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Edvo-Kit #
315

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In Search of the Sickle Cell Gene by Southern Blot

Experiment Objective:

In this experiment, students will perform a simulated Southern Blot to test a family for sickle cell disease and identify a mutation in the hemoglobin gene. Southern blotting is an important technique that is useful to both clinicians and researchers. Students will understand this genetic disorder, the relationship between genotype and phenotype, Mendelian inheritance, and the biology behind Southern blot.

See page 3 for storage instructions.

Table of Contents

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

Experiment Components

Component		Storage	Check ✓
DNA Samples for Electrophoresis			
A	Sickle cell gene sample	-20 °C Freezer	❑
B	Sickle cell trait (carrier) sample	-20 °C Freezer	❑
C	Normal gene sample	-20 °C Freezer	❑
D	Mother's DNA sample	-20 °C Freezer	❑
E	Child's DNA sample	-20 °C Freezer	❑
F	Father's DNA sample	-20 °C Freezer	❑
Components for Membrane Transfer			
•	Pre-cut Nylon Membranes (7 x 7 cm)	Room Temp.	❑
•	Pre-cut Filter Papers (7 x 7 cm)	Room Temp.	❑
•	NaCl	Room Temp.	❑
•	NaOH	Room Temp.	❑
Other Reagents & Supplies			
•	UltraSpec-Agarose™ Powder	Room Temp.	❑
•	Concentrated Electrophoresis Buffer	Room Temp.	❑
•	Practice Gel Loading Solution	Room Temp.	❑
•	Concentrated Blue-Blot DNA Stain™ Solution	Room Temp.	❑

This experiment contains enough reagents for 5 lab groups.

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.

Experiment Requirements

- Concentrated HCl (12 N)
- Distilled Water
- Horizontal Gel Electrophoresis Apparatus (Cat. #502 recommended)
- D.C. Power Supply
- Microwave or Hot Plates
- Balance
- Incubator Oven
- Automatic Pipettes with Tips
- Graduated Cylinders
- 250 mL and 500 mL Flasks or Beakers
- Small Trays or Containers
- Forceps
- Scissors
- Plastic Wrap
- Tape
- Several Packs of Paper Towels
- Latex or Vinyl Lab Gloves
- Safety Goggles
- Filter Paper (Optional)

Background Information

SICKLE CELL DISEASE

Sickle cell disease (SCD) is an inherited blood disorder that affects around 5 million people worldwide. The disease is caused by small changes in an individual's genetic coding that interfere with their body's ability to create hemoglobin – a protein that transports oxygen. Changes to hemoglobin primarily affect red blood cells. Individuals with SCD have red blood cells that are misshaped, stiff, sticky and short-lived (Figure 1). As a result, these individuals often have anemia – a medical condition characterized by low red blood cell numbers and low blood oxygen levels. Many people with SCD also experience periodic blood clots, that block the flow of oxygen to other important organs, and have compromised spleens. These conditions result in a number of symptoms ranging from chronic tiredness to heart attacks.

Normal Red Blood Cell
- Disked-shaped
- Flexible
- Smooth
- Lifespan of ~120 days

Sickle Cell
- C-shaped
- Stiff
- Sticky & prone to clotting
- Lifespan of ~15 days

Figure 1

Symptoms of sickle cell disease usually appear 5-6 months after birth. In children, the disorder can cause delayed growth and development, yellowing of the skin and eyes, secondary infections like pneumonia, and episodes of pain called "pain crisis". If untreated these symptoms worsen with time. Adults with severe SCD can experience chronic pain, fatigue, aseptic necrosis (localized bone death), vision loss, as well as life-threatening lung and heart injuries. In less severe cases, the main symptoms are tiredness, dizziness, difficulty breathing, and a weakened immune system.

Medical researchers are currently working to find a cure for this disease. One option is a bone marrow transplant. However, this requires a closely matched marrow donor, several rounds of chemotherapy, and years of taking immunosuppressive drugs. Consequently, transplants are saved for severe cases of SCD in otherwise healthy and young patients. Another potential cure is gene therapy - using biotechnologies like CRISPR to introduce a healthy copy of the mutated hemoglobin gene into a patient's genome (see Box 1). Such strategies are promising but still being carefully tested. In the meantime, proper medical care and lifestyle changes can help an individual with SCD avoid pain crisis, lessen other symptoms, and prevent complications.

Box 1:
In the summer of 2019 Victoria Gray underwent an experimental treatment where researchers collected bone marrow cells, edited their genetic material to produce fetal hemoglobin, destroyed most of her unedited bone marrow cells, and then reintroduced the edited cells. The initial results were promising – Ms. Gray observed a dramatic reduction in most SCD symptoms and her blood tests showed healthy hemoglobin and red blood cell levels. However, scientists are still carefully monitoring the long term effects of the treatment. Because Victoria Gray was the first U.S. patient to be treated using CRISPR, her story was widely published.

Early and accurate identification of SCD is essential to treating the disease but can be challenging. This is because affected individuals show a wide range of symptoms and because these symptoms are not distinct to the disease. Luckily, many places in the US now automatically screen newborns for sickle cell disease. This is sometimes done by measuring the level of hemoglobin in a blood sample or by observing red blood cells under a microscope. However, early in a baby's life, the

production of a different type of hemoglobin called fetal hemoglobin may mask these physical traits of the disease. A more accurate test is to look at the individual's DNA for the root source of the disorder.

