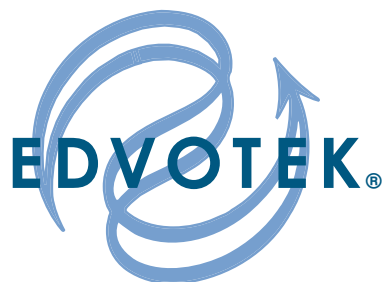


The Biotechnology Education Company®



334
EDVO-Kit #

PCR-based VNTR Human DNA Typing

Storage:
See Page 3 for specific instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is for students to isolate human DNA and compare DNA polymorphisms between individuals

All 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.

The Biotechnology Education Company® • 1-800-EDVOTEK • www.edvotek.com

**Revised &
Improved
Experiment
Instructions**

Two options are presented in this experiment for sources from which to obtain human DNA: 1) hair, or 2) cheek cells. Special caution is required when handling human cheek cells. Swabs used for harvesting cheek cells should be soaked in a 15% bleach solution after the cells are suspended in the buffer.

The PCR process and *Taq* DNA polymerase are covered by patents owned by Hoffman-LaRoche, Inc.

Table of Contents

	Page
Experiment Components	3
Experiment Requirements	4
Background Information	5
Experiment Procedures	
Experiment Overview	10
Laboratory Safety	11
Module I: Isolation of DNA	
I-A: Isolation of DNA from Human Hair	12
I-B: Isolation of DNA from Human Cheek Cells	14
Module II: Amplification of the D1S80 Locus	16
Module III: Electrophoresis	
Agarose Gel Preparation	18
Conducting Agarose Gel Electrophoresis	22
Staining with InstaStain® Ethidium Bromide	24
Study Questions	26
Instructor's Guidelines	27
Notes to the Instructor	28
Pre-Lab Preparations	30
Batch Agarose Gel Preparation	34
Experiment Results and Analysis	35
Study Questions and Answers	36
Appendices	
PCR Experimental Success Guidelines	37
Polymerase Chain Reaction Using Three Waterbaths	39
Preparation and Handling of PCR Samples With Wax	40
Staining with InstaStain® Methylene Blue	41
Material Safety Data Sheets	43

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



EDVO-Kit # 334 PCR-based VNTR Human DNA Typing

Experiment Components

CONTENTS

Component Quantities:

Experiment # 334 contains material for up to 24 human DNA typing reactions.

Sample volumes are very small. For liquid samples, it is important to quickly spin the tube contents in a microcentrifuge to obtain sufficient volume for pipetting. Spin samples for 10-20 seconds at maximum speed.

	Storage
A D1S80 primer mix	-20°C Freezer
B Tris Buffer	-20°C Freezer
C 200 base pair ladder	-20°C Freezer
D Chelating Agent	Room Temperature
E 10x PBS	Room Temperature
F Tubes with PCR reaction pellets™	Room Temperature
Each PCR reaction pellet™ contains	
• dNTP Mixture	
• <i>Taq</i> DNA Polymerase Buffer	
• <i>Taq</i> DNA Polymerase	
• MgCl ₂	
G Control DNA	-20°C Freezer
H Proteinase K	Room Temperature

REAGENTS & SUPPLIES:

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- 10x Gel Loading Solution
- InstaStain® Ethidium Bromide
- Microcentrifuge Tubes (0.5 ml snap-top - not for boiling)
- Microcentrifuge Tubes (1.5 ml screw-cap - use for boiling)
- PCR tubes (0.2 ml - for thermal cyclers with 0.2 ml template)
- Calibrated transfer pipets
- Wax beads (for waterbath option or thermal cyclers without heated lid)

All 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.

EDVOTEK - The Biotechnology Education Company®
 1-800-EDVOTEK • www.edvotek.com
 24-hour FAX: (301) 340-0582 • email: edvotek@aol.com



EVT 003106K

EDVO-Kit # 334 PCR-based VNTR Human DNA Typing

Requirements

*If you do not have a thermal cycler, PCR experiments can be conducted, with proper care, using three waterbaths. However, a thermal cycler assures a significantly higher rate of success.

