EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

EDVO-Kit #

311

DNA Fingerprinting III:
Southern Blot Analysis

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

This experiment introduces students to Southern Blot analysis used in DNA Fingerprinting for a hypothetical paternity determination.

Table of Contents

This experiment contains enough reagents for 5 groups.

Experiment Components

Storage

Biotin labeled samples, ready for electrophoresis — **Store components A-I in the freezer.**

A Standard DNA fragments
B Mother DNA cut with Enzyme 1
C Mother DNA cut with Enzyme 2
D Child DNA cut with Enzyme 1
E Child DNA cut with Enzyme 2
F Father 1 DNA cut with Enzyme 1
G Father 1 DNA cut with Enzyme 2
H Father 2 DNA cut with Enzyme 1
I Father 2 DNA cut with Enzyme 2

Reagents for Southern Blot and Non-Isotopic Detection

J	Detection buffer, 20x concentrated	**Refrigerator**
K	Shielding Buffer	**Refrigerator**
L	Termination buffer, 20x concentrated	**Refrigerator**
M	NBT/BCIP Tablets	**Freezer**
N	Streptavidin-Alkaline Phosphatase, SAAP	**Freezer**

Other Reagents & Supplies — **Room temp**

- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- Nylon Membranes (7 x 14 cm)
- Precut filter paper (7 x 14 cm)

STORAGE OF PERISHABLES

This experiment includes perishable components which were sent on wet ice. Store these components at -20°C (-4°F). Please note what type of freezer you have and store components accordingly.

Frost-free Freezer

Most refrigerator/freezers in homes are frost free. This means the freezer goes through warming cycles to eliminate frost (defrost cycle). If using this type of freezer, keep the enzymes in the foam chest (with the ice brick) in which they were sent. This will help maintain the enzymes at -20°C when the freezer goes through the defrost cycle.

Non Frost-free Freezer

These older model freezers, which are still sold but are harder to find, do not go through warming cycles. Therefore, ice will build up on freezer walls over time. If using this type of freezer, check to make sure that it maintains temperature at -20°C.

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- UV Transilluminator (for optional staining)
- Incubator (37° C, 65° C, and 80° C)
- Microwave, hot plate or burner
- 100 ml graduated cylinder
- 10 ml graduated cylinder, or 5 or 10 ml pipets and pumps
- 250 ml flasks or beakers
- Hot gloves or beaker tongs
- Safety goggles and disposable laboratory gloves
- Plastic wrap
- Paper towels
- Forceps
- Plastic trays (non-acrylic, to hold 7 x 14 cm membrane)
- Distilled or deionized water (approximately 12-15 liters)
- Ice (for pre-lab)
- NaCl
- NaOH
- Concentrated HCl

DNA Fingerprinting

DNA fingerprinting (also called DNA typing) allows for the unambiguous identification of the source of DNA. The method has become very important in forensic laboratories where it is used to provide evidence in paternity and criminal cases. In contrast to the more conventional procedures, such as blood typing that can only exclude a suspect, DNA fingerprinting can provide positive identification with great accuracy.

When first introduced, DNA fingerprinting involved the electrophoretic analysis of DNA fragment sizes generated by restriction enzymes. With the advent of the Polymerase Chain Reaction (PCR), specific regions of DNA are amplified and analyzed by gel electrophoresis or other procedures.

This experiment will focus on restriction enzymes and Southern blot analysis as applied to DNA fingerprinting.

ABOUT RESTRICTION ENZYMES

Restriction enzymes are endonucleases that catalyze the cleavage of phosphodiester bonds within both strands of DNA. They require Mg^{+2} for activity and generate a 5 prime (5') phosphate and a 3 prime (3') hydroxyl group at the cleavage sites. The distinguishing feature of restriction enzymes is that they cleave DNA at very specific base sequences that are recognition sites. Restriction enzymes are produced by many different species of bacteria (including blue-green algae). Over 3,000 restriction enzymes have been discovered and catalogued.

Restriction enzymes are named according to the organism from which they are isolated. This is done by using the first letter of the genus followed by the first two letters of the species. Only certain strains of particular species produce restriction enzymes that follow the species designation in the name. Roman numerals are used to designate the different restriction enzymes produced by the same organism.

Specific double-stranded DNA sequences (usually 4 to 8 base pairs in length) are used by restriction enzymes as recognition sites. Cleavage occurs within or near these sites that are indicated by arrows (see examples below). Recognition sites are usually symmetrical, i.e., both DNA strands in the site have the same base sequence when read 5' to 3'.

```
      ↓
5'-GAATTC-3'  ——>  5'-G        AATTC-3'
3'-CTTAAG-5'  ——>  3-CTTAA        G-5'
           ↑
```

As shown, *Eco* RI causes staggered cleavage at its site. The resulting ends of DNA fragments are called "sticky" or "cohesive" due to the single-stranded regions at the ends that are complementary.

DNA Fingerprinting Analysis

Certain restriction enzymes, such as *Hae* III, introduce cuts that are directly opposite each other. This type of cleavage generates "blunt" ends.

```
      ↓
5'-GGCC-3'  ——>  5'-GG   CC-3'
3'-CCGG-5'  ——>  3'-CC   GG-5'
      ↑
```

The recognition sites of another subset of restriction enzymes contain variable base positions. For example, *Ava* I recognizes:

```
   ↓
5'-CPyCGPuG-3'      (Py = pyrimidine = C or T and
3'-GPuGCPyC-5'            Pu = purine = G or A)
         ↑
```

Keep in mind that A pairs with T and G pairs with C. Consequently, there are four possible sequences *Ava* I recognizes. Recognition sites of this type are called degenerate recognition sites.

Another group of restriction enzymes have recognition sites that are divided by a certain number of totally variable bases. For example, *Bgl* I recognizes:

```
            ↓
5'-GCCNNNNNGGC-3'     (N = A, G, C  or T)
3'-CGGNNNNNCCG-5'
      ↑
```

There are 625 possible sequences that *Bgl* I can cleave. The only bases the enzyme truly recognizes are the six G-C flanking base pairs at both ends of the palindrome. The *Bgl* I recognition site is hyphenated and is separated by 5 base pairs.

The size of the DNA fragments generated by restriction enzyme cleavage depends on the distance between recognition sites. In general, bigger DNA affords a greater probability that a given recognition site will occur. The number of times a restriction enzyme cleaves double-stranded DNA is determined as 4^N where N is the number of bases that make up the restriction enzyme recognition site. For *Eco* RI, N = 6 bases, therefore $4^6 = 4096$. This number designates the frequency that *Eco* RI will cut DNA.

ABOUT AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis is a procedure used to analyze DNA fragments generated by restriction enzymes. Agarose gels consist of microscopic pores that act as a molecular sieve. Samples of DNA are loaded into wells formed in the gel during gel casting. Since DNA has a strong negative charge at neutral pH, it migrates through the gel towards the positive electrode. In general, DNA fragments are separated by the gel according to their size. The smaller the fragment, the faster it migrates. After electrophoresis, DNA fragments can be visualized by staining the gel with dyes. Restriction enzyme cleavage of relatively small DNA molecules, such as plasmids and viral DNAs, usually produce discrete banding patterns of the DNA frag-

DNA Fingerprinting Analysis

ments. However, cleavage of large DNA, such as human chromosomal DNA, generates many differently sized fragments that exceed the resolving capacity of agarose gels. Consequently, the cleaved DNA is visualized as a smear after staining and has no obvious banding patterns.

ABOUT SOUTHERN BLOT ANALYSIS

Southern blots facilitate RFLP analysis of complex DNA. After electrophoresis, the gel is sequentially treated in HCl and NaOH. The HCl treatment introduces apurinic sites in DNA that makes phophodiester bonds at these sites labile and introduces nicks in double-stranded DNA. Apurinic sites result when a purine base (adenine) is cleaved as in an A/T base pair. The NaOH treatment disrupts hydrogen bonds between the base pairs. The sequential acid and base treatments generate small single stranded DNA fragments from large DNAs . This facilitates transfer of DNA fragments from the gel to the nylon membrane.

