

Edvo-Kit #

207

Edvo-Kit #207

Southern Blot Analysis

Experiment Objective:

In this experiment, students perform a modified Southern blot that will introduce them to the key steps of this important biotechnology all while helping resolve a paternity case.

See page 3 for storage instructions.

Version 207.200317

EDVOTEK®

Table of Contents

	Page
Experiment Components	3
Experiment Requirements	4
Background Information	5
Experiment Procedures	
Experiment Overview and General Instructions	8
Module I: Agarose Gel Electrophoresis	9
Module II: Denaturation and Transfer	11
Module III: Detection and Analysis	14
Study Questions	15
Instructor's Guidelines	
Notes to the Instructor	16
Pre-Lab Preparations	17
Experiment Results and Analysis	19
Study Questions and Answers	20
Appendix A: Bulk Preparation of Electrophoresis Buffer and Agarose Gels	21

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

Technical Support

1.800.EDVOTEK
Mon. - Fri. 8 AM to 5:30 PM EST



Please Have the Following Info:

- Product Number & Description
- Lot Number on Box
- Order/Purchase Order #

1.800.EDVOTEK • info@edvotek.com • www.edvotek.com

www.edvotek.com

- Online Catalog
- Order Products
- Experiment Protocols
- Tech Support
- Resources!



EDVOTEK and The Biotechnology Education Company are registered trademarks of EDVOTEK, Inc. UltraSpec-Agarose are trademarks of EDVOTEK, Inc.



1.800.EDVOTEK • Fax 202.370.1501 • info@edvotek.com • www.edvotek.com

Duplication of any part of this document is permitted for non-profit educational purposes only. Copyright © 1989-2022 EDVOTEK, Inc., all rights reserved. Version 207.200317

Experiment Components

Component	Storage	Check ✓
DNA Samples for Electrophoresis		
A Standard DNA Fragments	-20 °C Freezer	<input type="checkbox"/>
B Mother's DNA cut with Enzyme	-20 °C Freezer	<input type="checkbox"/>
C Child's DNA cut with Enzyme	-20 °C Freezer	<input type="checkbox"/>
D Father 1's DNA cut with Enzyme	-20 °C Freezer	<input type="checkbox"/>
E Father 2's DNA cut with Enzyme	-20 °C Freezer	<input type="checkbox"/>
Components for Membrane Transfer		
• Pre-cut Nylon Membranes (7 x 7 cm)	Room Temp.	<input type="checkbox"/>
• Pre-cut Filter Papers (7 x 7 cm)	Room Temp.	<input type="checkbox"/>
Other Reagents & Supplies		
• UltraSpec-Agarose™ Powder	Room Temp.	<input type="checkbox"/>
• Concentrated Electrophoresis Buffer	Room Temp.	<input type="checkbox"/>
• Practice Gel Loading Solution	Room Temp.	<input type="checkbox"/>
• Concentrated Blue-Blot DNA Stain™ Solution	Room Temp.	<input type="checkbox"/>
• 1 mL Pipette	Room Temp.	<input type="checkbox"/>
• Transfer Pipettes	Room Temp.	<input type="checkbox"/>
• 100 mL Graduated Cylinder	Room Temp.	<input type="checkbox"/>

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)
- NaCl
- NaOH
- 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)