- Thermal cycler (EDVOTEK Cat. # 532 highly recommended) or three waterbaths*
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- UV Transilluminator or UV Photodocumentation system
- UV safety goggles
- Automatic micropipets (5-50 μ l) with tips
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Disposable vinyl or latex laboratory gloves
- Ice buckets and ice
- Distilled or deionized water
- 15% Bleach solution

If you need answers to questions, call our

Technical Service Department

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



FAX: (301) 340-0582

web: www.edvotek.com

email: edvotek@aol.com

Now with
Online Ordering

www.edvotek.com

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

Please have the following information:

- The kit number and title
- Kit lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date



VNTR Human DNA Typing

Polymorphic DNA refers to chromosomal regions that vary widely from individual to individual. By examining several of these regions within the genomic DNA obtained from an individual, one may determine a "DNA Fingerprint" for that individual. DNA polymorphisms are now widely used for determining paternity/maternity, kinship, identification of human remains, and the genetic basis of various diseases. The most far-reaching application, however, has been in the field of criminal forensics. DNA from crime victims and offenders can be matched to crime scenes, often affecting the outcome of criminal and civil trials.

The beginning of DNA fingerprinting occurred in the United Kingdom in 1984, following the pioneering work of Dr. Alex Jeffreys at the University of Leicester. Analysis by Jeffreys led to the apprehension of a murderer in the first DNA fingerprinting case in September 1987. The first U.S. conviction occurred on November 6, 1987 in Orlando, FL. Since then, DNA analysis has been used in thousands of convictions. Additionally, over 100 convicted prison inmates have been exonerated from their crimes, including several death row inmates.

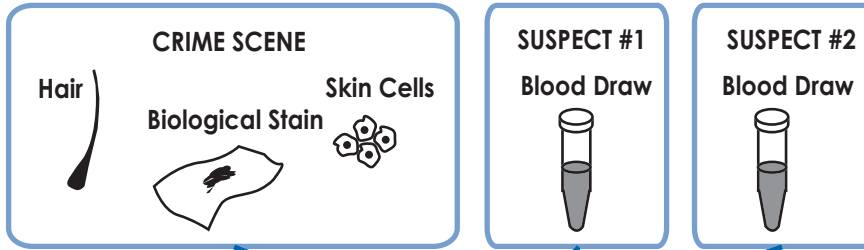
In 1990, the Federal Bureau of Investigation (FBI) established the **Combined DNA Index System (CODIS)**, a system which allows comparison of crime scene DNA to DNA profiles in a convicted offender and a forensic (crime scene) index. A match of crime scene DNA to a profile in the convicted offender index indicates a suspect for the crime, whereas a match of crime scene DNA to the forensic index (a different crime scene) indicates a serial offender. CODIS has now been used to solve dozens of cases where authorities had not been able to identify a suspect for the crime under investigation.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved.

EVT 003106K



VNTR Human DNA Typing



The first step in forensic DNA fingerprinting is the collection of human tissue from the crime scene or victim. These tissues include blood, hair, skin, and body fluids. The sample, often present as a stain, is treated with a detergent to rupture (lyse) cell membranes and obtain DNA for

Treat to release DNA



Perform PCR to amplify specific polymorphic regions



further analysis (Figure 1). One early method, called Restriction Fragment Length Polymorphism (RFLP) analysis, involves digesting the DNA with restriction enzymes, separating the fragments by agarose gel electrophoresis, transferring the DNA to a membrane, and hybridizing the membrane with probes to polymorphic regions. This method is statistically very accurate, but requires relatively large amounts of DNA and takes several days to complete. Because of the time and DNA requirements, the RFLP method is no longer used in forensics, but remains in use in certain medical genetics-based tests.

Crime Scene	Suspect #1	Suspect #2
—	—	—
—	—	—
—	—	—
—	—	—
—	—	—

Suspect #2 matches Crime Scene

In forensics, the polymerase chain reaction (PCR) is now used to amplify and examine highly polymorphic DNA regions. These are regions that vary in length from individual to individual and fall into two categories: 1) Variable Number of Tandem Repeats (VNTR) and 2) Short Tandem Repeats (STR). A VNTR is a region that is variably composed of a 15-70 base pair sequence, typically repeated 5-100 times. An STR is similar to a VNTR except that the repeated unit is only 2-4 nucleotides in length. By examining several different VNTRs or STRs from the same individual, investigators obtain a unique DNA fingerprint for that individual which is unlike that of any other person (except for an identical twin).