In Southern blots a replica of the gel DNA bands is transferred to a nylon membrane. This is done by placing the nylon membrane on the gel after electrophoresis and transferring the fragments to the membrane by capillary action or by electrotransfer. Transferred DNA becomes permanently adsorbed to the nylon and can be manipulated easier than a gel.

Analysis of transferred DNA requires hybridization of the specific gene(s) with probe(s) that are small single stranded piece of DNA. Initially radioactively labeled probes where used for analysis, but more recently (as in this experiment) non-isotopic detection systems are routinely used. In forensic RFLP analysis the probes are single stranded DNA fragments that contains base sequences that are complementary to the variable arrays of tandem repeats found in human chromosomes. A solution containing the probe is incubated with the membrane that contains the single stranded transferred DNA fragments. Under the proper conditions, the probe will only base pair (hybridize) to DNA fragments that contain the complementary sequences. The nylon membrane used in Southern blot analysis is washed to remove excess probe. Only fragments that are hybridized to the probe will be detected as discrete DNA bands that serve as a set of fingerprints.

ABOUT DNA FINGERPRINTING

No two individuals have exactly the same pattern of restriction enzyme recognition sites. This is due to the large number of alleles present in human DNA . Alleles are alternate forms of a gene that result in alternative expressions of genetic traits. Chromosomes occur in matching pairs, one of maternal and the other of paternal origin. The two copies of a gene at a given chromosomal locus represent a composite of parental genes that constitute part of an individual's unique genotype. It follows that alleles have differences in their base sequences that result in frequency variations of restriction enzyme recognition sites. Other differences in base sequences between individuals can occur because of mutations and deletions that create or eliminate restriction recognition sites. Figure 1 shows how a "silent mutation" that can eliminate a restriction enzyme recognition site without altering the protein product.

Experiment Procedure

DNA Fingerprinting Analysis

No two individuals have exactly the same pattern of restriction enzyme recognition sites. This is due to the large number of alleles present in human DNA . Alleles are alternate forms of a gene that result in alternative expressions of genetic traits. Chromosomes occur in matching pairs, one of maternal and the other of paternal origin. The two copies of a gene at a given chromosomal locus represent a composite of parental genes that constitute part of an individual's unique genotype. It follows that alleles have differences in their base sequences that result in frequency variations of restriction enzyme recognition sites. Other differences in base sequences between individuals can occur because of mutations and deletions that create or eliminate restriction recognition sites. Figure 1 shows how a "silent mutation" that can eliminate a restriction enzyme recognition site without altering the protein product.

TGTTTA|TGTTTA|TGTTTA|.....variable number

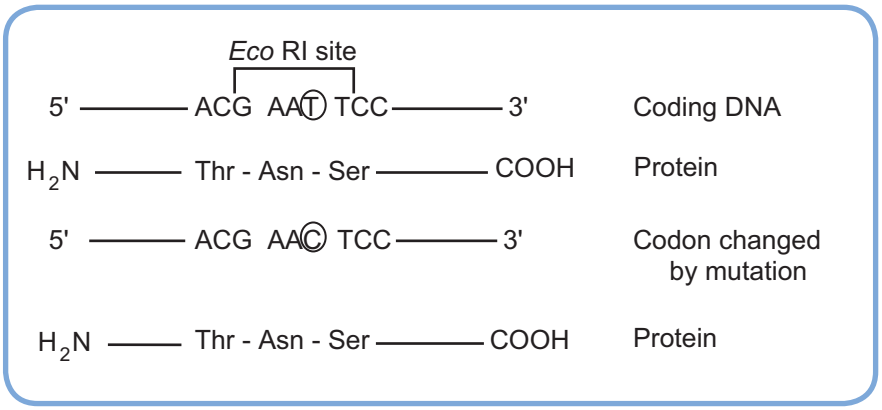

Figure 1: Effect of a point mutation results in an altered amino acid code.

When tandum arrays are flanked by restriction recognition sites, the length of the repeat will determine the size of the restriction enzyme fragment generated. Variations in the length of these fragments between individuals in a population are known as restriction fragment length polymorphisms (RFLP). RFLPs are a manifestation of the unique molecular genetic profile, or "fingerprint", of an individual's DNA. As shown in Figure 2, there are different sizes of repetitive sequences.

In forensic, DNA samples are extracted and purified from small specimens of skin, blood, semen, or hair roots collected at the crime scene and are routinely amplified by the polymerase chain reaction (PCR) . Small amounts of DNA suitable for analysis can also be obtained from dried stains of semen or blood. Prior to the discovery of PCR, the restriction enzyme, Hae III was used for DNA fingerprinting analysis. RFLP analyses was performed on DNA samples collected at the crime scene and were compared to DNA fingerprints obtained from suspects. If RFLP patterns matched it was reasonable to assume that the suspect was at the crime scene. In practice, thirteen different probes containing various repetitious sequences are used in order to satisfy certain statistical criteria for positive identification. In addition, the same DNA sample is digested with a different restriction enzymes and probed to firmly establish an individual's unique DNA fingerprint..

DNA Fingerprinting Analysis

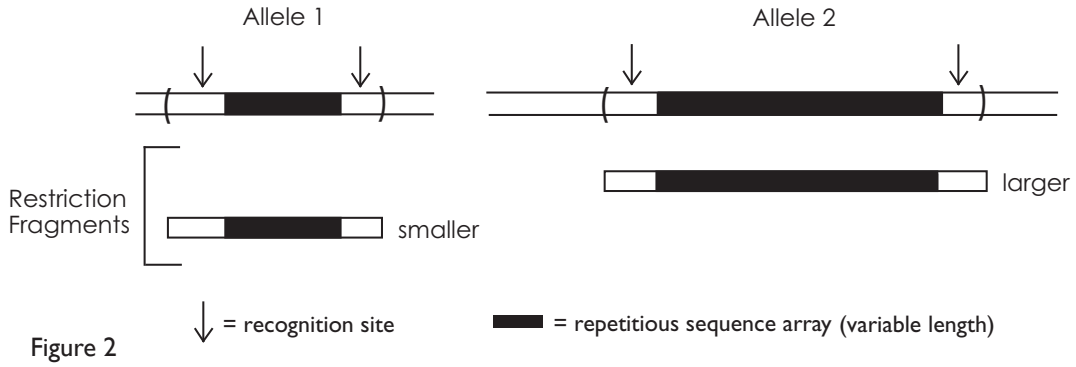

Figure 2

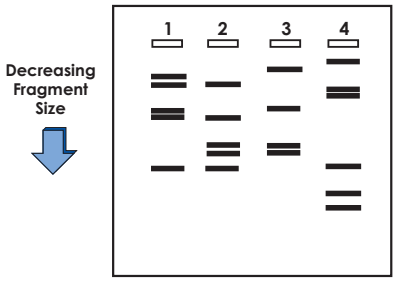

Figure 3

Lane 1 Mother
Lane 2 Child
Lane 3 Father
Lane 4 Unrelated

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, comparison of DNA fragmentation patterns obtained from the mother and child will give 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. Because of allelic differences, not all of the bands present in the parents 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.

ABOUT THE POLYMERASE CHAIN REACTION

The Polymerase Chain Reaction (PCR) has two important advantages over RFLP-based DNA fingerprinting analysis. The first is the sensitivity of PCR that allows for DNA fingerprinting identification using much smaller amounts of DNA. The second advantage is the speed of PCR analysis, which allows critical questions to be answered quickly as compared to Southern Blot analysis.

