SYBR® Safe DNA Stain	❑

Experiment #PLTW-PBS 418 is designed for 10 gels.

STORAGE:

Store QuickStrip™ samples and SYBR® Safe Stain in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

REAGENTS & SUPPLIES

Store the following components at room temperature.

- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer (50x) ❑
- Practice Loading Dye ❑
- FlashBlue™ DNA Stain ❑

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipettes with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 mL flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel staining/destaining)
- UV Transilluminator or Blue Light visualization system (use if staining with SYBR® Safe)
- UV safety goggles (use if staining with SYBR® Safe)
- White light visualization system (use if staining with FlashBlue™)
- Distilled or deionized water
- Computer with Internet access
- Laboratory journal

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.

Introduction

Previously, you researched the functions of cholesterol in the body and the roles LDL and HDL play in regulating cholesterol in the blood. Remember that LDL transports cholesterol to the cells. In some families the risk of heart disease is increased due to a genetic mutation that leads to very elevated levels of LDL in the blood. The genetic defect causes the LDL receptor on cells to be deformed and inefficient at binding LDL. The inefficient uptake or binding of LDL by the receptor results in elevated LDL in the bloodstream. This in turn leads to the accumulation of a fatty substance called plaque in the blood vessels. The plaque accumulation in the arteries can cause blockages in the blood flow which results in heart attacks or strokes.

One genetic disorder affecting the LDL receptor is called familial hypercholesterolemia. In many cases the defect is due to a single mutation in the receptor gene and is inherited as an autosomal dominant trait. Often this mutation is referred to as the FH mutation, because it is the mutation that is most closely associated with familial hypercholesterolemia. Whereas diet and exercise play a huge role in regulating cholesterol levels, more invasive interventions such as medications may be needed to keep this genetic disorder under control.

To detect the FH mutation, DNA is obtained from the patient's blood or saliva; the section of DNA containing the LDL receptor gene is then amplified by the polymerase chain reaction (PCR). The amplified DNA is analyzed to see if there is a mutation. To analyze the DNA, investigators use restriction enzymes to cut the DNA in specific places. By examining the sizes of the DNA fragments obtained after exposing the DNA to the restriction enzymes, it is possible to detect mutations or changes in the DNA. This detection is possible because of Restriction Fragment Length Polymorphism or RFLP. RFLP simply means that when different DNA samples are exposed to the same restriction enzyme, the DNA fragments produced by the enzyme may be different lengths. The different lengths are due to differences in the DNA sequences of the two samples; the DNA sequence differences are a polymorphism. A mutation or change in the DNA sequence can change where the enzyme cuts the DNA, so the DNA fragments are different sizes, or are greater or lesser in number, than in the normal DNA. The resulting DNA fragments can be visualized and analyzed through gel electrophoresis.

In past activities you have analyzed the results of gel electrophoresis experiments. In this activity you will actually use DNA electrophoresis to separate and analyze DNA fragments. Use your final gel to determine if members of Aki's family have familial hypercholesterolemia.

Background Information

Cholesterol is a complex lipid essential for the survival of all animal cells. Its primary function is the stabilization of plasma and organellar membranes. The structure of cholesterol, shown in Figure 1, demonstrates the four-ring backbone found in steroid hormones such as testosterone and estrogens. Cholesterol is also a precursor for vitamin D and for bile salts, which facilitate the digestion of lipids in the intestine.

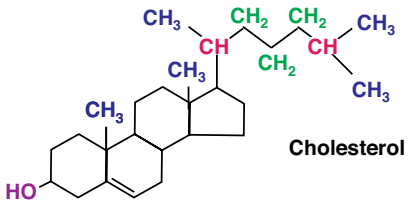


Figure 1:
Chemical Structure of Cholesterol.

Cholesterol is synthesized in the liver and is absorbed in the intestine from ingested food. It is circulated in body fluids in spherical bodies known as lipoprotein particles. These lipoproteins are classified according to their densities, which are determined by gradient centrifugation. Cholesterol processed by the liver is packaged into particles known as very low–density lipoproteins (VLDL), which are processed in the bloodstream to form low-density lipoproteins (LDL). High-density lipoproteins (HDL) take up cholesterol from LDL and peripheral tissues and transport it back to the liver for repackaging or excretion (Figure 2). Because HDL removes cholesterol from the circulation, this is often referred to as "good cholesterol". LDL, in contrast, transport cholesterol from the liver to arteries and is often termed "bad cholesterol".