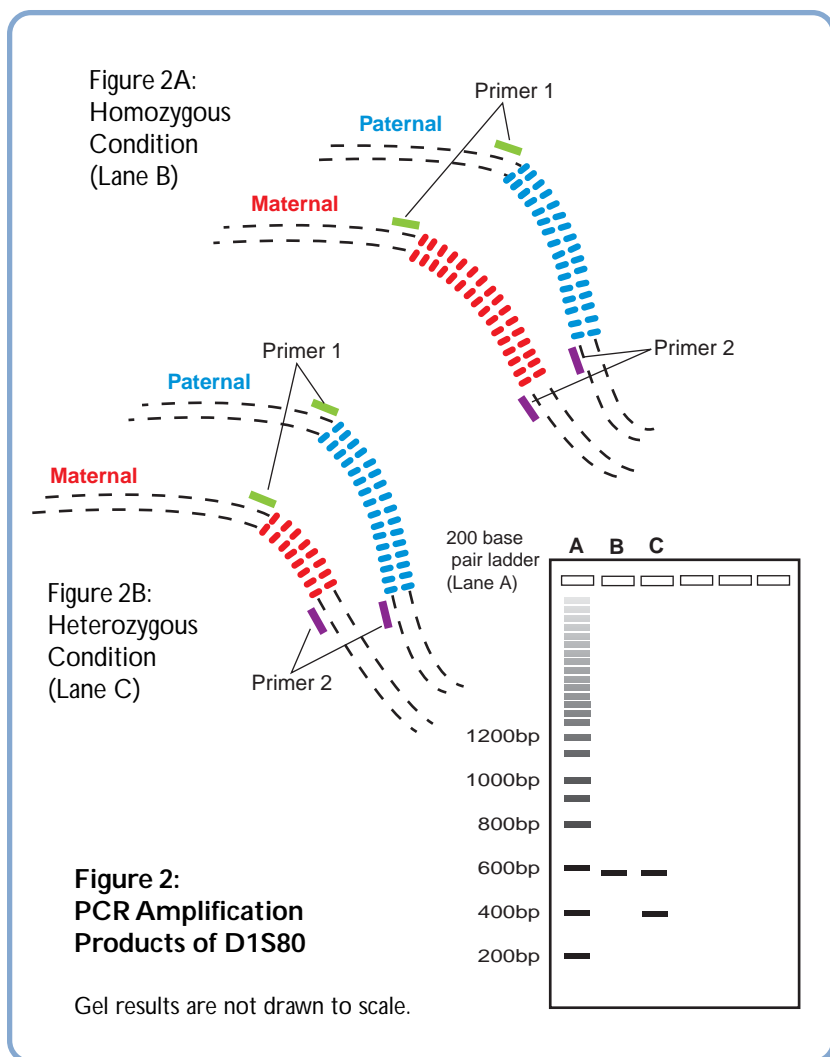
Figure 1: Comparison of crime scene DNA to DNA from suspects.



VNTR Human DNA Typing

One VNTR known as D1S80, is present on chromosome 1 and contains a 16-nucleotide sequence which is variably repeated between 16 and 40 times.

An individual who is homozygous for the D1S80 genotype will have equal repeat numbers on both homologues of chromosome 1, displaying a single PCR product following gel analysis (Figure 2A). More commonly, a person will be heterozygous, with differing D1S80 repeat numbers. Amplification of DNA from heterozygous individuals will result in two distinct PCR products (Figure 2B). For most applications, law enforcement agencies now use STRs as they are more easily amplified and thus require less starting DNA.

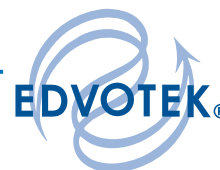


that inhabits hot springs, is stable at very high temperatures. Also included in the PCR reaction mixture are two synthetic oligonucleotides (15-30 nucleotides) known as "primers" and the extracted DNA known as the "template". The region of DNA to be amplified is known as the "target".