PCR amplification requires the use of a thermostable DNA polymerase, such as *Taq* polymerase. Purified from a bacterium known as Thermus Aquaticus that inhabits hot springs, *Taq* polymerase is commonly used in PCR because it remains stable at near-boiling temperatures. Also included in the PCR reaction are the four deoxynucleotides (dATP, dCTP, dGTP, and dTTP) and two synthetic oligonucleotides primers, typically 15-30 base pairs in length. These components, together with the DNA to be amplified, are incubated in an appropriate buffer that contains Mg^{+2}. The primers are designed to correspond to the DNA to be amplified also referred to as the target. The PCR

DNA Fingerprinting Analysis

reaction mixture is subjected to sequential heating/cooling cycles at three different temperatures in a thermal cycler (Figure 4).

- In the first step, the template is heated to near boiling (94° C - 96° C.) to denature (melt) the target DNA. This step, known as "denaturation" disrupts the hydrogen bonds between the two complimentary DNA strands and causes their separation.

- In the second step, the reaction mixture is cooled to a temperature that is typically in the range of 45° C- 65° C. In this step, known as "annealing", the two primers that are in great excess to the template, bind to the separated DNA strands.

- In the third step, known as "extension", the temperature is raised to 72°C. At this temperature the *Taq* polymerase is maximally active and adds nucleotides to the two hybridized primers to synthesize the new complimentary strands.

DNA fingerprinting analysis has become increasingly significant in court cases involving murder, rape, physical battery, and other types of crimes. Jurors are often asked to determine the validity of DNA evidence, resulting in both acquittals and convictions of suspected criminals. To ensure greater accuracy, scientists have incorporated standardization procedures in DNA fingerprint analysis. Standard DNA fragments are used to determine the exact size of individual DNA fragments in a DNA fingerprint. It is generally accepted that DNA fingerprints are identical only in the case of identical twins.

DNA Fingerprinting Analysis

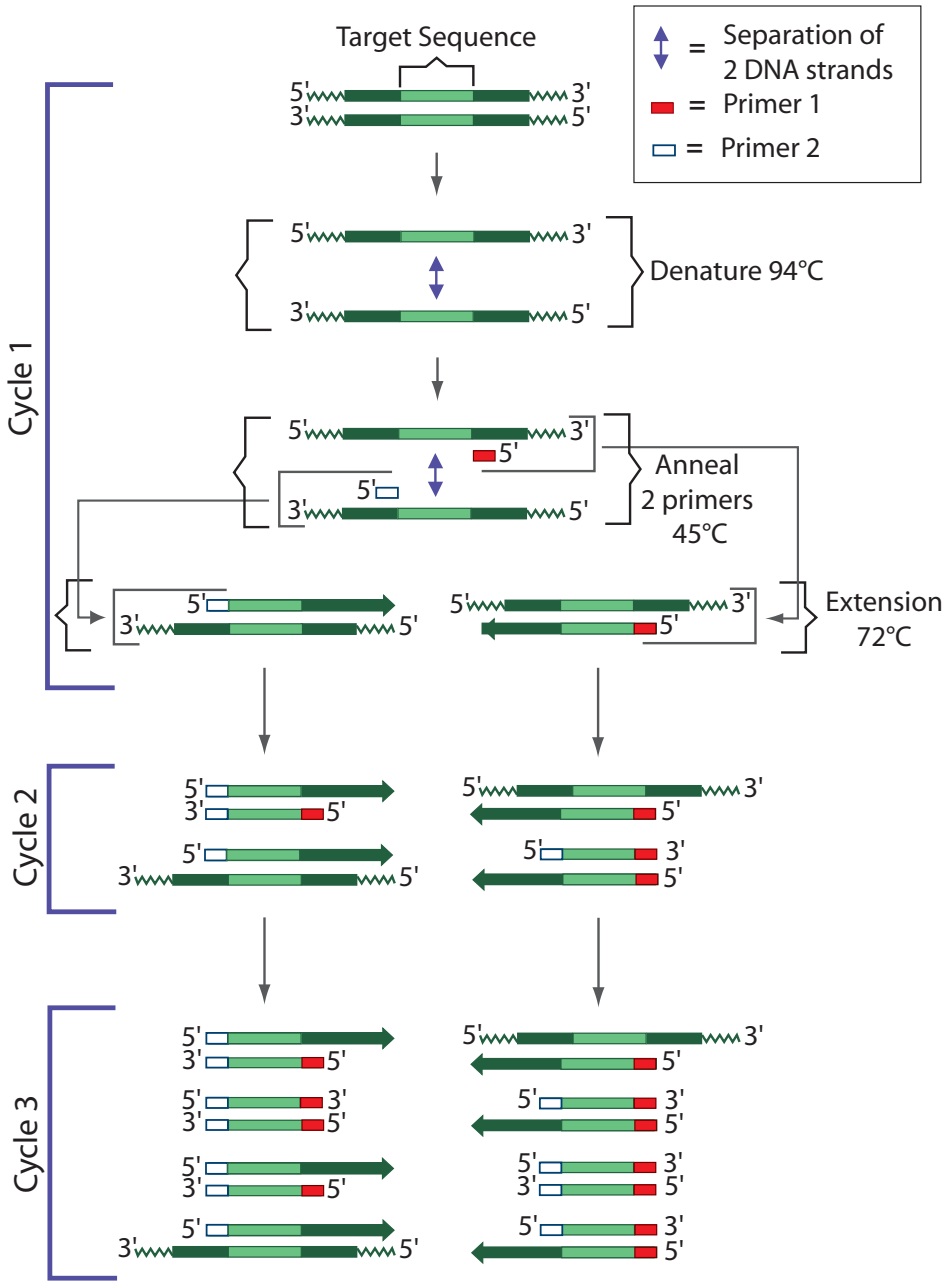

Figure 4: Polymerase Chain Reaction

DNA Fingerprinting Analysis

ABOUT THIS EXPERIMENT

The objective of the experiment is to match DNA fingerprinting patterns and identify the biological father. In an actual DNA based paternity determination, biotinylated probes would be used to detect complementary DNA sequences transferred to the membrane. The detection system employed would be identical to that used in this experiment.

In this experiment predigested and biotinylated DNA will be used to facilitate classroom instruction of Southern blots. In the hypothetical paternity case DNA from the mother, child and two possible fathers were extracted from blood samples. DNA samples were cleaved under the same conditions with two restriction enzymes in separate reactions.

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The following are the experiment steps:

1. Biotin labeled precut DNA samples are separated by gel electrophoresis.

2. DNA fragments are transferred to a nylon membrane by Southern blot analysis. Biotin groups will serve as labels for the transferred DNA.

3. To detect the presence of the biotinylated DNA a two step procedure will be employed. In the first step, non specific sites on the membrane will be blocked.

4. For the second step, a covalently linked streptavidin-alkaline phosphatase complex (SAAP) will be incubated with the nylon membrane. The SAAP will bind to the biotinylated DNA. Binding to DNA occurs by the streptavidin-biotin interaction. Since the alkaline phosphatase is covalently linked to the streptavidin, it will also bind to the biotinylated DNA.

5. The membrane is incubated with a substrate for alkaline phosphatase, 5-Bromo-4-chloro-3-indolyl phosphate (BCIP). This reaction causes Nitro Blue tetrazolium (NBT) to become reduced. Upon reduction NBT is converted into an insoluble blue product that precipitates on the nylon membrane indicating the position of the transferred DNA.

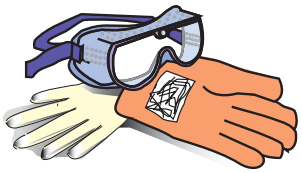
There is no blood or human tissues used in this experiment.

Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

This experiment introduces students to Southern Blot analysis used in DNA Fingerprinting for a hypothetical paternity determination.

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. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOK RECORDINGS:

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.