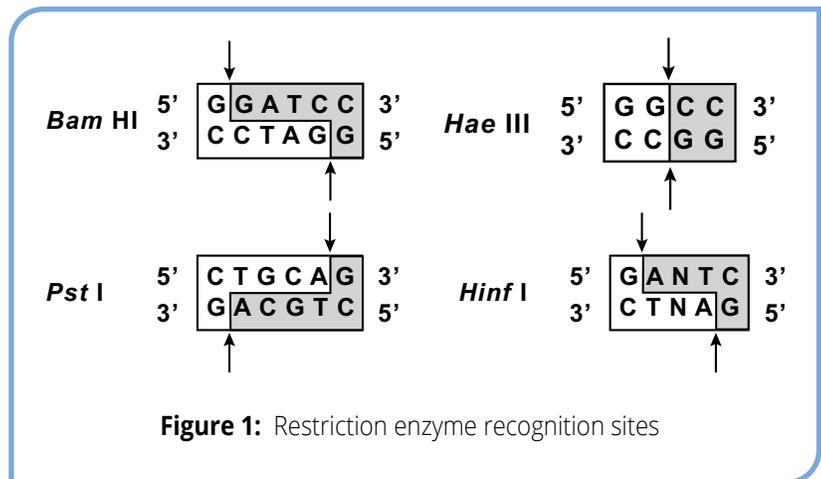
Background Information

DNA FINGERPRINTING

The human genome is 99.9% identical between individuals. However, there are regions of variation within our genome called polymorphisms. Each of us inherits a unique combination of polymorphisms from our parents. By focusing on several polymorphic regions, scientist can uncover a person's "DNA fingerprint". Like a regular fingerprint, a DNA fingerprint can be used to identify and distinguish between individuals. Because polymorphisms are inherited, DNA fingerprints can also be used to determine family relationships.

DNA fingerprinting was first used as a forensic tool in the United Kingdom, following the pioneering work of Dr. Alex Jeffreys. Fingerprint analysis by Jeffreys led to the apprehension of a murderer in the first forensic DNA case in 1987. Since then, fingerprinting has been used in thousands of convictions. Additionally, hundreds of convicted prison inmates have been exonerated from their crimes. DNA fingerprinting is also used to determine parentage, identify missing people, identify human remains, and diagnose certain inherited disorders.

Today there are many ways to observe similarities and differences in people's DNA. DNA fingerprinting is a general term that applies to them all. Three of the most popular technologies are sequencing, polymorphic chain reactions (PCR), and Southern blots. In this experiment, you will perform a Southern blot on restriction enzyme digested DNA. You will then analyze the resulting DNA fingerprints of a mother, child, and two possible fathers in order to resolve a paternity case.



USE OF RESTRICTION ENZYMES IN DNA FINGERPRINTING

Restriction enzymes are endonucleases which catalyze the cleavage of phosphodiester bonds within both DNA strands. This process, called digestion, results in DNA molecules that are cut at very specific sites. These cut sites occur in or near palindromic sequences of bases called recognition sites, which are generally 4 to 8 base pairs in length. The two most commonly used restriction enzymes in DNA fingerprinting are *Hae* III and *Hinf* I, which are 4-base and 5-base cutting enzymes. The examples in the figure 1 show recognition sites for various restriction enzymes.

In general, the longer the DNA molecule, the greater the probability that a given recognition site will occur. Human DNA is very large and contains approximately three billion base pairs. A restriction enzyme having a 6-base pair recognition site, such as *Bam* HI, would be expected to cut human DNA into approximately 750,000 different fragments. Because many restriction enzyme recognition sites are also polymorphic, allowing restriction enzymes to digest a DNA sample results in a unique mixture of many differently sized DNA fragments. These variations, called Restriction Fragment Length Polymorphism (RFLP), are ideal for DNA fingerprinting.

For simple samples (such as plasmid DNA and viral DNA) RFLPs can be visualized as distinct bands on an agarose gel using electrophoresis and DNA staining. However, for large and complex DNA (such as human DNA), gel electrophoresis and DNA staining results in a smear with no obvious banding pattern. This is because the digestion of these samples generates so

many RFLPs that they exceed the resolving capacities of the agarose. Southern blots overcome this challenge by pairing restriction enzyme digestion with a probe hybridization step that highlights a much smaller subset of RFLPs.

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 (Figure 2). These manipulations and modifications are described in detail below:

- 1. Collect and Extract:** In a forensic setting, blood or other tissue samples are collected from a crime scene or victim and cell samples are collected from potential suspects. For paternity determinations, cell samples are obtained from the mother, child, and possible fathers. These samples are then treated with a reagent mixture that contains a detergent to rupture the cell membranes and obtain the DNA for further analysis.
- 2. 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.
- 3. 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.
- 4. 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.