AN INHERITED DISORDER

Researchers have identified around 400 different DNA changes that can lead to SCD. The most common is an A to T single nucleotide mutation that alters the amino acid sequence of a protein known as beta-globin. More specifically, this DNA coding change causes cells to build beta-globin proteins that contain the amino acid valine instead of glutamic acid (Figure 2). Because these two amino acids have different charges this alters key properties a person's beta-globin and, in turn, their hemoglobin (Figure 3). Other subtypes of the disease are caused by similar nucleotide changes in the same gene.

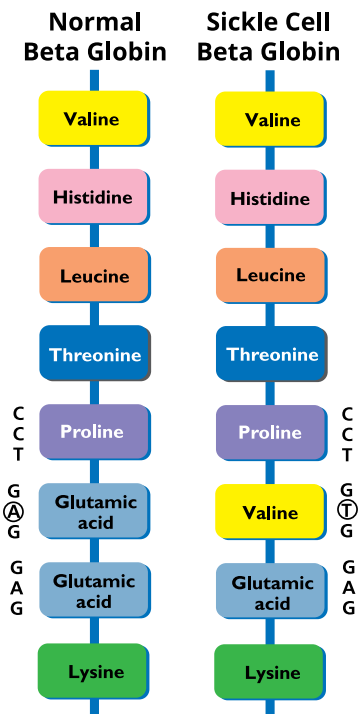


Figure 2

The beta-globin gene is found on chromosome 11 and contains the body's only set of instructions for making an essential part of most hemoglobin proteins. Because it is located on an autosomal (or paired) chromosome each individual has two copies – one that they inherited from their mother and one that they inherited from their father. However, humans need only one "normal" beta-goblin gene to produce a sufficient supply of healthy hemoglobin. In such a case, the normal gene is called "dominant" because its effects can compensate for a mutated gene. Similarly, SCD is called a "recessive" disease because symptoms only appear when someone has two mutant versions of the beta-globin gene.

A recessive disease like SCD can be difficult to trace through a family tree because the disease can "skip" multiple generations. This happens whenever there is a generation of carriers – heterozygous individuals with a dominant normal gene that masks the continued presence of the disease-causing mutation. At the same time, this disease is caused by a single gene located on an autosomal chromosome which makes its inheritance pattern easier to predict.

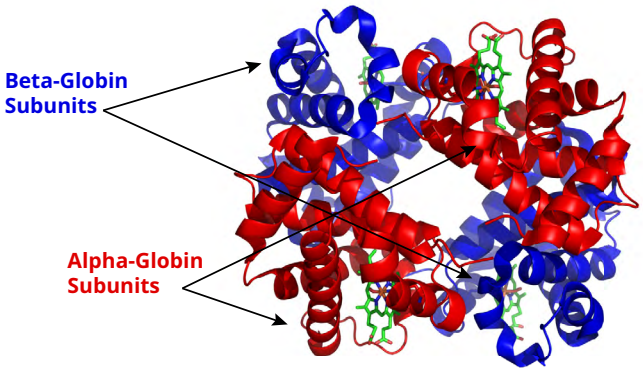


Figure 3

Source: Structure of Haemoglobin by Richard Wheeler. Zephyris at the English language Wikipedia / CC BY-SA (http://creativecommons.org/licenses/by-sa/3.0/). Modified from the original with arrows and text labels.

Inheritance of a single gene can be illustrated with a two-by-two grid known as a Punnett Square (Figure 4). Punnett Squares diagram alleles - alternative forms of a gene that are created by mutations. 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 and the recessive allele by a lower-case letter. 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.

Genetic counselors use Punnett squares to predict the probability that two healthy adults will have a child with sickle cell disease. For example, in the situation depicted in Figure 4, both potential parents are heterozygous.

These individuals will have few or no symptoms of the disease and may not even know they have the mutation. However, they have a 25% chance of having a child with the disease – the homozygous recessive genotype. They also have a 25% chance of having a healthy child with two normal genes – the homozygous dominant genotype. Finally, they have a 50% chance of having a heterozygous child like themselves.

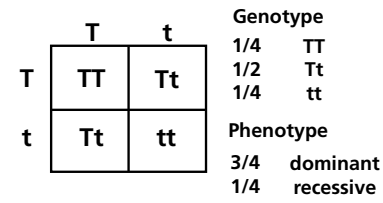


Figure 4

How can couples who are considering children determine whether their beta-globin genotype is homozygous dominant or heterozygous? How can young babies be screened for this disease when early blood tests may be inconclusive? By using biotechnologies that allow users to read – or at least deduce - the exact sequence of As, Ts, Gs, and Cs that make up an individual's genome. One such technology is called a Southern blot.