While cholesterol is essential for life, excess serum cholesterol can have serious negative consequences. The role of elevated blood cholesterol (especially LDL) in cardiovascular disease is well established. LDL can accumulate on arterial walls; LDL is then oxidized by molecules known as *free radicals* that are released from arterial wall membranes. This oxidation process results in the accumulation of inflammatory cells, resulting in the formation of a fatty substance known as plaque. Amassing of plaque can eventually lead to occlusions that restrict blood flow to the heart or brain, resulting in heart attack or stroke.

Correlation between cardiovascular diseases and elevated blood cholesterol, serum cholesterol levels are now routinely tested, both in clinical laboratories and, more recently, home diagnostic tests. Testing can determine levels of total cholesterol, HDL, and LDL. Levels of another potentially harmful lipid known as triglyceride, are also often determined. Elevated cholesterol levels indicate the need for cholesterol reduction by diet and other lifestyle changes and often, cholesterol-reducing medication. New drugs known as *statins* inhibit cholesterol synthesis in the liver. Some studies have even suggested that statins may prevent the onset of elevated cholesterol, prompting some to take these drugs as a preventative measure. Statins have been shown to cause few side effects and may actually lower the risk of other conditions such as kidney and Alzheimer's disease.

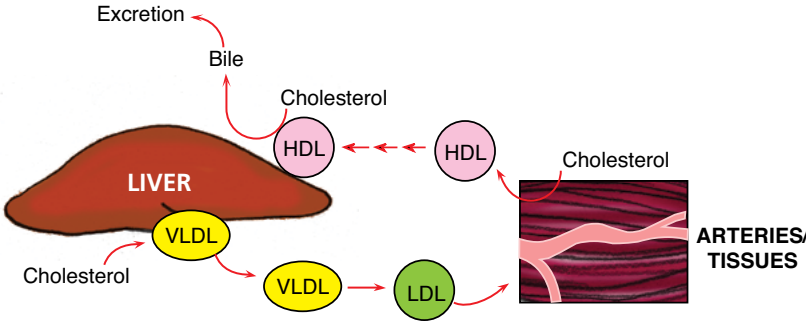


Figure 2: Cholesterol Metabolism.

As mentioned, most circulating cholesterol is found in LDL particles. Animal cells take up LDL from the circulation by a specific receptor. Individuals with a condition known as familial hypercholesterolemia (FH) possess mutations in the gene for the LDL receptor and thus are unable to efficiently remove LDL from the circulation. The result of this deficient uptake is that LDL remains in the circulation and accumulates on arterial walls. Patients who are heterozygous for this mutation still have one functional gene and therefore possess 50% the normal level of receptors of unaffected individuals. Patients who are homozygous for the mutation, however, completely lack the LDL receptor and therefore possess extremely elevated levels of serum cholesterol, often greater than 600 mg per 100 mL of serum (150-200 mg per 100 mL of serum is considered normal). These patients, if untreated, usually die in childhood of coronary artery disease.

FH results from any of hundreds of different mutations in the FH gene. In some ethnic groups, however, the disease may be passed on as a single mutation. The mutation may be detected by a combination of genetic-based diagnostics, such as Polymerase Chain Reaction (PCR) along with Restriction Fragment Length Polymorphism (RFLP) analysis.

In PCR, a specific region of DNA, usually within or near a disease-causing gene, is first amplified. PCR (Figure 4) uses an enzyme known as *Taq* DNA polymerase. This enzyme, purified from a bacterium that is found in hot springs, is stable at high temperatures. In the first step of PCR (denaturation), DNA complimentary strands are separated at 94°C, while the *Taq* DNA polymerase remains stable. In the second step (annealing), the sample is cooled to a temperature between 42°C and 65°C. This "cooling" allows hybridization of a set of two small (15-30) synthetic oligonucleotides, known as "primers", to the target region to be amplified. In this experiment, the target is the FH gene. In the third step (extension), the temperature is raised to 72°C and the *Taq* DNA polymerase then adds nucleotides to the primers to complete each new complimentary strand of the target. These three steps constitute one PCR "cycle". This process is typically repeated from 25-40 cycles, amplifying the target exponentially (Figure 4). PCR is performed in a thermal cycler, which is programmed to heat or cool the PCR reaction at the designated temperature for each step.