Following 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

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

AGAROSE GEL REQUIREMENTS FOR THIS EXPERIMENT

- Recommended gel size: 7 x 14 cm
- Number of sample wells required: 10
- Agarose gel concentration: 0.8%

PREPARING THE AGAROSE GEL

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
2. Place a well-former template (comb) in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.
3. To a 250 ml flask or beaker, add agarose powder and buffer as indicated in the Reference Tables (Appendix A) provided by your instructor. Swirl the mixture to disperse clumps of agarose powder.
4. With a marking pen, indicate the level of the solution volume on the outside of the flask.
5. Heat the mixture using a microwave oven or burner to dissolve the agarose powder.
6. Cool the agarose solution to 60°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume marked in step 4.

Important Note

Continue heating until the final solution appears clear (like water) without any undissolved particles. Check the solution carefully. If you see particles, the agarose is not completely dissolved.

After the agarose is cooled to 60°C:

7. Place the bed on a level surface and pour the cooled agarose solution into the bed.
8. Allow the agarose to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.
9. After the gel is solidified, be careful not to damage or tear the wells while removing the rubber dams or tape and comb(s) from the gel bed.
10. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
11. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer (refer to Table B on the instruction sheet from the Appendix provided by your instructor).

Experiment Procedure

Module I: Agarose Gel Electrophoresis

LOADING THE SAMPLES

Make sure the gel is completely submerged under buffer before loading the samples and conducting electrophoresis.

Gel with 2 rows, 6 wells per row:
(Model #M12 with two standard 6-tooth combs)
Load 35-38 µl of each sample.

First Row

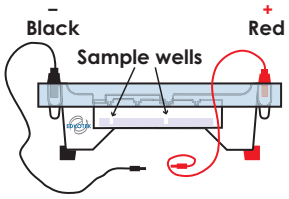
Lane	Tube	
1	A	Standard DNA fragments
2	B	Mother DNA cut with Enzyme 1
3	C	Mother DNA cut with Enzyme 2
4	D	Child DNA cut with Enzyme 1
5	E	Child DNA cut with Enzyme 2

Second Row

Lane	Tube	
1	A	Standard DNA fragments
2	F	Father 1 DNA cut with Enzyme 1
3	G	Father 1 DNA cut with Enzyme 2
4	H	Father 2 DNA cut with Enzyme 1
5	I	Father 2 DNA cut with Enzyme 2

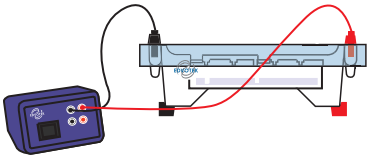
Reminder:

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



RUNNING THE GEL

1. After the DNA samples are loaded, properly orient the cover and carefully snap it onto the electrode terminals.
2. Insert the plugs of the black and red wires into the corresponding inputs of the power source.
3. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor.
4. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.
5. After the electrophoresis is completed, disconnect the power and remove the gel from the bed. (See Appendix C for optional staining step.)
6. Proceed to Module II - Soutnern Blot Analysis.



Electrophoresis can be completed in 15-20 minutes under optimal conditions. For Time and Voltage recommendations, refer to Table C (from Appendix A).

Module II: Southern Blot Analysis

Quick Reference:

The depurination procedure must be brief (no longer than 8 minutes). Prolonged exposure to HCl completely depurinates DNA strands. Subsequent treatment with a denaturation solution would fragment the completely depurinated DNA molecules into very short oligonucleotides, which are poor targets for probes.

During this procedure, the bromophenol blue tracking dye in the gel will change color from blue to greenish blue to yellow. After 8 minutes, the dye should be greenish to slightly yellow in color.

OVERVIEW

During this procedure you will perform the Southern blot, which involves the transfer of DNA fragments from the agarose gel to a nylon membrane. After the transfer, the membrane will be baked for a short time to fix the DNA to the membrane.

After electrophoresis, the gel is treated in HCl and NaOH. The HCl treatment introduces apurinic sites in DNA which makes phophodiester bonds at these sites labile and therefore introduces nicks in double-stranded DNA. Apurinic sites result when the purine base is removed, such as an adenine residue from the A/ T base pair. The NaOH treatment disrupts the the interstrand hydrogen bonds between the base pairs. The sequential acid and base treatments result in the formation of small fragments from large DNA fragments. This facilitates the transfer of DNA fragments to the nylon membrane. This procedure also causes double-stranded DNA restriction fragments to be converted into their single-stranded form.

Experiment Procedure

DEPURINATION/DENATURATION
(Approximately 1 hour)

1. After electrophoresis, place one agarose gel per tray containing 200 ml of 0.25 N HCl at room temperature. Allow the depurination to proceed for a maximum of 8 minutes. Stop depurination if the dye becomes completely yellow before 8 minutes.
2. Carefully discard the HCl solution - do not reuse.
3. Rinse the gel with several changes of 500 ml distilled or deionized water.
4. Add 200 ml of DNA denaturation solution (0.5M NaOH/0.6M NaCl) and soak the agarose gel for 15 minutes.

   Because of the density of the solution, the gel will float, so periodically shake the tray to immerse the gel in solution.
5. Discard the denaturation solution and add a fresh second 200 ml of DNA denaturation solution.
6. Continue to soak the gel for 30 minutes.

Module II: Southern Blot Analysis

SETTING UP THE SOUTHERN BLOT TRANSFER
(Approximately 30 minutes)

6. Place a sheet of plastic wrap on a flat level lab bench.

7. Remove the gel from the tray when the second 30 minute incubation in the NaOH/NaCl denaturation solution is complete. Save this solution to wet the nylon membrane in step 11 below.

8. Place the gel (well side down) directly on the plastic wrap. By inverting the gel, the smooth surface is on top for contact with the membrane.

9. Wearing gloves and using forceps, trim the nylon membrane to the size of the gel. If your gel is 7 x 14 cm, no trimming is necessary.

   Note: The membranes are very thin white sheets that may be sandwiched between one or two sheets of protective covering. If you are not sure which is the membrane, check with your instructor.

10. Carefully pick up a membrane at the edges with two forceps.

11. Slightly bend the membrane in the middle and slowly wet the membrane, from the middle toward the edges, in the DNA Denaturation Solution which is in the tray from step 7.

12. Release the membrane and gently submerge. Allow it to become thoroughly saturated with DNA Denaturation Solution for 5 minutes.

13. Remove the saturated membrane from the DNA Denaturation Solution and place it on top of the inverted agarose gel.

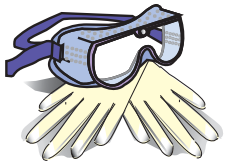
14. Place a piece of filter paper, which has been cut to the same size as the membrane, on top of the membrane.

15. Roll a 5 or 10 ml pipet across the filter paper. This will remove air bubbles.

16. Carefully place a stack of paper towels approximately 1.5 to 2 inches thick on top of the filter paper.

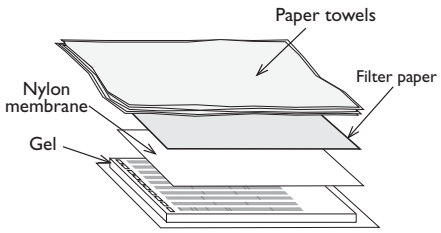
17. Place an empty 400 ml beaker in an empty tray on top of the paper towels for weight. **Do not use anything heavier than an empty beaker to provide weight**.

18. Allow the blot to progress 3-4 hours or overnight.



WEAR GLOVES - DO NOT TOUCH THE NYLON MEMBRANE WITH BARE HANDS.

Areas of the membrane touched by ungloved hands will leave oil residues and will not bind DNA during transfer. Many gloves contain powder, which will increase the background on the membrane. Put on gloves and wash them under tap water to remove any residual powder. Handle the membrane with clean forceps.