DETECTION USING SOUTHERN BLOTS

Southern Blots are a popular way for scientists to search an individual's genome for a specific DNA sequence of interest and then determine its size and part of its nucleotide sequence. The method's name comes from its inventor (Dr. Edward Southern) and from one of the procedure's key steps (transferring or "blotting" DNA from an agarose gel to a nylon membrane). This procedure is advanced and demanding - It uses several different biotechnologies, involves multiple steps, and can take up to 18 hours to complete! However, its accuracy, specificity, and accessibility make it a valuable genetics tool.

During a Southern Blot, an individual's DNA is manipulated and modified in several ways so that differences in DNA can be observed by the human eye. These manipulations and modifications are described in detail below:

1. **Digest:** Long strands of DNA are broken into smaller fragments using proteins called restriction enzymes that act like molecular scissors. Because these enzymes cut double-stranded DNA molecules at very specific sequences, the size of the DNA fragments created by this step is directly related to which enzyme was used and the nucleotide sequence of the DNA.

2. **Separate:** The mixture of digested DNA is separated by size using agarose gel electrophoresis. The DNA is added into depressions within a gel and an electrical current is then passed through the gel. Because DNA has a strong negative charge, the current drives the restriction fragments through the gel towards the positive electrode. Small DNA fragments move through the gel easily, but large DNA fragments have a more difficult time. This causes differently sized fragments to separate and form discrete "bands" within the gel.

3. **Denature:** The gel is first submerged in an acid solution (often HCl). This acid treatment "nicks" the DNA molecules that are in the gel by removing certain nucleotide bases called purines. Next, the gel is submerged in a base solution (often NaOH). This base treatment disrupts the bonds between all the base pairs causing the double-stranded DNA to divide into single-stranded halves. Timing is key during this step. Overexposure – especially to the acid – can cause the DNA molecules to break down into very short pieces that are much more difficult to visualize.

4. **Transfer:** DNA is transferred from the gel to a nylon membrane using a small electric current or using capillary action. This experiment will use capillary action by creating a stack comprised of the gel, a nylon membrane, dry filter paper, and paper towels which causes buffer to flow from the gel - a region of high water potential - to the paper towels - a region of low water potential. The DNA follows the current but then gets stuck on the membrane whose minor positive charge serves to both attract and capture negatively charged molecules like DNA.

5. **Fix:** The DNA is securely attached to the membrane in the same pattern that it was on the gel. This is done by exposing the membrane to UV rays, incubating it in a vacuum, or baking it for 2 hours at 80°C.

6. **Hybridize:** The membrane is incubated in a solution containing small fragments of single-stranded DNA called probes. Probes have a very specific sequence of nucleotides, which is chosen by the experimenter, as well as an attached fluorescent or radioactive molecule that serves as a label. During incubation probes bind to any DNA molecules on the membrane that have complementary - or close to complementary - sequences. This "DNA-to-DNA" binding, called hybridization, is strong. It's also very selective. Only a small fraction of the hundreds of thousands of differently sized DNA fragments fixed on the membrane will contain a complementary sequence. Consequently, when the membrane is then washed only a few bands of hybridized probes stay attached to the membrane.

7. **Visualize:** Finally, the membrane is treated to highlight the probe labeled DNA bands. As the majority of southern blot probes are radioactively labeled this is usually done using x-ray film. However, it can also be accomplished by exposing the membrane to UV light (in cases where fluorescently labeled probes are used) or by soaking the membrane in color-changing solutions (in cases where chromogenically labeled probes are used). Scientists can then determine key parts of an individual's genotype based on which bands appear and their size.

In this experiment, you will use a Southern Blot to determine the phenotypes and genotypes of the DuSang family. The child in this family has been unusually tired recently. Also, the parents have noticed a yellowing around the child's mouth and eyes. Because these are early signs of SCD and because both parents have distant family members with the disease, they have decided to go to a genetic counselor for testing.

Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will perform a simulated Southern Blot to test a family for sickle cell disease and identify a mutation in the hemoglobin gene. Southern blotting is an important technique that is useful to both clinicians and researchers. Students will understand this genetic disorder, the relationship between genotype and phenotype, Mendelian inheritance, and the biology behind Southern blot.

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. Exercise caution when using any electrical equipment in the laboratory.
4. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

- Record (draw) your observations, or photograph the results.

After the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Module I
Agarose Gel Electrophoresis
45-60 min.