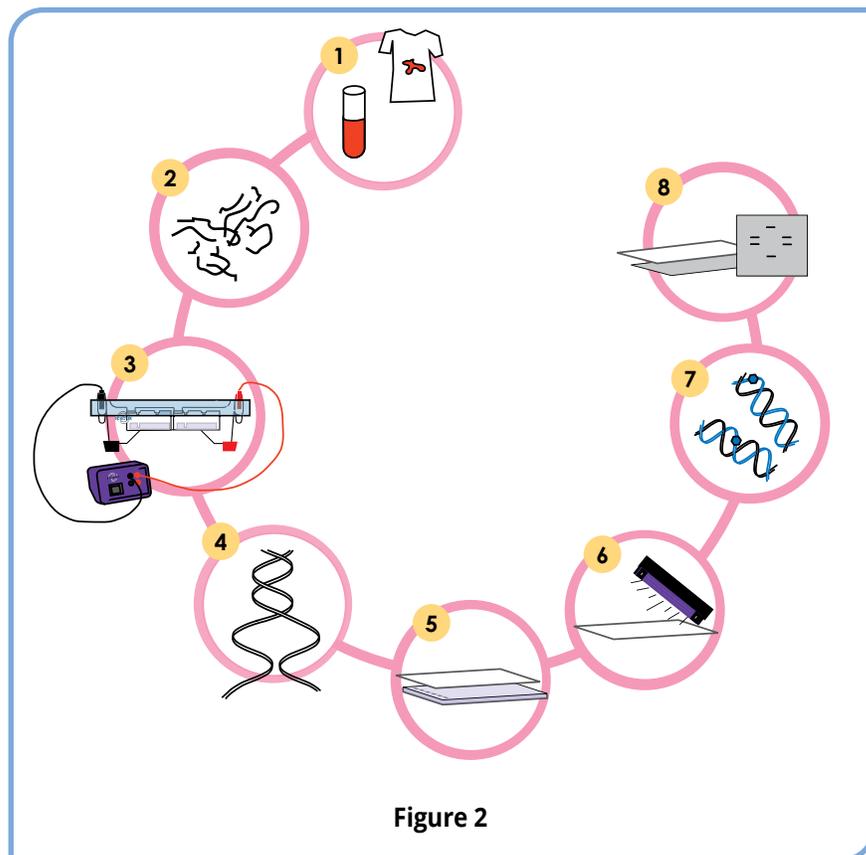


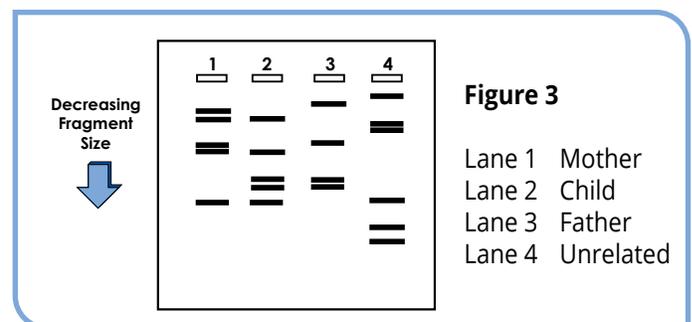
Figure 2

5. **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.
6. **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.
7. **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.
8. **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.

PATERNITY ANALYSIS

Humans are diploid organisms with 23 pairs of chromosomes. This means that every DNA region comes in two copies - one that was inherited from the individual's mother and one that was inherited from their father. These two copies can be identical (in which case the individual is said to be "homozygous" at that location) or different (in which case the individual is said to be "heterozygous" at that location).

For paternity determinations, DNA is obtained from the mother, child, and possible fathers. A child's DNA is a composite of both parent DNA. Therefore, a comparison of DNA RFLP patterns obtained from the mother and child will be a partial match. DNA bands in the child's fingerprint that are not present in the mother's profile must match with those from the biological father. Not all of the bands present in the parent's fingerprint will appear in the child's fingerprint. However, as shown in Figure 3, DNA bands that appear in the child's fingerprint must be found in the fingerprint from either the father or mother.



Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment students perform a modified Southern blot that will introduce them to the key steps of this important biotechnology all while helping resolve a paternity case.