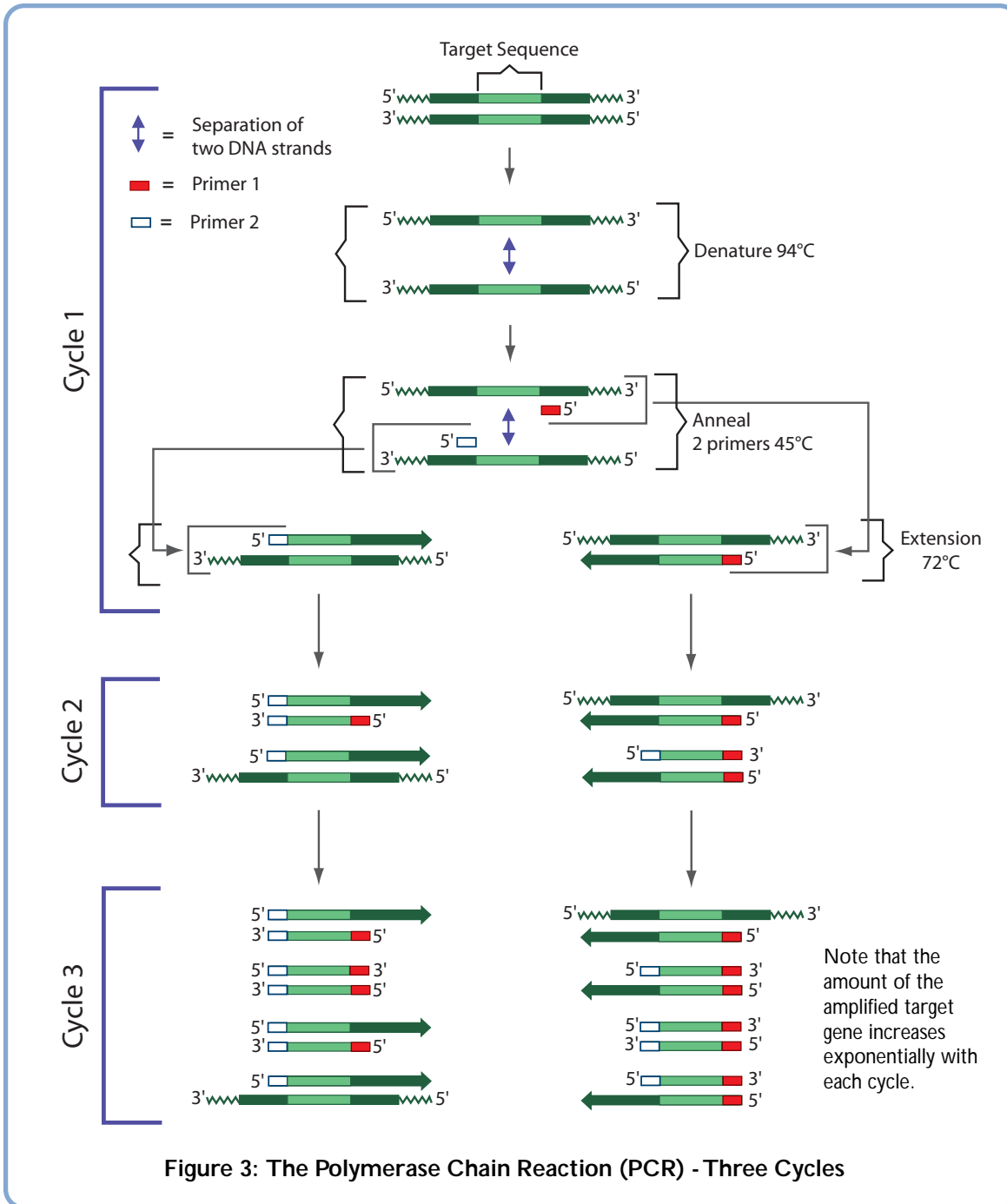
Background Information

As mentioned previously, DNA Fingerprinting now uses the polymerase chain reaction (PCR). PCR was invented in 1984 by Kary Mullis at the Cetus Corporation in Emeryville, California. Dr. Mullis was awarded a Nobel Prize for his work in 1993. The enormous utility of PCR is based on its ease of use and its ability to amplify DNA.