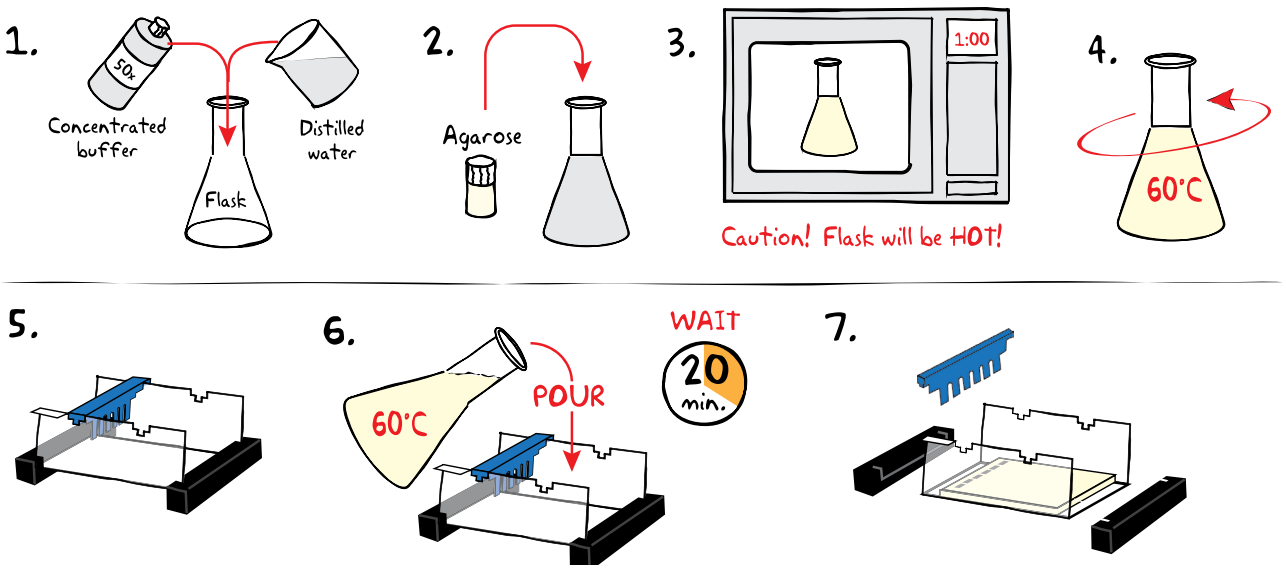
↓

Module II
Denaturation and Transfer
90 min + incubation (3 hours or overnight) + 40 min.

↓

Module III
Detection and Analysis
20 min.

Module I: Agarose Gel Electrophoresis



CASTING THE AGAROSE GEL

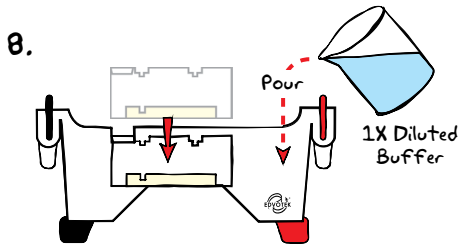

Wear gloves and safety goggles

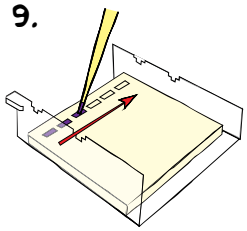
1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **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. **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.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

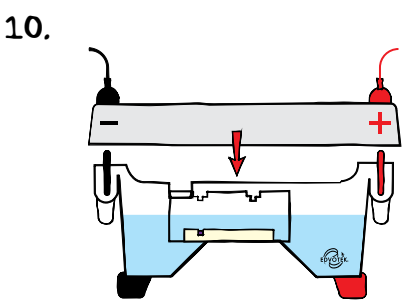
Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 mL	29.4 mL	0.23 g	30 mL
7 x 14 cm	1.2 mL	58.8 mL	0.46 g	60 mL

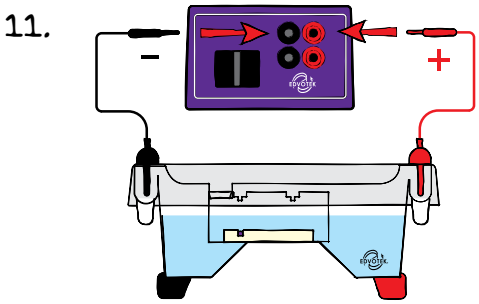
Module I: Agarose Gel Electrophoresis, continued





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





NOTE:
To save classroom time, the DNA samples in this experiment have already been digested with the restriction enzymes and mixed with loading buffer. They are ready to be loaded into the gel.

RUNNING THE GEL

8. **PLACE** the gel (still 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.
9. **LOAD** 18-20 µL of each DNA sample according to Table 1, at right.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3.5 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and *proceed immediately* to Module II instructions for **DENATURATION AND TRANSFER**.

Table 1: Gel Loading

Lane	Tube	Sample
1	A	Sickle cell gene sample
2	B	Sickle cell trait (carrier) sample
3	C	Normal gene sample
4	D	Mother's DNA sample
5	E	Child's DNA sample
6	F	Father's DNA sample