LABORATORY SAFETY

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:

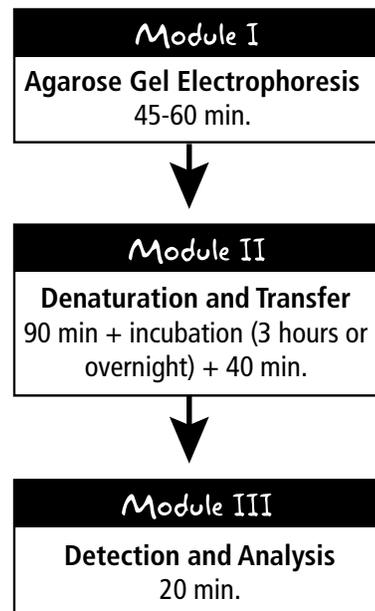
- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

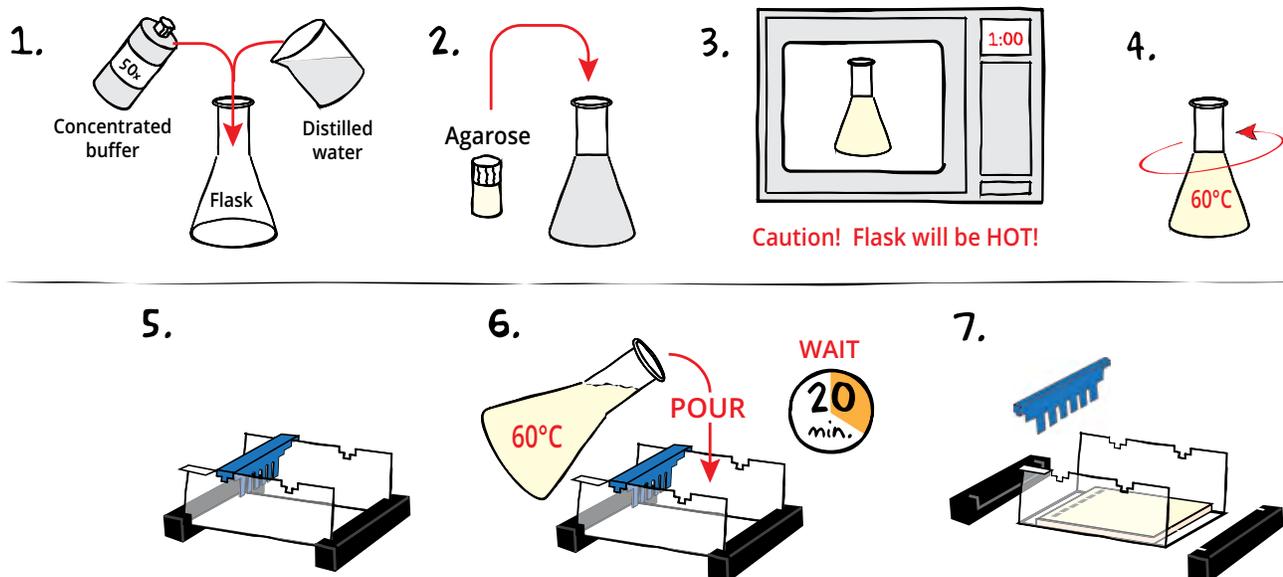
- Record (draw) your observations, or photograph the results.