OPTIONAL STOPPING POINT

Experiment Procedure

Module II: Southern Blot Analysis

19. After incubating 3-4 hours or overnight, remove the tray, beaker, and all the paper towels.

20. Wearing rinsed gloves and using forceps, flip the stack (paper towels and filter paper) over to lie on the paper towels.

21. Using a pencil, draw through each sample well and trace its position on the nylon membrane and identify lane one.

22. Using forceps, remove the membrane from the gel. Note the thickness and consistency of the dehydrated gel.

    The gel can be discarded since all further processing takes place with the nylon membrane.

23. Take the membrane and lay it on a dry paper towel with the **DNA side up** (the side of the membrane which was in direct contact with the gel).

24. Using a pencil, label the DNA side of the membrane with your lab group number or initials onto the corner of the membrane. The bromophenol blue dye can be seen on the membrane.

25. For optimal results, place the membrane between two small sheets of filter paper and place into an 80°C oven for 30 minutes.

26. Proceed to the non-isotopic detection procedure.

OPTIONAL STOPPING POINT

The dried membrane can be stored at room temperature, away from moisture and between two sheets of filter paper until you are ready to continue.

Module III: Non-isotopic Detection of DNA

NON-ISOTOPIC DETECTION OF DNA
(Approximately 4 hours)

During this experiment, you will process the membrane to visualize DNA bands. **There are no stopping points in this module.**

MEMBRANE SHIELDING

The purpose of this procedure is to shield non-specific binding sites on the nylon membrane. This helps to reduce the overall background. **Use only one membrane per tray during processing.**

1. Rehydrate the baked membrane for 5 minutes in 100 ml of diluted Detection Buffer (J), in a tray. **Keep the DNA side of the membrane UP.** Place only one membrane per tray.

2. While the membrane is rehydrating, warm 50 ml of Membrane Shielding buffer (K) to 65°C in the incubation oven or on a hot plate.

   - Use a graduated cylinder to measure 50 ml of Shielding Buffer (K) and place into a 400 ml beaker.
   - Place in the 65°C incubation oven, or exercise caution if warming the solution on a hot plate set at 65˚C.
   - Do not allow the temperature to exceed 65˚C. If the temperature exceeds 65˚C, the solution may become thickened (like gelatin) and difficult to disperse evenly over the membrane.

3. Stabilize the membrane with forceps. Pour and discard the Detection buffer from the tray. Do not allow the membrane to drop into the sink.

4. Slowly add 50 ml of the warmed Membrane Shielding Buffer (K), to the tray, pouring the buffer very gently over the top of the membrane. The membrane should still have the DNA side facing up.

5. Cover the tray with the lid and place into a 60° C - 65˚C incubation oven for 1 hour. **(NOTE: Temperature should not exceed 65° C.)**

Module III: Non-isotopic Detection of DNA

DETECTION

6. After the one-hour incubation, remove the lid from the tray and place it upside down on the lab bench.
7. Turn down the oven to 37˚C for color development which begins with step 18.
8. Using forceps to hold the membrane in the tray, carefully pour off the Shielding buffer.
9. Place the membrane on the underside of the tray lid, set aside.
10. Rinse out the inside of the tray with distilled water. Shake out the tray to remove excess water. Do not dry with paper towels (lint may increase background).
11. Place the membrane back into the tray, DNA side up. Use only one membrane per tray!
12. In a clean 50 ml Erlenmeyer flask, add 8 microliters of SAAP (N) to 10 ml of diluted Detection buffer. Mix well. Cover the membrane in the tray with this solution.
13. Incubate at room temperature for 10 minutes. Occasionally rock the tray from side to side to ensure that the membrane is covered with solution.
14. Remove the membrane from the tray and place it on the underside of the lid, as before.
15. Wash out the tray thoroughly with distilled water. Remove any excess water.
16. Place the membrane back into the tray, DNA side up. Rinse off the lid with distilled water.
17. Wash the membrane extensively to remove any unbound and non-specifically bound SAAP. First, wash in 400 ml of diluted Detection Buffer for 20 minutes with occasional rocking. Discard the solution.

Step 17:
A shaking platform can be used to rock the tray and disperse the wash solution.

18. Second, wash in 200 ml of diluted Detection Buffer for 15 minutes with occasional rocking. Discard the solution.
19. Third, wash in 200 ml of diluted Detection Buffer for 15 minutes with occasional rocking. **Prepare color development solution during this wash.** Discard the solution.
20. Ten minutes before the completion of the third membrane wash, prepare the Color Development solution.
    - Dissolve the BCIP/NBT tablets in exactly 40 ml of distilled water.
    - Vortex until completely dissolved.
    - This is enough solution for all five groups and must be used within one hour.

Experiment Procedure

Module III: Non-isotopic Detection of DNA

COLOR DEVELOPMENT

21. After the incubation from step 19 is complete, carefully remove the membrane from the tray and place it on the underside of the tray lid.

22. Add 8 ml of complete Color Development solution to the tray.

23. Place the membrane **DNA SIDE DOWN** into the tray and cover with a lid or plastic wrap. Make sure the membrane is in complete contact with the color development solution.

24. Place the tray inside a 37°C incubation oven in the dark. Allow the reaction to proceed for several hours.

25. To determine the extent of the reaction, you should examine the membrane after 30 to 60 minutes. Blue bands corresponding to the position of the DNA fragments should be clearly visible. If all of the predicted bands are visible, proceed to the termination phase (step 27). If the bands are not visible, continue the incubation.

26. Re-examine after 3 to 4 hours. All the bands should be visible. If you have trouble visualizing any of the bands, a prolonged development time is permissible. However, the background on the membrane may become darker. If any bands can be seen, even though they may be faint, it is recommended that you proceed to Termination (step 27).

Steps 21-24 Alternative:

Use a sealable, leak-proof bag to place the membrane in for color development. Add the complete color development solution, remove any air pockets, and seal the bag. Place it on a tray in the incubation oven.

TERMINATION

27. Place the membrane on the tray lid. Completely discard the Color Development solution and thoroughly rinse the tray with distilled water.

25. Transfer the membrane to the rinsed tray. Soak the membrane in 100 ml of diluted Termination solution (L) for 5 minutes. Protect the membrane from strong direct light.

26. Allow the membrane to dry in the dark between 2 sheets of filter paper at room temperature. Alternatively, dry for 5 minutes in a 80°C oven.

27. After recording the results, the membrane can be stored in a dark place for several months. Prolonged exposure to bright light will increase the background.

Study Questions

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

1. Why do different individuals such as siblings have different restriction enzyme recognition sites?
2. What is the function of probes in DNA paternity analysis?
3. Why is there more than one single locus probe used in an actual paternity DNA test?
4. What advantage does Southern Blot Analysis offer for genetic testing?

Instructor's Guide

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

EDUCATIONAL RESOURCES

Electrophoresis Hints, Help and Frequently Asked Questions

EDVOTEK Experiments are designed for maximum success in the classroom setting. However, even the most experienced students and teachers occasionally encounter experimental problems or difficulties. The EDVOTEK web site provides several suggestions and reminders for conducting electrophoresis, as well as answers to frequently asked electrophoresis questions.

Technical Service Department

Mon - Fri
9:00 am to 6:00 pm ET

FAX: 202.370.1501
Web: www.edvotek.com
email: info@edvotek.com

Please have the following information ready:

- **Experiment number and title**
- **Kit lot number on box or tube**
- **Literature version number (in lower right corner)**
- **Approximate purchase date**

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Visit our web site for information about EDVOTEK's complete line of "hands-on" experiments for biotechnology and biology education.

Notes to the Instructor:

APPROXIMATE TIME REQUIREMENTS

- **Module I**
  The experiment begins with agarose gel electrophoresis of biotin-labeled samples, ready for electrophoresis. The total time for Module 1 is approximately 3 hours.