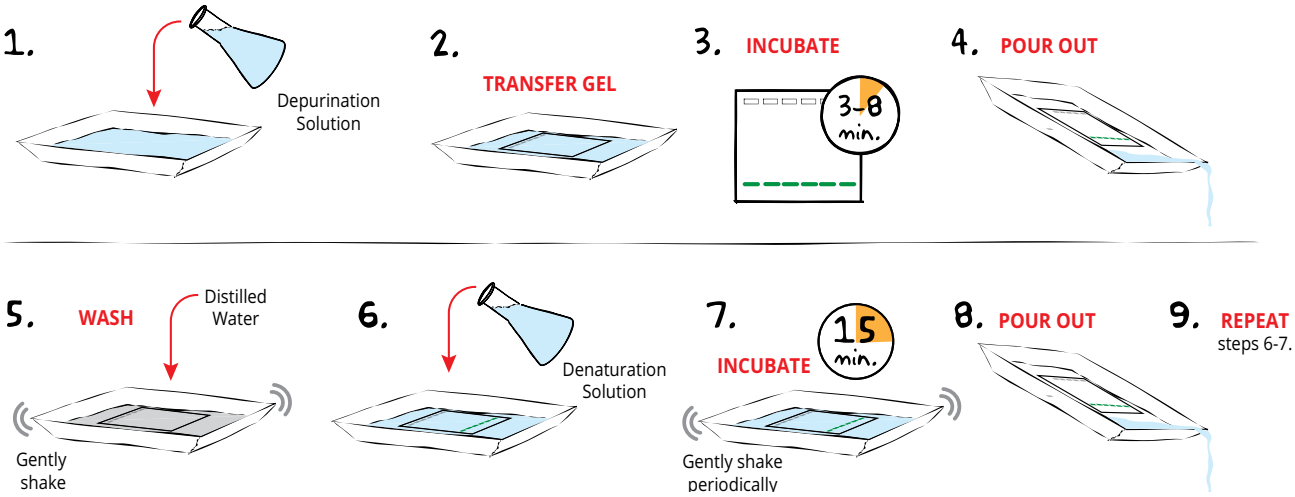
Table B: 1x Electrophoresis Buffer (Chamber Buffer)

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

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

Volts	Electrophoresis Model: M6+ Min. / Max.	M12 (new) Min. / Max.	M12 (classic) & M36 Min. / Max.
150	15/20 min.	20/30 min.	25 / 35 min.
125	20/30 min.	30/35 min.	35 / 45 min.
75	35 / 45 min.	55/70 min.	60 / 90 min.

Module II: Denaturation and Transfer



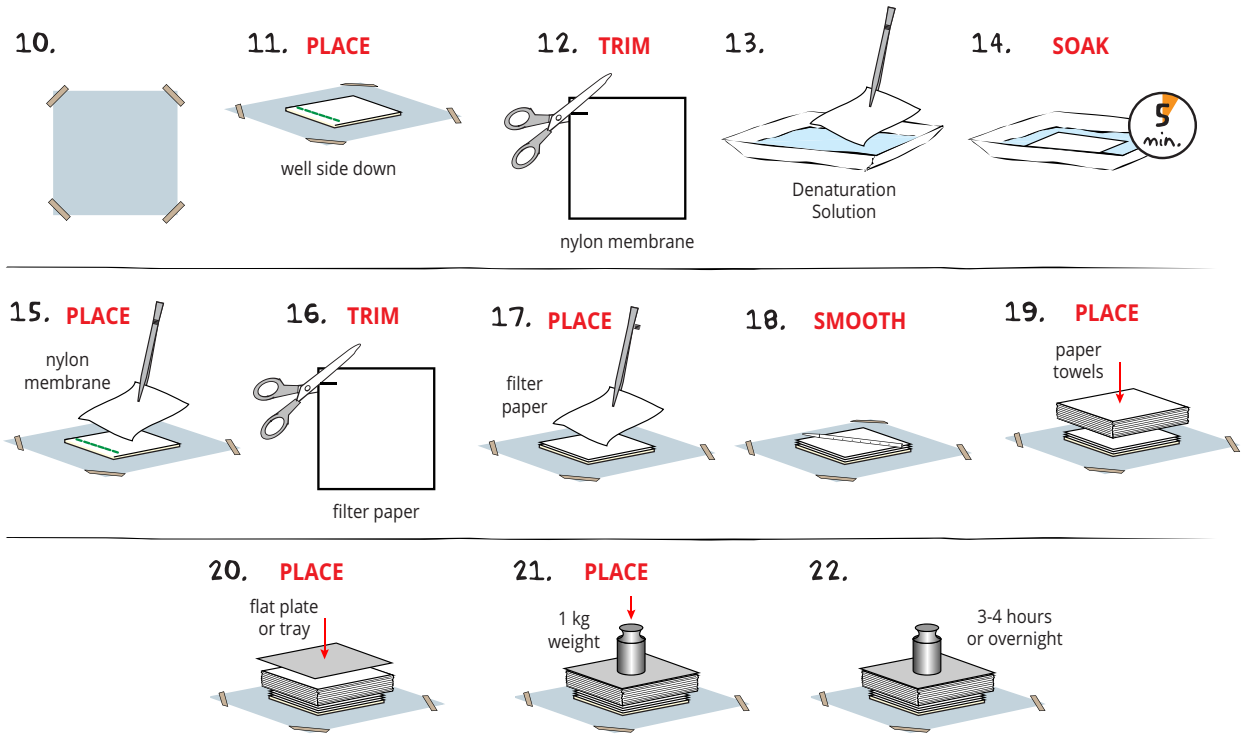
DEPURINATION

1. **ADD** 200 mL of depurination solution (0.25 N HCl) to a plastic tray.
2. **TRANSFER** the gel from Module I to this tray. Make sure the gel is immersed in the solution.
3. **INCUBATE** at room temperature for 3-8 minutes. **STOP** incubation and continue to step 3 as soon as the blue tracking dye in the gel changes to a greenish yellow. **WATCH** the time and this color change *carefully* as over-incubation will fragment the DNA and make later detection difficult.
4. Immediately, **POUR OUT** the depurination solution.
5. **WASH** the gel by adding 200 mL of distilled water to the tray, gently shaking the tray, and then pouring the water out. **REPEAT** this wash 2 times.