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



CASTING THE AGAROSE GEL

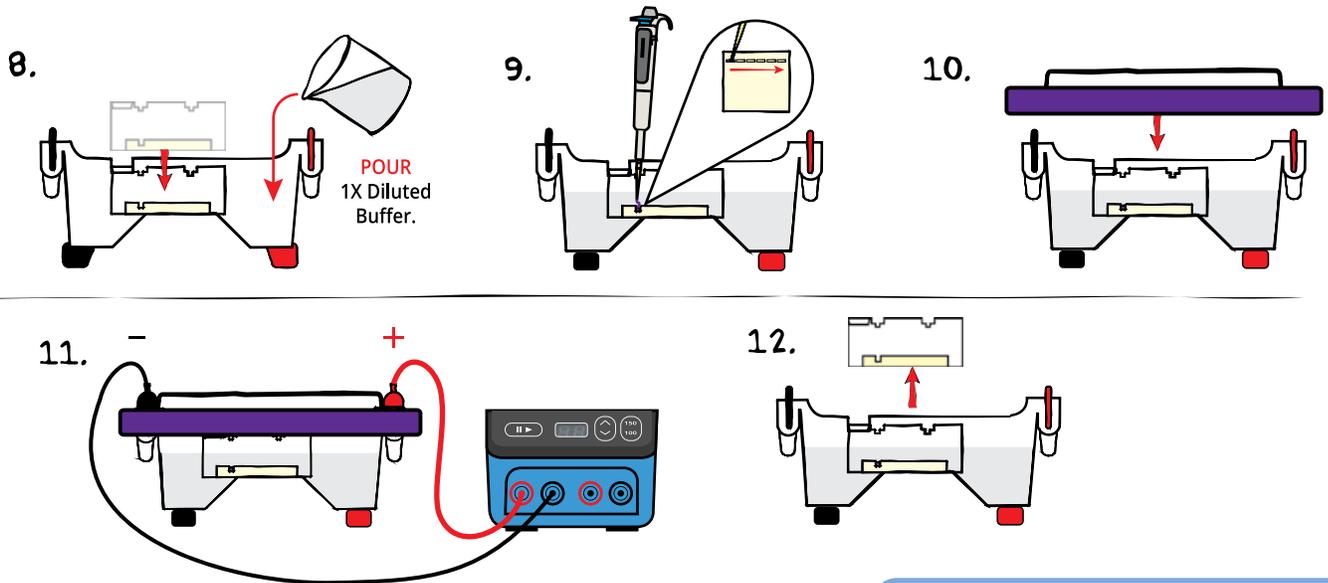
- DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
- MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
- DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** 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).
- COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat.
- 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.
- 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.
- 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™ Gels				
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	0.24 g	30 mL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL

*Recommended gel volume for the EDGE™ Integrated Electrophoresis System. (Cat. #500).

Module I: Agarose Gel Electrophoresis



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** 35-38 μL of each DNA sample according to Table 1.
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 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**.

REMINDER:

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

Table 1: Gel Loading

Lane	Tube	Sample
1	A	Standard DNA Fragments
2	B	Mother's DNA cut with Enzyme
3	C	Child's DNA cut with Enzyme
4	D	Father 1's DNA cut with Enzyme
5	E	Father 2's DNA cut with Enzyme

Table B
1x Electrophoresis Buffer (Chamber Buffer)

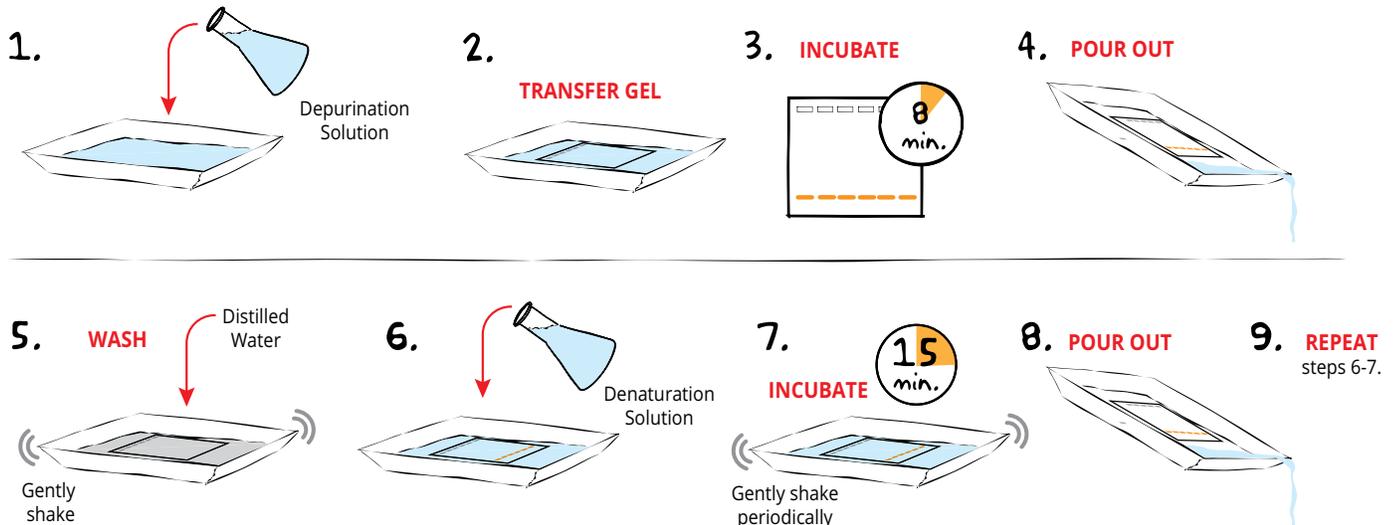
EDVOTEK Model #	Total Volume Required	Dilution	
		50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