- **Module II**
  The Southern blot should be set up immediately following electrophoresis. It will require two-days: 2 hours on Day 1 to prepare the blot which can progress overnight. On Day 2, the blot is disassembled and the membrane is baked for 30 minutes in an oven.

- **Module III**
  This is the non-isotopic detection phase of the experiment. Once detection begins it must be completed during the same time period. It will require approximately 4 hours.

- **Pre-lab preparations**
  Pre-lab preparations and dispensing of biologicals and reagents take approximately 1-2 hours.

- **Agarose Gel preparation**
  Whether you choose to prepare the gel(s) in advance or have the students prepare their own, allow approximately 30-40 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification. See section "Options for Preparing Agarose Gels" below.

Quick Reference: Approximate Time Requirements

1. Agarose Gel Electrophoresis
   3 hours
2. Southern Blot Transfer
   Day 1: 2 hours
   Day 2: 45 min.
3. Non-isotopic Detection of DNA
   4 hours

Table C
Time and Voltage Recommendations

Volts	EDVOTEK Electrophoresis Model M6+ Minimum / Maximum	EDVOTEK Electrophoresis Model M12 & M36 Minimum / Maximum
150	15 / 20 min	25 / 35 min
125	20 / 30 min	35 / 45 min
70	35 / 45 min	60 / 90 min
50	50 / 80 min	95 / 130 min

- **Conducting Electrophoresis**
  The approximate time for electrophoresis will vary from 15 minutes to 2 hours. Generally, the higher the voltage applied, the faster the samples migrate. However, depending upon the apparatus configuration and the distance between the two electrodes, individual electrophoresis units will separate DNA at different rates. Follow manufacturer's recommendations. Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.

OPTIONS FOR PREPARING AGAROSE GELS

A minimum of 10 sample wells per gel are required. If using the EDVOTEK Model #M12 electrophoresis unit, cast a 7 x 14 cm gel with two 6-well combs. Alternatively, use a 10-well comb accessory (Double Comb, Cat. # 683).

Notes to the Instructor:

There are several options for preparing agarose gels for the experiment.

1. Individual Gel Casting:
   Each student lab group can be responsible for casting their own individual gel prior to conducting the experiment.

2. 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 store gels at -20°C. 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 hot, molten agarose before placing the gels into the apparatus for electrophoresis. This will prevent the gels from sliding around in the trays and the chambers.

3. Batch Gel Preparation:
   A batch of agarose gel can be prepared for sharing by the class. To save time, a larger quantity of UltraSpec-Agarose can be prepared for sharing by the class. See instructions for "Batch Gel Preparation".

GEL CONCENTRATION AND VOLUME

The agarose gel concentration required for this experiment is 0.8% weight by volume.

OPTIONAL GEL STAINING AFTER ELECTROPHORESIS

Optional gel staining can be performed with InstaStain® Ethidium Bromide (Cat. #2001, not included). InstaStain® EtBr.

1. Stain the gel according to InstaStain product instructions (Appendix C). If photographic documentation equipment is available, photograph the stained gel for a permanent record.

2. After viewing and/or photography of the stained gel, proceed to the Southern blot.

Caution: Ethidium Bromide is a listed mutagen. Disposal of the InstaStain® EtBr cards, which contain only a few micrograms of ethidium bromide, is minimal compared to the large volume of liquid waste generated by traditional ethidium bromide staining procedures. Disposal of InstaStain® cards and gels should follow institutional guidelines for chemical waste.

Note: The DNA samples have been intentionally spiked with chromosomal DNA to show a smeared pattern when stained with Ethidium bromide. Specific "probed" bands will be visible on the membrane once the DNA has been transferred and the membrane has been processed with the detection agents.

Pre-Lab Preparations

MODULE I: AGAROSE GEL ELECTROPHORESIS

The experiment begins with agarose gel electrophoresis of biotin-labeled samples, ready for electrophoresis.

A	Standard DNA fragments
B	Mother DNA cut with Enzyme 1
C	Mother DNA cut with Enzyme 2
D	Child DNA cut with Enzyme 1
E	Child DNA cut with Enzyme 2
F	Father 1 DNA cut with Enzyme 1
G	Father 1 DNA cut with Enzyme 2
H	Father 2 DNA cut with Enzyme 1
I	Father 2 DNA cut with Enzyme 2

Note: The membranes are very thin white sheets that may be sandwiched between one or two sheets of printed or plain protective covering. If you are not sure which sheet is the membrane, please call EDVOTEK Technical Service (1-800-338-6835).

MODULE II: SOUTHERN BLOT ANALYSIS

General Preparations (for 5 blots)

1. For each gel, gather the following:
   - 1 piece of pre-cut nylon membrane (cut to fit your gel size)
   - 1 piece of pre-cut blotting filter paper (cut to fit your gel size)
   - 20-30 paper towels (towels should be large enough to cover the gel)
2. On day two of the Southern Blot transfer, set up an 80°C oven.

Preparation of Solutions

1. Prepare 1.0 liter of approximately 0.25 N HCl. Mix together:

21	ml	Concentrated HCl (12 N)
979	ml	Distilled/deionized water

2. Prepare 2.0 liters of the alkaline/salt DNA denaturation solution, 0.5 M NaOH/0.6 M NaCl.

1.8	L	Distilled or deionized water
40.0	g	NaOH pellets
70.0	g	NaCl

Add NaOH and NaCl to the water. Use a magnetic stir plate to dissolve. Add distilled water to a final volume of 2.0 liters.

Pre-Lab Preparations

MODULE III: NON-ISOTOPIC DETECTION OF DNA

Once detection begins it must be completed during the same time period. It will require approximately 4 hours. DO NOT MIX THE SAAP SOLUTION (N) OR THE NBT/BCIP TABLETS (M) UNTIL INDICATED IN THE EXPERIMENT. All other required buffers can be prepared prior to the lab.

1. Prepare the Detection Buffer solution from the 20X concentrated stock (J) by mixing the following components:

250.0	ml	20x concentrated Detection Buffer (J)
4.75	L	Distilled or deionized water.

   Mix well. Each blot will require 0.9 L of the diluted Detection Buffer.

2. The Shielding Buffer (K) is not concentrated, and should be used without dilution. Do not warm it until just before you need it. Prepare just enough for the blot which is ready for processing. Detailed instructions for warming are outlined in the Student Experiment Procedure.

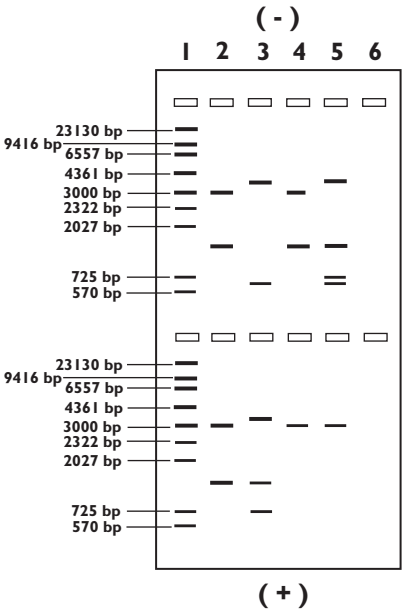
3. Prepare the Termination Solution from 20x concentrated Termination Solution (L). For 5 gels, prepare 500 ml of solution in a graduated cylinder, as follows:

25	ml	20 x concentrated Termination Solution (L)
475	ml	Distilled or deionized water.

   Mix well. Each group will require 50 ml of diluted Termination Solution.

4. On the day of the lab, set the incubation oven(s) at 37° C, 65° C, and 80°C.

Experiment Results and Analysis



Actual results will yield broader bands of varying intensities. The idealized schematic shows the relative positions of the bands, but the results are not depicted to scale.

The largest fragments will migrate the slowest, the smallest will migrate the fastest.