DENATURATION

6. **ADD** 200 mL of denaturation solution (0.5 M NaOH/6 M NaCl) to the tray.
7. **INCUBATE** at room temperature for 15 minutes. The density of the solution will cause the gel to float, periodically **SHAKE** the tray to keep it immersed.
8. **POUR OUT** the denaturation solution.
9. **REPEAT** steps 6 and 7 with new denaturation solution. **DO NOT POUR OUT** this second volume of denaturation solution and keep the tray—you will need both for steps 13 and 14.

Module II: Denaturation and Transfer, continued



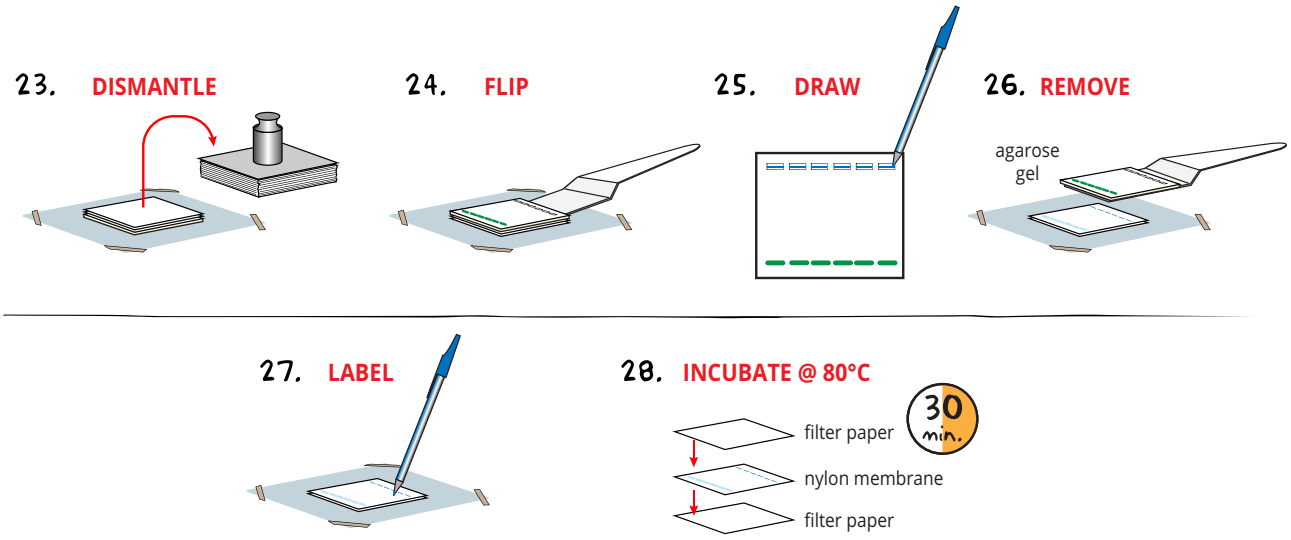
SOUTHERN BLOT TRANSFER

Oils from your hands and powders from gloves can interfere with the transfer. For steps 10-20 wear rinsed and dried lab gloves and for steps 12-15 use two clean forceps.

10. **PREPARE** your lab bench by spreading out a small sheet of plastic wrap. To keep the wrap tight and stable, **TAPE** the edges to the bench.
11. **PLACE** your gel well side down onto the plastic wrap so that the exposed (top side) of the gel is smooth.
12. Using forceps and scissors, carefully **TRIM** the nylon membrane to the size of the gel. A membrane that is larger than the gel will compromise the transfer process.
13. Carefully **SLIDE** the membrane out of the blue cover and **TRANSFER** to the denaturation solution from step 9. For best results, **ADD** the membrane to the solution in a U shape so that the middle comes in contact with the solution first. The solution can then slowly spread out to both edges.
14. **SOAK** the membrane for 5 minutes.
15. Using forceps, **REMOVE** the membrane from the tray and **PLACE** it on top of the inverted agarose gel.
16. **TRIM** the filter paper to the same size as the gel and membrane.
17. **PLACE** the filter paper on top of the nylon membrane.
18. **REMOVE** any air bubbles from the top of the gel, membrane, and filter paper using a large pipette tip (5 mL–10 mL) or similar cylindrical object.
19. **PLACE** a 5-6 cm stack of paper towels on top of the filter paper.
20. **PLACE** an empty tray or flat plate on top of the paper towels.
21. **PLACE** a 1 kg weight on top—an empty 500 mL beaker works well.
22. **ALLOW** the transfer to progress for 3-4 hours or overnight.

NOTE:
When handling the nylon membrane, be gentle and try to touch only the outer edges.