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

Volts	Electrophoresis Model	
	EDGE™	M12 & M36
	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

*Gels that have previously been removed from their trays should be "anchored" back to the tray with a few drops of molten agarose before placing into the electrophoresis chamber. This will prevent the gels from sliding around in the trays and the chambers.

Module II: Denaturation and Transfer



DEPURINATION

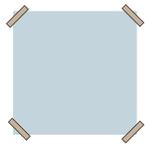
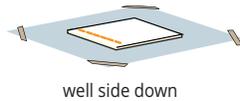
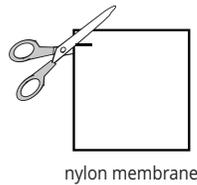
1. **ADD** 200 mL of depurination solution (0.25 N HCl) to a plastic tray.
2. **TRANSFER** the gel from Module I to this tray. Make sure the gel is immersed in the solution.
3. **INCUBATE** at room temperature for 8 minutes. **WATCH** the time *carefully* as over-incubation will fragment the DNA and 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

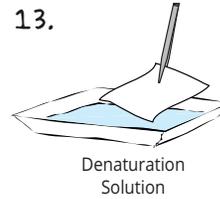
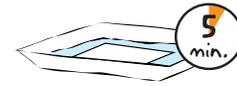
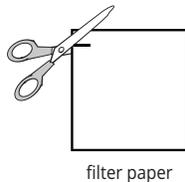
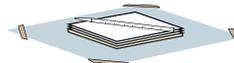
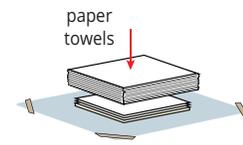
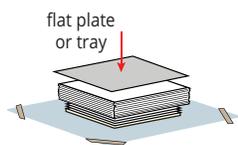
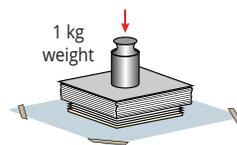
6. **ADD** 200 mL of denaturation solution (0.5 M NaOH/0.6 M NaCl) to the tray.
7. **INCUBATE** at room temperature for 15 minutes. The density of the solution will cause the gel to float, periodically **SHAKE** the tray to keep it immersed.
8. **POUR OUT** the denaturation solution.
9. **REPEAT** steps 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

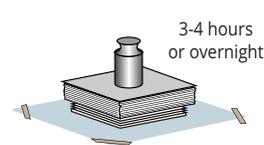
10.

11. **PLACE**12. **TRIM**

13.

14. **SOAK**15. **PLACE**16. **TRIM**17. **PLACE**18. **SMOOTH**19. **PLACE**20. **PLACE**21. **PLACE**

22.