Typically, following PCR of the target region within a gene, the amplified DNA is further examined using RFLP analysis. An RFLP is defined as a variation in the number of restriction sites in a specific DNA region in diseased vs. healthy individuals. The amplified DNA is digested with a specific restriction enzyme. If the patient possesses a mutation in the LDL receptor gene, the digestion pattern will differ from the pattern obtained from unaffected individuals.

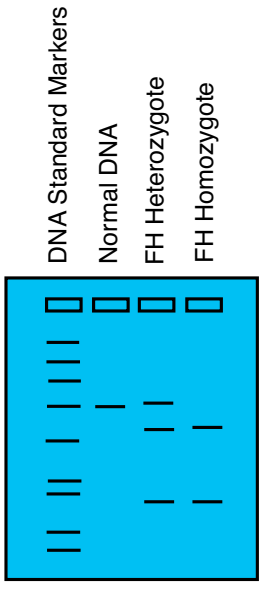


Figure 3:
Agarose gel that demonstrates normal, heterozygote and homozygote FH gene.

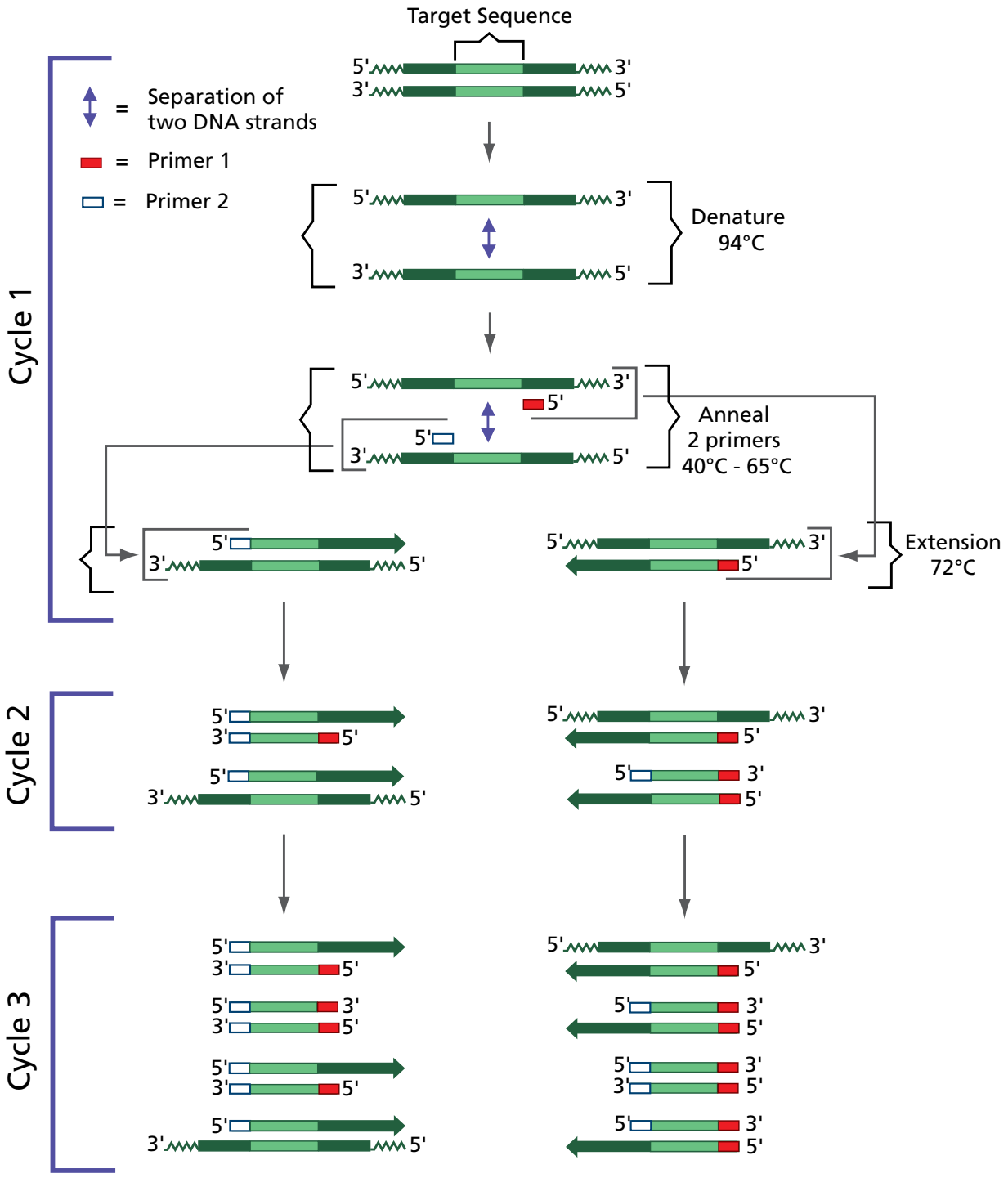
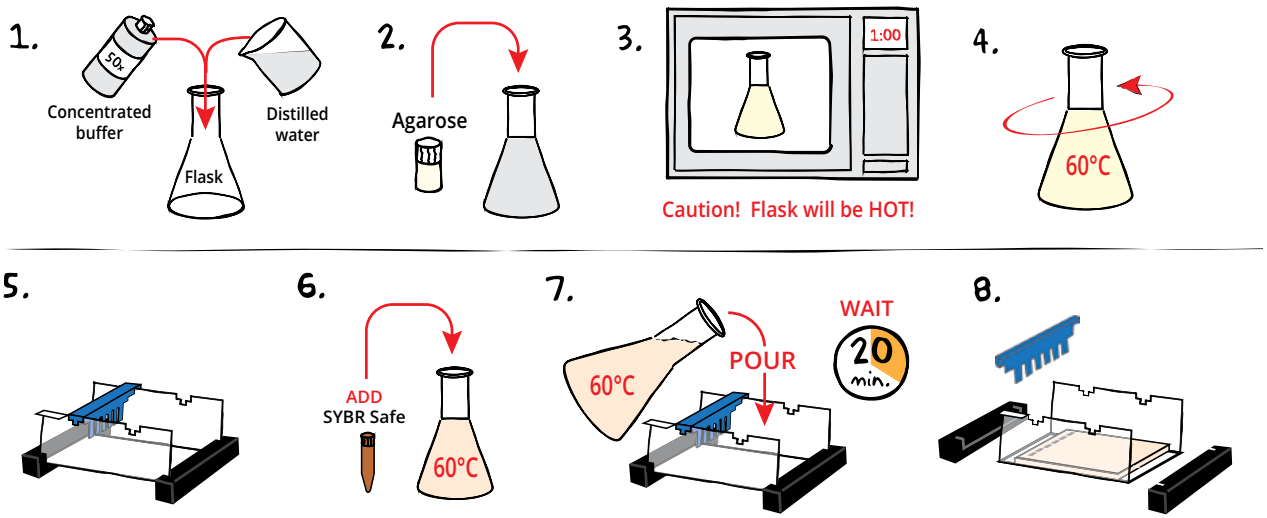


Figure 4: DNA Amplification by the Polymerase Chain Reaction

Part 1: Agarose Gel Electrophoresis



NOTE: If you are casting your own gels, review the following instructions. If you are using pre-cast gels, proceed to Step 8.

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** agarose to 60 °C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. Before casting the gel, **ADD** diluted SYBR® Safe to the 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** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

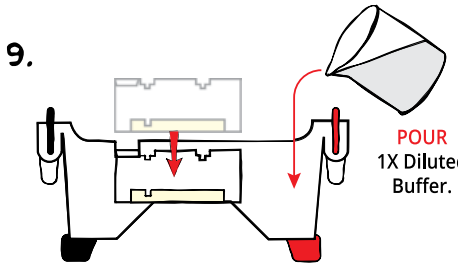
Wear gloves and safety goggles

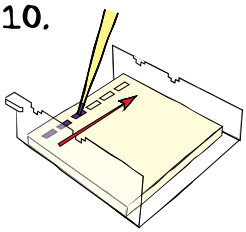
Reminder: This experiment requires 0.8% agarose gels cast with 8 wells.