Option 1: Gel with 2 rows, 6 wells per row:

First Row Lane	Tube	
1	A	Standard DNA fragments
2	B	Mother DNA cut with Enzyme 1
3	C	Mother DNA cut with Enzyme 2
4	D	Child DNA cut with Enzyme 1
5	E	Child DNA cut with Enzyme 2

Second Row Lane	Tube	
1	A	Standard DNA fragments
2	F	Father 1 DNA cut with Enzyme 1
3	G	Father 1 DNA cut with Enzyme 2
4	H	Father 2 DNA cut with Enzyme 1
5	I	Father 2 DNA cut with Enzyme 2

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

Notes:

Appendices

Appendices

A Agarose Gel Preparation For Southern Blot Analysis

B Quantity Preparations for Agarose Gel Electrophoresis

C Staining and Visualization of DNA with InstaStain® Ethidium Bromide Cards

Material Safety Data Sheets

Appendix
A

0.8% Agarose Gel Electrophoresis Reference Tables

If preparing a 0.8% gel with concentrated (50x) buffer, use Table A.1

If preparing a 0.8% gel with diluted (1x) buffer, use Table A.2

Table A.1 Individual 0.8%* UltraSpec-Agarose™ Gel

Size of Gel (cm)	Amt of Agarose (g)	+ Concentrated Buffer (50x) (ml)	+ Distilled Water (ml)	= Total Volume (ml)
7 x 7	0.23	0.6	29.4	30
7 x 10	0.39	1.0	49.0	50
7 x 14	0.46	1.2	58.8	60

Table A.2 Individual 0.8%* UltraSpec-Agarose™ Gel

Size of Gel (cm)	Amt of Agarose (g)	+ Diluted Buffer (1x) (ml)
7 x 7	0.23	30
7 x 10	0.39	50
7 x 14	0.46	60

* 0.77 UltraSpec-Agarose™ gel percentage rounded up to 0.8%

Table B Electrophoresis (Chamber) Buffer

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

For DNA analysis, the recommended electrophoresis buffer is Tris-acetate-EDTA, pH 7.8. The formula for diluting EDVOTEK (50x) concentrated buffer is one volume of buffer concentrate to every 49 volumes of distilled or deionized water. Prepare buffer as required for your electrophoresis unit.

Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.1 for 0.8% agarose gels. The time for electrophoresis will vary from approximately 15 minutes to 2 hours depending upon various factors. Conduct the electrophoresis for the length of time determined by your instructor.

Table C.1 Time and Voltage Guidelines (0.8% Gel)

	EDVOTEK Electrophoresis Model M6+	EDVOTEK Electrophoresis Model M12 & M36
Volts	Minimum / Maximum	Minimum / Maximum
150	15 / 20 min	25 / 35 min
125	20 / 30 min	35 / 45 min
70	35 / 45 min	60 / 90 min
50	50 / 80 min	95 / 130 min

Agarose Gel Preparation - Quantity Preparations

Appendix B

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.

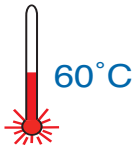
Table D
Bulk Preparation of Electrophoresis Buffer

Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
60		2,940		3000 (3 L)

Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.1.

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. The volume required is dependent upon the size of the gel bed and DNA staining method which will be used. Refer to Appendix A or B for guidelines.
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.

Table E.I
Batch Preparation 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

Note: The UltraSpec-Agarose™ kit component is often labeled with the amount it contains. In many cases, the entire contents of the bottle is 3.0 grams. 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.

Appendix

C

Staining and Visualization of DNA InstaStain® Ethidium Bromide Cards

Wear Gloves and Goggles

Do not stain gel(s) in the electrophoresis chamber.

1. After electrophoresis, place the gel on a piece of plastic wrap on a flat surface. Moisten the gel with a few drops of electrophoresis buffer.

2. Wearing gloves, remove and discard the clear plastic protective sheet, and place the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel.

3. With a gloved hand, firmly run your fingers over the entire surface of the InstaStain® Ethidium Bromide card. Do this several times.

4. Place the gel casting tray and a small empty beaker on top to ensure that the InstaStain® Ethidium Bromide card maintains direct contact with the gel surface.

   Allow the InstaStain® Ethidium Bromide card to stain the gel for 3-5 minutes.

   Note: Staining time is optimized for 0.8-1.0% gels. Gels of higher concentrations will take longer to stain.

5. After 3-5 minutes, remove the InstaStain® Ethidium Bromide card. Transfer the gel to a ultraviolet (300 nm) transilluminator for viewing. Be sure to wear UV protective goggles.

Caution: Ethidium Bromide is a listed mutagen.

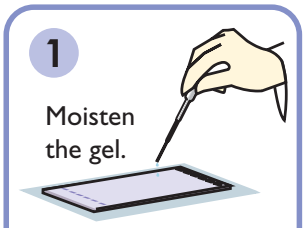
Disposal of InstaStain® Ethidium Bromide:

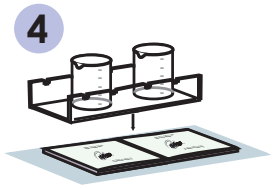
Disposal of InstaStain® Ethidium Bromide cards and gels should follow institutional guidelines for solid chemical waste.

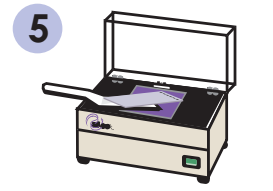
Additional Notes About Staining:

- If bands appear faint, or if you are not using EDVOTEK UltraSpec- Agarose™, gels may take longer to stain with InstaStain® Ethidium Bromide. Repeat staining and increase the staining time for an additional 3-5 minutes.

- DNA markers should be visible after staining even if other DNA samples are faint or absent. If markers are not visible, troubleshoot for problems with the electrophoretic separation.







Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Agarose

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 1121 5th Street NW Washington DC 20001

Emergency Telephone Number: 202.370.1500

Telephone Number for information: 202.370.1500

Date Prepared: 4/5/12

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				
CAS #9012-36-6				

Section III - Physical/Chemical Characteristics

Boiling Point: For 1% solution 194 F		Specific Gravity (H 0 = 1): 2	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Insoluble - cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics N.D. = No data

Flash Point (Method Used): No data	Flammable Limits	LEL: N.D	UEL: N.D.		

Extinguishing Media: Water spray, dry chemical, carbon dioxide, halon or standard foam

Special Fire Fighting Procedures: Possible fire hazard when exposed to heat or flame

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

Hazardous Polymerization	May Occur		Conditions to Avoid: None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Inhalation: No data available Ingestion: Large amounts may cause diarrhea

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Sweep up and place in suitable container for disposal

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece.

Ventilation	Local Exhaust	Special	
	Mechanical: Gen. dilution ventilation	Other	

Protective Gloves: Yes Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Impervious clothing to prevent skin contact

Work/Hygienic Practices: None

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): 50x Electrophoresis Buffer

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 1121 5th Street NW Washington DC 20001

Emergency Telephone Number: 202.370.1500

Telephone Number for information: 202.370.1500

Date Prepared: 4/5/12

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point: No data		Specific Gravity (H_20 = 1):	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Appreciable, (greater than 10%)

Appearance and Odor: Clear, liquid, slight vinegar odor

Section IV - Physical/Chemical Characteristics N.D. = No data

Flash Point (Method Used): No data	Flammable Limits	LEL: N.D.	UEL: N.D.

Extinguishing Media: Use extinguishing media appropriate for surrounding fire.

Special Fire Fighting Procedures: Wear protective equipment and SCBA with full facepiece operated in positive pressure mode.

Unusual Fire and Explosion Hazards: None identified

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

Hazardous Polymerization	May Occur		Conditions to Avoid: None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): None

Carcinogenicity: None identified NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Irritation to upper respiratory tract, skin, eyes

Medical Conditions Generally Aggravated by Exposure: None

Emergency First Aid Procedures: Ingestion: If conscious, give large amounts of water

Eyes: Flush with water Inhalation: Move to fresh air Skin: Wash with soap and water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing. Mop up spill and rinse with water, or collect in absorptive material and dispose of the absorptive material.