The PCR amplification (Figure 3) uses an enzyme known as *Taq* polymerase. This enzyme, originally purified from a bacterium



VNTR Human DNA Typing



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/ laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

VNTR Human DNA Typing

In the first step of the PCR reaction, the template complementary DNA strands are separated (denatured) from each other at 94°C, while the *Taq* polymerase remains stable. In the second step, known as annealing, the sample is cooled to an intermediate temperature (usually 40° - 65°C) to allow hybridization of the two primers to the two strands, one to each of the two strands of the target DNA sequence. In the third step, known as extension (also sometimes called DNA synthesis), the temperature is raised to 72°C and the *Taq* polymerase adds nucleotides to the primers to complete the synthesis of the new complementary strands.

These three steps - denaturation, annealing, and extension - constitute one PCR "cycle". This process is typically repeated for 30-40 cycles, amplifying the target sequence exponentially. PCR is performed in a thermal cycler, an instrument that is programmed to rapidly heat, cool, and maintain samples at designated temperatures for varying amounts of time.

The objectives of this experiment are to isolate human DNA and compare DNA polymorphisms between individuals by PCR amplification and gel electrophoresis. In this experiment, each student will 1) extract his/her DNA from hair or cheek cells, 2) amplify DNA at the D1S80 locus by PCR, and 3) examine the PCR products on agarose gels.

Experiment Overview

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. If you will be conducting PCR using a thermal cycler without a heated lid, also read the Appendix entitled "Preparation and Handling PCR Samples with Wax".

If you will be using three waterbaths to conduct PCR, read the two appendices entitled "Polymerase Chain Reaction Using Three Waterbaths" and "Handling samples with wax overlays".

3. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT OBJECTIVE:

The objective of this experiment is for students to isolate human DNA and compare DNA polymorphisms between individuals

BRIEF DESCRIPTION OF EXPERIMENT:

In this experiment, each student will extract his/her DNA from hair or cheek cells, amplify DNA at the D1S80 locus by PCR, and examine the PCR products on agarose gels. DNA purified from a cultured cell line is included and may be used as a positive control. Objectives of this experiment are the isolation of human DNA and the comparison of DNA polymorphisms between individuals by PCR amplification and gel electrophoresis.

GEL SPECIFICATIONS

This experiment requires a gel with the following specifications:

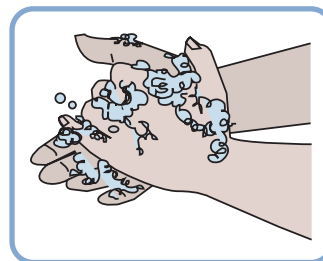
- Recommended gel size 7 x 14 cm (long tray)
- Number of sample wells required 6
- Placement of well-former template first set of notches
- Gel concentration required 1.0%



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

Laboratory Safety

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Special caution is required when handling human cheek cells. Swabs used for harvesting cheek cells should be soaked in a 15% bleach solution after the cells are suspended in the buffer. Swabs can be disposed in solid waste after soaking in the bleach solution.
3. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
4. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
5. Exercise caution when using any electrical equipment in the laboratory.
 - Although electrical current from the power source is automatically disrupted when the cover is removed from the apparatus, first turn off the power, then unplug the power source before disconnecting the leads and removing the cover.
 - Turn off power and unplug the equipment when not in use.
6. EDVOTEK injection-molded electrophoresis units do not have glued junctions that can develop potential leaks. However, in the unlikely event that a leak develops in any electrophoresis apparatus you are using, IMMEDIATELY SHUT OFF POWER. Do not use the apparatus.
7. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



(Preferred method)

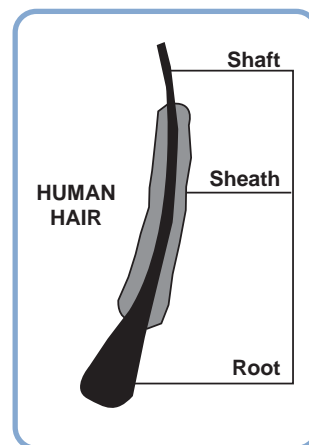
Module I-A: Isolation of DNA from Human Hair

Experiment Procedures

This experiment can alternatively be performed using DNA isolated from cheek cells (see page 14).

A sufficient volume of cells is required to ensure that there is enough DNA to yield positive DNA fingerprinting results. To maximize success, carefully read and follow all experimental instructions.

WARNING!
Use only screw-cap tubes when boiling for DNA isolation. Do not use snap-top tubes when boiling.