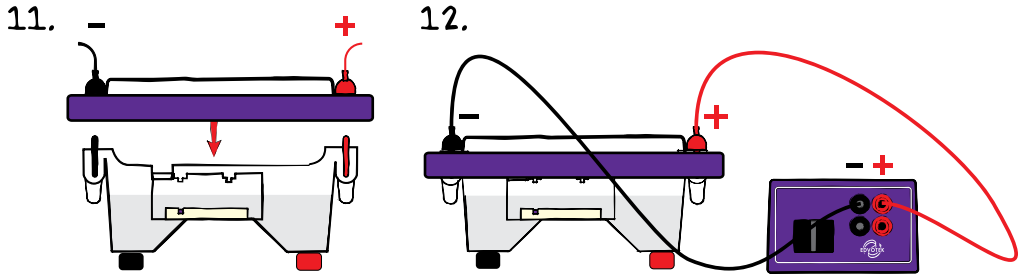
Table A — Individual 0.8% UltraSpec-Agarose™ Gel with SYBR® Safe Stain

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume	Diluted SYBR® (Step 6)
7 x 7 cm	0.6 mL	29.4 mL	0.23 g	30 mL	30 µL
7 x 10 cm	1.0 mL	49.0 mL	0.39 g	50 mL	50 µL
7 x 14 cm	1.2 mL	58.8 mL	0.46 g	60 mL	60 µL

Part 1: Gel Electrophoresis of Restriction Fragments, continued







Wear gloves and safety goggles

9. **PLACE** gel (on the tray) into 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 sample (35 µL) into the well in the order indicated by Table 1.
11. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
12. **CONNECT** 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.

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

Table 1: Gel Loading

Lane 1	Tube A	DNA Standard Marker
Lane 2	Tube B	Normal DNA Sample (ff)
Lane 3	Tube C	FH Control (FF)
Lane 4	Tube D	Changman, Aki's Father
Lane 5	Tube E	Jia, Aki's Sister
Lane 6	Tube F	Aki
Lane 7	Tube G	Justin, Aki's Brother-in-law
Lane 8	Tube H	Amy, Aki's Niece

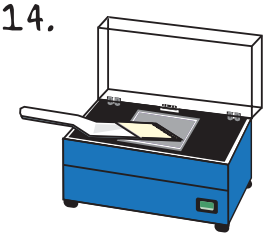
Table B: 1x Electrophoresis Buffer (Chamber Buffer)

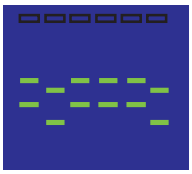
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE	300 mL	6 mL	294 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

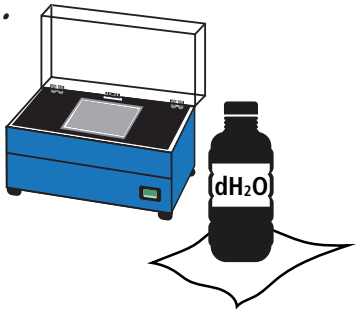
Table C: Time and Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model: M6+	M12 & M36
Volts	Min. / Max.	Min. / Max.
150	15/20 min.	25 / 35 min.
125	20/30 min.	35 / 45 min.
75	35 / 45 min.	60 / 90 min.

Part 1: Gel Electrophoresis of Restriction Fragments, continued

14. 

15. 

16. 

VISUALIZING THE SYBR® GEL

14. **SLIDE** 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.