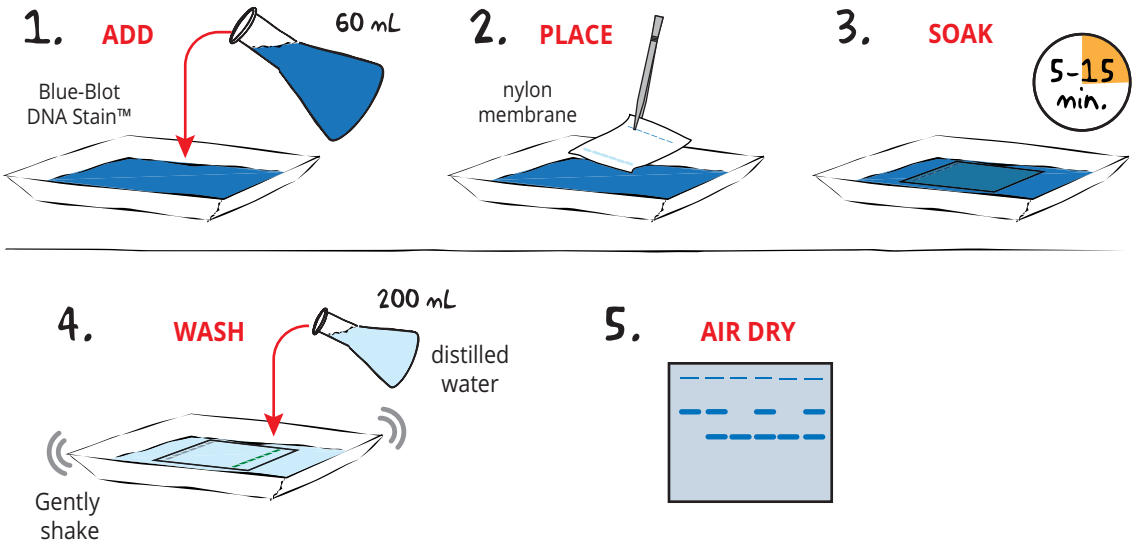
Module II: Denaturation and Transfer, continued



23. **DISMANTLE** the stack above the filter paper by removing the weight, tray, and paper towels.
24. **FLIP** the remaining stack (filter paper-membrane-gel) so that the gel is now facing up. This can be done with a spatula or with forceps and rinsed gloves.
25. Use a pen to **DRAW** through the six sample wells on the gel and mark their position on the nylon membrane.
26. Carefully **REMOVE** and **DISCARD** the gel with forceps or a spatula.
27. **VERIFY** that the transfer has occurred by the presence of the blue tracking dye on the membrane. Using a pen, gently **LABEL** this side of the membrane with your groups ID. This is the side that the DNA is on so keep the label small.
28. For best results, **PLACE** the membrane between two sheets of filter paper or paper towels and **INCUBATE** at 80 °C for 30 minutes.

OPTIONAL STOPPING POINT:
Once you have performed the fixation step (Step 28), then the membrane can be stored until you are ready to continue to Module III. Keep it in between the two sheets of filter paper, at room temperature, and away from moisture.

Module III: Detection and Analysis



DETECTION

1. **ADD** 60 mL of Blue-Blot DNA Stain™ solution to a sealable bag or tray.
2. **PLACE** the membrane from Module II in the bag or tray. If you are using a tray, keep the DNA side facing up.
3. **SOAK** the membrane for 5-15 minutes making sure that the membrane stays covered by the stain solution.
4. **WASH** the membrane by adding 200 mL of distilled water, gently shaking the bag or tray, and then pouring the water out. **REPEAT** until the DNA bands are clearly visible against a light blue background.
5. **REMOVE** the membrane from the water and **AIR DRY**. **STORE** dry membranes in your lab notebook.

ANALYSIS QUESTIONS

EXAMINE the banding pattern.

6. How many bands do you observe in each column?

COMPARE the banding pattern created by the Mother's, Child's, and Father's DNA samples to the three controls.

7. Describe the genetic makeup (genotype) of each sample. Which samples are homozygous dominant, heterozygous, and homozygous recessive?
8. Do any of the family members have sickle cell disease?
9. If the father and mother have another child, what is chance that the child will have sickle cell disease? What is the chance that the child will have the sickle cell mutation?

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Create a Punnett Square for a couple where one parent has two normal beta-globin genes and one parent has sickle cell disease. What are the chances that this couple will have a child with the disease? What are the chances that they will have a child who is a carrier?

2. What are the key steps of a typical Southern Blot?

3. Brainstorm reasons why early detection is such an important part of treating sickle cell disease.

Instructor's Guide

NOTES TO THE INSTRUCTOR

In this experiment Module I must be immediately followed by Module II. Module III can be completed any time as the DNA is highly stable once it is fixed on the nylon membrane.

Southern blots are time intensive. This experiment saves you significant class time by sending predigest DNA fragments and by combining the hybridization and visualization steps. If your class would like to run a full Southern blot check out our kit "DNA Fingerprint by Southern Blot" (Cat. # 311).

Preparation for:	What to do:	When?	Time Required:
Module I: Agarose Gel Electrophoresis	(Optional) Prepare diluted electrophoresis buffer	One day before Module I (or up to two weeks with proper storage)	40 min.
	(Optional) Prepare molten agarose and pour gels		
	Hand out supplies	Day of Module I	10 min.
Module II: Denaturation and Transfer	Prepare solutions	Up to 24 hours before Module II	15 min.
	Hand out supplies	Day of Module II	10 min.
	(Optional) Set incubator to 80 °C	Anytime before performing Module II	5 min.
Module III: Detection and Analysis	Prepare Blue-Blot™ Stain	Up to 24 hours before Module III	10 min.