1. Isolate 3-4 hairs containing a sheath, a barrel-shaped structure (often white in color) encircling the shaft near the base of the hair (see figure at right). If necessary, sheaths can be cut from the remainder of the hair shaft.

The preferred source is hairs from the eyebrows. They are short in length and can be readily placed in the bottom of the tube.

2. Place the hairs in the bottom of a 1.5 ml screw-cap tube.
3. Obtain the lysis solution from your instructor.
This lysis solution contains 25 mM Tris-HCl pH 8.0, 5% chelating agent, 50 µg/ml proteinase K. The chelating agent removes Mg (required by DNA degrading nucleases and DNA polymerases). The small beads need to be suspended in the buffer prior to delivery to the cell suspension. Proteinase K will digest proteins found in solution.
4. Use the calibrated transfer pipet or a micropipet with disposable tip to transfer the lysis solution:
 - Mix the lysis solution by vortexing or pipeting up and down.
 - Before the chelating agent settles, quickly remove 150 µl and add it to the tube containing the hair.
5. Make sure the hair sheaths are completely submerged in solution and are not stuck on the sides of the tube.
6. Place tube in a float and place a 56°C waterbath for 15 minutes.
7. Remove the tube from the waterbath and allow it to cool for 30 seconds.
8. Vortex the tube for 15 seconds.



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

(Preferred method)

Module I-A: Isolation of DNA from Human Hair, continued

WARNING!

Make sure you are using a screw-cap tube for boiling your sample. Do not use snap-top tubes when boiling.

9. Check again that the hair sheaths are completely submerged in solution and are not stuck on the sides of the tube.
10. Place tube in a float and place in boiling water for 10 minutes.

Boiling is required to obtain cell lysis. The DNA is not degraded by boiling and nucleases will not degrade the DNA because the Mg is chelated (trapped).

11. Remove tube from water and cool on ice for 2 minutes.
12. Vortex for 10 seconds.
13. Spin in a microcentrifuge for 30 seconds. Centrifuge the cell suspension carefully.

If a microcentrifuge is not available, allow the chelating agent to settle in the tube for 3 minutes.

14. Carefully remove 50 μ l of supernatant and transfer it to a clean 0.5 ml microcentrifuge tube.

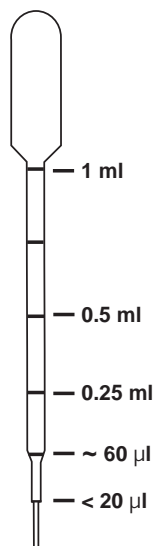
Transfer the DNA (supernatant) to the clean microcentrifuge tube very carefully. It is the step prior to the PCR reaction. If any chelex beads (as few as a couple) are transferred, they can easily trap the Mg cation which is required by the *Taq* DNA polymerase as a cofactor for catalysis.

15. Place the tube on ice.
16. Proceed with steps as outlined in Module II: Amplification of the D1S80 locus.

**OPTIONAL STOPPING POINT**

The supernatant may be stored at -20°C until the experiment is continued.

This experiment can alternatively be performed using DNA isolated from hair (see page 12).



A sufficient volume of cells is required to ensure that there is enough DNA to yield positive DNA fingerprinting results. To maximize success, carefully read and follow all experimental instructions.

Special caution is required when handling human cheek cells. Swabs used for harvesting cheek cells should be soaked in a 15% bleach solution after the cells are suspended in the buffer. Swabs can then be disposed in solid waste after soaking in the bleach solution.

1. Obtain cells by swabbing the inside of the mouth with a cotton-tipped applicator. Twirl the applicator while vigorously swabbing both cheeks, between the gum line and under the tongue .
2. Place the cotton head in 2 ml of PBS (in a labeled 15 ml conical tube) and twirl back and forth vigorously for 30 seconds to dislodge cells. Press the cotton head against the walls of the conical tube to squeeze out as much liquid as possible.
3. Place the cotton-tipped swab in a beaker or container filled with 15% bleach solution to soak and disinfect.
4. Using a fresh applicator, repeat steps 1 and 2. Twirl the applicator to add cells to the same tube containing the 2 ml of PBS.
5. Place the second cotton-tipped swab in the 15% bleach solution to soak and disinfect.
6. With a calibrated transfer pipet, transfer 2 ml of the cells in PBS into a 2 ml screw-cap microtest tube.
7. Spin at 5000 - 6000 g in a microcentrifuge (with appropriate counter-balance) for 1 minute.