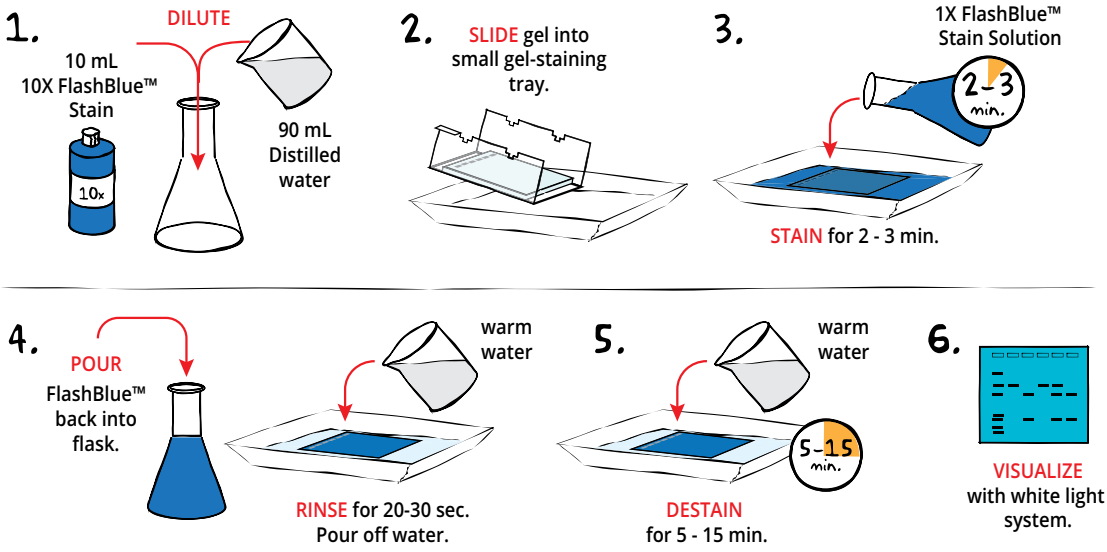
15. **PHOTOGRAPH** results.

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.

Part 2: Staining Agarose Gels Using FlashBlue™ (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 FlashBlue™, 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 149 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.

Instructor's Guide

INTRODUCTION

In this activity students will test members of Aki's family for familial hypercholesterolemia using gel electrophoresis. Students have performed gel electrophoresis in previous activities and have analyzed gel pictures. This will be the first time they are actually setting up and running the gel.

Before the activity consider reviewing the action of restriction enzymes and the science of the polymerase chain reaction (PCR). PCR is used to make copies of a specific segment of DNA. Primers specific to that region are used to identify the area to be copied. In this case, PCR has been used to amplify part of the FH gene of the simulated DNA. The simulated DNA has been predigested with restriction enzymes. Restriction enzymes recognize specific sequences in the DNA and cut in designated places along the strand. Remind students that they simulated this process when they completed the paper DNA fingerprint to determine whose DNA was found at the scene of Anna Garcia's death. The resulting pieces of DNA are run on a gel and visualized as distinct bands. Because restriction enzymes recognize specific sequences, they are able to identify differences in normal and mutated DNA. These base pair differences are referred to as single nucleotide polymorphisms (SNPs).

LAB SETUP

Prepare the gels ahead of time or, if time permits, allow the students to mix and prepare the agarose gels. Instructions are provided in the EDVOTEK kit and are outlined in the Gel Electrophoresis section. Note, however, that for this version of the lab, you will need to make 8 well gels instead of 6 well gels.

This kit contains two staining options: SYBR® Safe and FlashBlue™. Only one of these dyes should be used at a time. If the instructor chooses to use SYBR® Safe, it MUST be added to the molten agarose during gel casting.

In this simulation, students are told they are testing the DNA of Aki's family members. Samples are ready to load and have been custom packaged to fit the scenario. The kit comes with control samples that correspond to homozygous recessive and homozygous dominant, labeled normal DNA sample and FH control, respectively. Note that FH is a dominant disorder. Only one affected allele is required for a person to express the disorder.

Each student group can run one agarose gel and should receive the following materials:

- One QuickStrip™ of DNA samples A-H
- Automatic micropipette and tips
- 0.8% Agarose gel with 8 wells
- Electrophoresis buffer
- Electrophoresis gel chamber and DC power supply
- SYBR® Safe or FlashBlue™ gel stain

PART 1 PREPARATIONS: AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel per student group. 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.

Prepare SYBR® Safe Stain

1. Following the instructions in Appendix B, prepare 1x Electrophoresis Buffer by combining 10 µL of 50X Concentrated Buffer with 490 µL of distilled water.