SOUTHERN BLOT TRANSFER

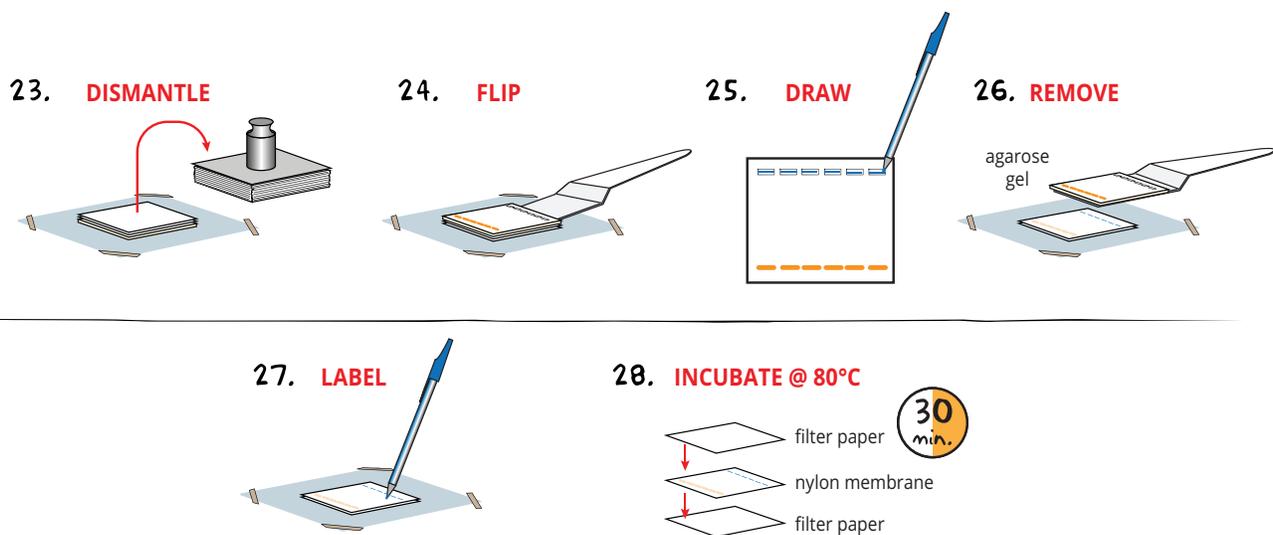
Oils from your hands and powders from gloves can interfere with the transfer. For steps 10-20 wear rinsed and dried lab gloves and for steps 12-15 use two clean forceps.

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



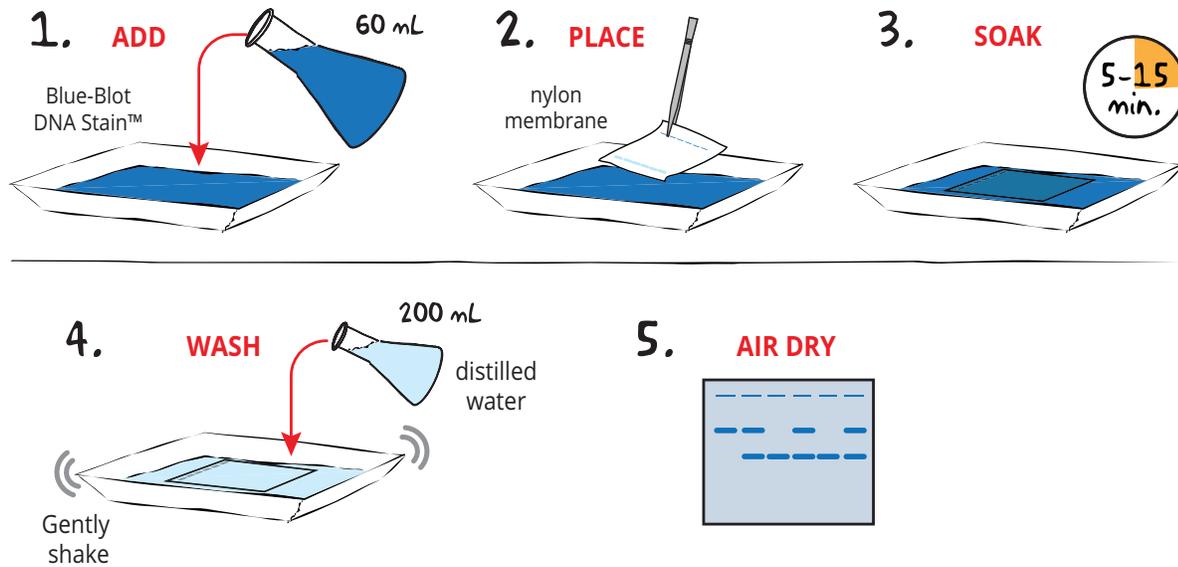
23. **DISMANTLE** the stack above the filter paper by removing the weight, tray, and paper towels.
24. **FLIP** the remaining stack (filter paper-membrane-gel) so that the gel is now facing up. This can be done with a spatula or with forceps and rinsed gloves.
25. Use a pen to **DRAW** through the six 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 on the membrane. Using a pen, gently **LABEL** this side of the membrane with your group's 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. What band(s) is present in both the Mother's DNA and Child's DNA?
8. What band(s) is present in the Child's DNA but not the Mother's DNA?
9. Based on your Southern blot results, who is the father?