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

Pre-Lab Preparations

MODULE I: 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. Both the nylon membrane and filter papers included in this experiment have been precut to fit 7x7 cm gels. To ensure a good transfer your class's gels must be 7x7 cm or smaller or must be easily cut to meet this size requirement.

Individual Gel Preparation:
Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module I 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. Electrophoresis buffer can also be prepared in bulk. See Appendix A.

Preparing Gels in Advance:
Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks. 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.

DNA Samples:
Components A through F are predigested DNA in a gel loading solution that are ready to be loaded directly into each group's gel. Each tube should contain ~110 µL of sample. Student groups can share these tubes or you may aliquot the solutions to additional tubes. In this case each group should receive ~20 µL of each sample.

NOTE:
Accurate pipetting is critical for maximizing successful experiment results. If students are unfamiliar with using micropipettes, we recommended performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this advanced level experiment.

FOR MODULE I
Each Group should receive:
- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- DNA Samples

Pre-Lab Preparations

MODULE II: DENATURATION AND TRANSFER

1. Prepare 1 L of depurination solution (0.25 N HCl) by mixing 21 mL of concentrated HCl (12 N) and 979 mL distilled water. Provide each group with 200 mL of this mixture. Label each beaker either as "Depurination Solution" or as "0.25 N HCl".
2. Prepare 2 L of denaturation solution (0.5 M NaOH/0.6 M NaCl) by adding 40 g of NaOH and 70 g of NaCl to 1.8 L of distilled water, mixing, and then brining to a final volume of 2 L. Provide each group with 400 mL of this mixture. Label each beaker either as "Denaturation Solution" or as "0.5 M NaOH/0.6 M NaCl".
3. Distribute the remaining Module II items. For the nylon membranes, keep both the upper and lower protective cover sheets around the membranes. Use rinsed and dried lab gloves for this step as powdered gloves my interfere with the transfer.
4. If you plan to do the optional fixation step, set an incubator to 80°C. (If this will be after an overnight transfer, wait until the second day of Module II.)

FOR MODULE II
Each Group should receive:
- Depurination Solution
- Denaturation Solution
- Distilled Water
- Tray large enough to hold a 7x7 cm gel
- Plastic wrap and tape
- 1 pre-cut nylon membrane (7x7 cm), protected by two blue sheets
- 1 pre-cut filter paper (7x7 cm)
- Stack of paper towels (around 20)
- 2 forceps, 1 spatula (optional), and lab gloves
- Pen
- Filter Paper*

* *Filter paper provides additional protection during the final fixation step. If you do not have filter paper students can carefully sandwich the membrane between two paper towels.*

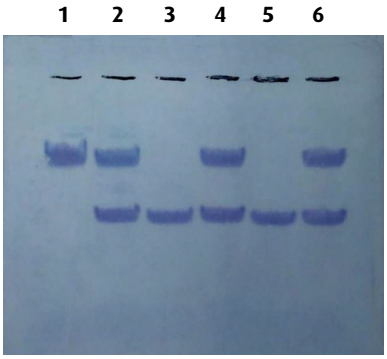
MODULE III: DNA DETECTION AND ANALYSIS

1. Prepare 300 mL of Blue-Blot DNA Stain™ by combining 30 mL Blue-Blot DNA Stain™ (10x) and 270 mL distilled water.
2. Provide each group with 60 mL of the solution.

FOR MODULE III
Each Group should receive:
- Tray or sealable bag large enough to hold a 7x7 membrane
- Diluted Blue-Blot DNA Stain™ Solution

Expected Results and Analysis

EXPECTED AGAROSE GEL RESULTS:



Lane	Sample
1	Sickle cell gene sample
2	Sickle cell trait (carrier) sample
3	Normal gene sample
4	Mother's DNA sample
5	Child's DNA sample
6	Father's DNA sample

ANSWERS TO MODULE III ANALYSIS QUESTIONS:

6. **How many bands do you observe in each column?**

   Refer to the gel results, above.

7. **Describe the genetic makeup (genotype) of each sample. Which samples are homozygous dominant, heterozygous, and homozygous recessive?**

   Homozygous Dominant:
   - Normal gene sample (Lane 3)
   - Child's DNA sample (Lane 5)

   Heterozygous:
   - Sickle cell trait (carrier) sample (Lane 2)
   - Mother's DNA sample (Lane 4)
   - Father's DNA sample (Lane 6)

   Homozygous Recessive:
   - Sickle cell gene sample (Lane 1)

8. **Do any of the family members have sickle cell disease?**

   All three individuals in this family do not have sickle cell disease.

9. **If the father and mother have another child, what is chance that the child will have sickle cell disease? What is the chance that the child will have the sickle cell mutation?**

   Both the father and mother are carriers which means that they have a 25% chance of having a child with the disease and a 50% chance of having a child that is also a carrier.

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

Appendix A
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.

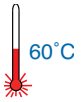
Table D — Bulk Preparation of Electrophoresis Buffer

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.

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. 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.***
7. 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.

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.

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

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