2. Add 390 µL of the 1X buffer from step 1 to the tube of SYBR® Safe and mix by tapping the tube several times. The diluted SYBR® Safe Stain is now ready to be used during agarose gel preparation.

Individual Gel Preparation

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Part 1 in the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water, agarose powder, and diluted SYBR® Safe Stain.

Batch Gel Preparation

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. See Appendix B.

Preparing Gels in Advance

Gels may be prepared ahead and stored for later use. Solidified gels can be store in the refrigerator for up to 2 weeks. Place 1-2 mL of electrophoresis buffer in a sealable bag with the gels to prevent them from drying out. Excessive buffer will cause SYBR® Safe to diffuse out of the gels.

Do not freeze gels at -20° C as freezing will destroy the gels.

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.

Preparing the QuickStrips™

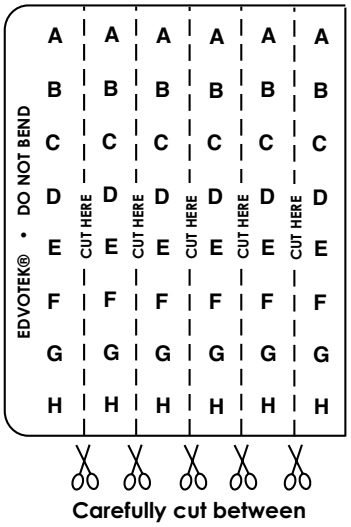
QuickStrip™ tubes consist of a microtiter block covered with a protective overlay. Each well contains pre-aliquoted DNA.

Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the protective overlay while separating the samples.

Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.

The wells of the QuickStrips™ contain the following and 35 µL of each sample should be loaded into the agarose gel in the following manner:

Tube A	Lane 1	DNA Standard Markers
Tube B	Lane 2	Normal DNA Sample
Tube C	Lane 3	FH control
Tube D	Lane 4	Changman
Tube E	Lane 5	Jia
Tube F	Lane 6	Aki
Tube G	Lane 7	Justin
Tube H	Lane 8	Amy



Carefully cut between each set of tubes

PART 2 PREPARATIONS: STAINING WITH FLASHBLUE™ (OPTIONAL)

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™.

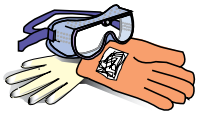
Agarose gels can be stained with diluted FlashBlue™ for 2-3 minutes and destained for only 15 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to develop in the destaining solution, resulting in dark blue DNA bands that contrast with a uniformly light 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.

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.

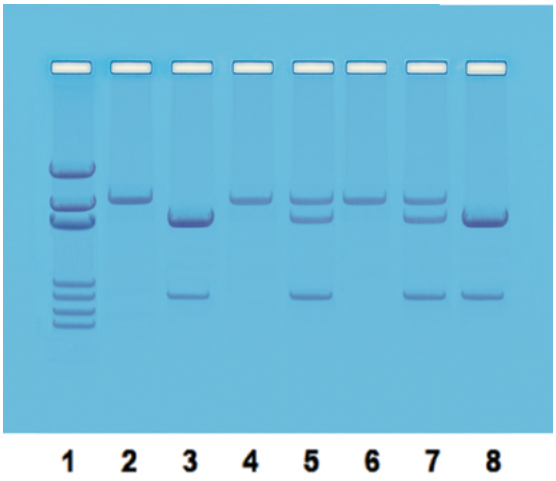
FOR PART 2
Each Student Group should receive:
- 10 mL 10X concentrated FlashBlue™
- Small plastic tray or weight boat
- Distilled or deionized water