Study Questions

1. What is polymorphic DNA? How is it used for identification purposes?
2. What are the key steps of a typical Southern blot?
3. What is the most likely cause of Restriction Fragment Length Polymorphisms?
4. What is the advantage of using a Southern blot when analyzing an individual's full genome over simply using restriction enzyme digestion followed by gel electrophoresis and DNA gel staining?

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.

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.

Technical Support

1.800.EDVOTEK

Mon. - Fri. 8 AM to 5:30 PM EST



Please Have the Following Info:

- Product Number & Description
- Lot Number on Box
- Order/Purchase Order #

1.800.EDVOTEK • info@edvotek.com • www.edvotek.com

www.edvotek.com

- Online Catalog
- Order Products
- Experiment Protocols
- Tech Support
- Resources!



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 pre-cut 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. Student groups can share these tubes or you may aliquot the solutions to additional tubes. In this case each group should receive 40 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 needs:

- 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 may interfere with the transfer.
4. Set an incubator to 80°C. (If this will be after an overnight transfer, wait until the second day of Module II.)

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 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.

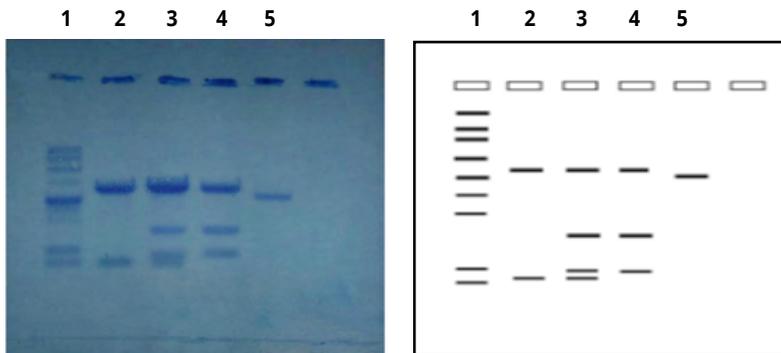
FOR MODULE III

Each group should receive:

- Tray or sealable bag large enough to hold a 7x7 membrane
- Diluted Blue-Blot DNA Stain™ Solution

Experiment Results and Analysis

EXPECTED AGAROSE GEL RESULTS:



Standard DNA Fragment Sizes:

23130 bp, 9416 bp, 6557 bp,
4361 bp, 3000 bp, 2322 bp,
2027 bp, 725 bp*, 570 bp*.

** These fragments are not
always visible due to their size.*

Lane Sample

- | | |
|---|--------------------------------|
| 1 | Standard DNA Fragments |
| 2 | Mother's DNA cut with Enzyme |
| 3 | Child's DNA cut with Enzyme |
| 4 | Father 1's DNA cut with Enzyme |
| 5 | Father 2's DNA cut with Enzyme |

ANSWERS TO MODULE III ANALYSIS QUESTIONS:

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

Refer to the gel results, above.

7. What band(s) is present in both the Mother's DNA and Child's DNA?

Both the top (largest) and bottom (smallest) band in the Child's DNA sample is also present in the Mother's DNA sample.

8. What band(s) is present in the Child's DNA but not the Mother's DNA?

The middle two bands in the Child's DNA sample are not present in the Mother's DNA sample.

9. Based on your Southern blot results, who is the Father?

Father 1

**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, 45 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 cm tray. **For this experiment, 7 x 7 cm gels are recommended.**

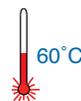


Table E Batch Prep of 0.8% UltraSpec-Agarose™					
Amt of Agarose	+	Concentrated Buffer (50X)	+	Distilled Water	Total Volume
3.0 g		7.5 mL		367.5 mL	375 mL

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.

7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks. Do not freeze gels.