Waste Disposal Method: Dispose in accordance with all applicable federal, state, and local enviromental regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation	Local Exhaust	Yes	Special	None	
	Mechanical (General)	Yes	Other	None	

Protective Gloves: Yes Eye Protection: Safety goggles

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Detection/Shielding Buffer

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 1121 5th Street NW Washington DC 20001

Emergency Telephone Number: 202.370.1500

Telephone Number for information: 202.370.1500

Date Prepared: 4/5/12

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Tris (Hydroxymethyl) Aminomethane ---------- No data ----------				
CAS # 77-86-1				

Section III - Physical/Chemical Characteristics

Boiling Point: NO data		Specific Gravity (H_20 = 1):	NO data
Vapor Pressure (mm Hg.)	NO data	Melting Point	NO data
Vapor Density (AIR = 1)	NO data	Evaporation Rate (Butyl Acetate = 1)	NO data

Solubility in Water: Soluble

Appearance and Odor: Clear liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used): Not flammable	Flammable Limits	LEL: NO data	UEL: NO data

Extinguishing Media: Use media appropriate for surrounding fire

Special Fire Fighting Procedures: Wear SCBA and protective clothing

Unusual Fire and Explosion Hazards: May emit toxic fumes

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: Heat
	Stable	X	

Incompatibility: Oxidizing materials, acids

Hazardous Decomposition or Byproducts: Carbon monoxide, carbon dioxide, and nitrogen oxides

Hazardous Polymerization	May Occur		Conditions to Avoid: N/A
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Moderately toxic by ingestion. May cause irritation to eyes, skin, and mucous membranes

Carcinogenicity: None NTP? No IARC Monographs? No OSHA Regulation? No

Signs and Symptoms of Exposure: Irritation of eyes or skin, coughing or respiratory irritation

Medical Conditions Generally Aggravated by Exposure: Unknown

Emergency First Aid Procedures: Skin/eye: Flush with water. Inhalation: remove to fresh air. Ingestion: Seek medical attention

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear protective clothing. Mop up with absorbant material and dispose of properly.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Store in a cool, dry place

Other Precautions: Avoid contact

Section VIII - Control Measures

Respiratory Protection (Specify Type): NOISH approved respirator

Ventilation	Local Exhaust		Special	NA	
	Mechanical (General)	Yes	Other	NA	

Protective Gloves: Rubber Eye Protection: Safety goggles

Other Protective Clothing or Equipment: Lab coat, eye wash, safety shower

Work/Hygienic Practices: Wash after handling

Material Safety Data Sheets

Full-size (8.5 x 11") pdf copy of MSDS is available at www. edvotek.com or by request.

EDVOTEK®

Material Safety Data Sheet
May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Termination Buffer

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202.370.1500**

Telephone Number for information: **202.370.1500**

Date Prepared: 4/5/12

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Ethylenediaminetetraacetic acid	No data	No data	No data	No data
C10-H14-08-33-3				
CAS # 139-33-3				

Section III - Physical/Chemical Characteristics

Boiling Point	NO data	Specific Gravity (H_20 = 1)	NO data
Vapor Pressure (mm Hg.)	NO data	Melting Point	NO data
Vapor Density (AIR = 1)	NO data	Evaporation Rate (Butyl Acetate = 1)	NO data

Solubility in Water: Soluble

Appearance and Odor: Clear liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		NO data	NO data

Extinguishing Media: Dry chemical, carbon dioxide, halon, water spray or standard foam

Special Fire Fighting Procedures: Move container from fire area if possible

Unusual Fire and Explosion Hazards: Thermal decomposition products may include toxic and hazardous oxides of carbon, nitrogen, and sodium.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Excessive heat, sparks or open flame
	Stable	X	

Incompatibility: Acids, Aluminum, metals, oxidizers (strong)

Hazardous Decomposition or Byproducts: Thermal decomposition products of toxic and hazardous oxides of carbon, nitrogen, &sodium.

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic): Moderate toxic by ingestion. Systemic toxicity may result. May chelate lead,magnesium,zinc,& trace metals if present in intestine,causing increased absorption

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
No data	No data	No data	No data

Signs and Symptoms of Exposure: Mucous membrane irritation, eye/skin irritation, irritating to gastrointestinal system.

Medical Conditions Generally Aggravated by Exposure: Renal or heart disease, potassium deficiency, insulin dependent diabetes, seizures or intracranial lesions

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Mop up with absorptive material. Containerize to dispose of properly.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Store away from strong oxidizers or heat. Avoid skin/eye contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece and organic vapor cartridge.

Ventilation			Special	
	Local Exhaust	Yes	Special	None
	Mechanical (General)	Dilution Vent. Sys.	Other	None

Protective Gloves		Eye Protection	
	Yes		Splash goggles

Other Protective Clothing or Equipment: Wear impervious clothing to prevent skin contact.

Work/Hygienic Practices: Emergency eye wash should be available

EDVOTEK®

Material Safety Data Sheet
May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): NBT

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202.370.1500**

Telephone Number for information: **202.370.1500**

Date Prepared: 4/5/12

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
N, N-Dimethylformamide	No data	No data	No data	No data
CAS # 68-12-2 H-C-O-N-(C-H3)2				
Nitro Blue Tetrazolium	No data	No data	No data	No data
CAS # 298-83-9 C4OH3OC12N1006 Contains methyl alcohol				

Section III - Physical/Chemical Characteristics

Boiling Point	NO data	Specific Gravity (H_20 = 1)	NO data
Vapor Pressure (mm Hg.)	NO data	Melting Point	NO data
Vapor Density (AIR = 1)	NO data	Evaporation Rate (Butyl Acetate = 1)	NO data

Solubility in Water: Soluble

Appearance and Odor: Yellow, hygroscopic, mobile liquid with a faint amine-like odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		NO data	NO data

Extinguishing Media: Dry chemical, carbon dioxide, water spray, or alcohol-resistant foam

Special Fire Fighting Procedures: Remove from fire if possible. Spray sides of container after fire is out. Apply from as far a distance as possible. Avoid breathing vapors. Keep upwind.

Unusual Fire and Explosion Hazards: Vapors are heavier than air and may travel considerable distance to source of ignition and flashback.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Incompatibles
	Stable	X	

Incompatibility: Metals, halogens, sodium compounds, strong oxidizers.

Hazardous Decomposition or Byproducts: Thermal decomposition products may include toxic oxides of carbon and nitrogen.

Hazardous Polymerization			Conditions to Avoid
	May Occur		Incompatibles
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic): Prolonged exposure may be fatal
Skin: dermatitis Eye: Conjuctivitis

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
No data	No data	No data	No data

Signs and Symptoms of Exposure: Skin: irritation, redness, itching Eyes: irritation, blurred vision
Ingestion/inhalation: headache, dizziness, nausea, abdominal pain, hypertension

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Inhalation: remove to fresh air, give artificial respiration if breathing has stopped
Eyes: flush with water 15-20 minutes; Skin: remove contaminated clothing, wash with soap

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear safety equipment. Mop up with absorbant material; containerize for later disposal
For larger spills use water to reduce vapors

Waste Disposal Method: Observe all federal, state and local regulations

Precautions to be Taken in Handling and Storing: Store away from incompatibles

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator

Ventilation			Special	
	Local Exhaust	Yes	Special	None
	Mechanical (General)	Dilution Vent. Sys.	Other	NA

Protective Gloves		Eye Protection	
	Yes		Splash goggles

Other Protective Clothing or Equipment: Wear impervious clothing to prevent skin contact.

Work/Hygienic Practices: Emergency eye wash should be available