Wear gloves and safety goggles

EXPECTED RESULTS

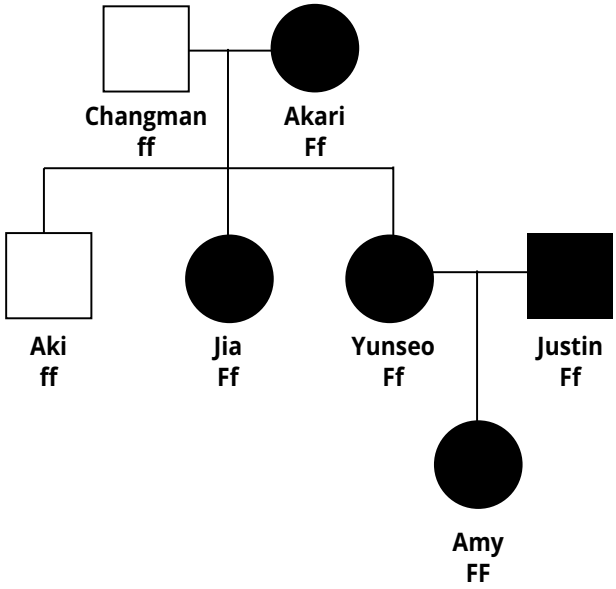
Load and run the gel as described in the directions provided with the kit. Stain the finished product and observe the resulting bands. Results are shown below.



Lane 1	DNA Standard Marker
Lane 2	Normal DNA Sample (ff)
Lane 3	FH Control (FF)
Lane 4	Changman, Aki's Father (ff)
Lane 5	Jia, Aki's Sister (Ff)
Lane 6	Aki (ff)
Lane 7	Justin, Aki's Brother-in-law (Ff)
Lane 8	Amy, Aki's Niece (FF)

Having the FH mutation introduces an additional restriction site for the enzyme used in the activity. Normal DNA is not cut by the included enzyme and thus the DNA runs as one fragment. The mutated FH gene DNA has an additional restriction site and thus it is cut into two pieces by the RE. Individuals who are heterozygous show on the gel as having both the bands for normal DNA combined with the abnormal DNA, as they have copies of both. These individuals show three bands on the gel.

Results are visualized on the pedigree below. In this case, individuals who are Ff are affected, so there are no half shaded circles or squares. The gel results reveal that Changman does not have the FH mutation and thus his genotype is ff. Jia is heterozygous for the FH gene. Since Jia has the disorder, we know she had to receive the F from a parent. Since her dad is homozygous recessive, she must have received the abnormal allele from her mother. Therefore, we need to determine Akari's genotype. Although she is affected, she cannot be FF as all of their children would be affected. We know this is not true as Aki does not have the disorder. Thus Akari is heterozygous. Gel results also show that Amy is homozygous dominant. Even though we did not test Yunseo, we know she must carry the F allele as her daughter received a copy from both parents. In theory, Yunseo could be FF or Ff; however, we know that Changman and Akari could not produce a child with FF. Thus Yunseo is heterozygous for FH.



In the case of familial hypercholesterolemia, individuals who are homozygous dominant contain more affected LH receptors that someone who is heterozygous, and thus, have incredibly high cholesterol values. Amy, the only member of the family to carry this genotype, has a total cholesterol of 600.

Appendix A
Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	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 properly.	Ensure that diluted SYBR® Safe was added to the gel.
	The gel was not stained for a sufficient period of time with FlashBlue™.	Repeat staining protocol.
	The background of gel is too dark after staining with FlashBlue™.	Destain the gel for 5-10 minutes in distilled water.
DNA bands were not resolved.	Tracking dye 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 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time	Re-stain the gel with FlashBlue™
There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.	The wrong percent gel was used for electrophoretic separation.	Be sure to prepare the correct percent agarose gel. For reference, the Ready-to-Load™ DNA samples should be analyzed using a 0.8% agarose gel.
There's not enough sample in my QuickStrip™.	The QuickStrip™ has dried out.	Add 40 µL water, gently pipet up and down to mix before loading.

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 for sharing by the class. Unused 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 Electrophoresis Buffer

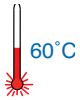
50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 mL		2,940 mL	3000 mL (3 L)

Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

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.

1. Use a 500 mL flask to prepare the diluted gel buffer.
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. 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 tube of diluted stain (see page 13) to the cooled agarose and mix well.
7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 mL for a 7 x 7 cm tray, 50 mL for a 7 x 10 cm tray, and 60 mL for a 7 x 14 cm tray. ***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.

Table E — Batch Prep of 0.8% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50X) (mL)	+	Distilled Water (mL)	Total Volume (mL)
3.0		7.5		382.5	390