Important: Check to see that the buffer is clear (not cloudy) and that there is a visible white pellet (approximately 7-8 mm in diameter). If the buffer is cloudy with little or no pellet, spin the tube(s) for an additional 30 - 60 seconds. If the buffer is clear with little or no pellet, obtain another swab and repeat steps 1-5.

8. Pipet off the supernatant, using care to avoid discarding the pellet.
9. Mix the tube of lysis solution by inverting it several times.

This lysis solution contains 25 mM Tris-HCl pH 8.0, 5% chelating agent, 50 µg/ml proteinase K. The chelating agent removes Mg (required by DNA degrading nucleases and DNA polymerases). The small beads need to be suspended in the buffer prior to delivery to the cell suspension. Proteinase K will digest proteins found in solution.



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/ laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

Module I-B: Isolation of DNA from Human Cheek Cells, continued

WARNING!

Make sure you are using a screw-cap tube for boiling your sample. Do not use snap-top tubes when boiling.

10. Continue to mix the lysis solution with a calibrated transfer pipet* by pipeting up and down several times.

Then quickly add 150 μ l of the lysis solution to the tube containing the pellet.

*Alternatively, use a micropipet with a disposable tip to measure 150 μ l of the lysis solution suspension. It may be necessary to cut the end of the tip to create a larger opening to allow the lysis solution to pass through more easily.

11. Resuspend the pellet in lysis solution by pipetting up and down several times, or by vortexing gently. Check to see that the pellet is fully suspended.
12. Place the tube in a float and place it in a 56°C waterbath for 15 minutes.
13. Remove the tube from the waterbath and cool for 30 seconds.
14. Vortex the tube for 15 seconds.
15. Lyse the cells by placing the tube in a float and placing it in a boiling waterbath for 10 minutes.
16. Allow the tube to cool for 2 minutes. Vortex for 10 seconds or tap the tube vigorously to mix.
17. Spin the tube in a microcentrifuge for 2 minutes to pellet the cell debris, leaving DNA in the supernatant.
18. Transfer 20-50 μ l of the supernatant to a clean 0.5 ml tube, using care to avoid disturbing the pellet.
- Transfer the DNA (supernatant) to the clean microcentrifuge tube very carefully. It is the step prior to the PCR reaction. If any chelex beads (as few as one or two) are transferred, they can easily chelate (trap) the Mg required by the *Taq* DNA polymerase as a cofactor for catalysis. Any carry-over of chelex to the PCR reaction will not yield results.
19. Place the sample (supernatant) on ice.
20. Proceed with steps as outlined in Module II.

**OPTIONAL STOPPING POINT**

The supernatant may be stored at -20°C until the experiment is continued.

Module II: Amplification of the D1S80 Locus

The PCR reaction pellet™ contains *Taq* DNA polymerase, the four deoxytriphosphates, Mg^{+2} and buffer.

Sample volumes are very small. For liquid samples, it is important to quickly spin the tube contents in a microcentrifuge to obtain sufficient volume for pipeting. Spin samples for 10-20 seconds at maximum speed.

PCR REACTION:

1. Label the tube containing the PCR reaction pellet™ with your initials.
2. Tap the reaction tube to assure the reaction pellet is at the bottom of the tube.
3. Add the following to the pellet:

D1S80 primer solution	20.0 μ l
Cell DNA (supernatant)	5.0 μ l

4. Gently mix the PCR reaction tube and quickly spin it in a microcentrifuge to collect all the sample at the bottom of the tube. Make sure the PCR reaction pellet™ is completely dissolved.
5. If your thermal cycler has a 0.5 ml template, proceed to step 6.
If your thermal cycler has a 0.2 ml template, carefully transfer the entire contents of your PCR reaction tube into a clean 0.2 ml PCR tube before proceeding to step 6.
6. If your thermal cycler is equipped with a heated lid, proceed directly to polymerase chain reaction cycling.
If your thermal cycler does not have a heated lid, or if you are cycling manually with three water baths, add one wax bead to the tube before proceeding to polymerase chain reaction cycling.

CONTROL REACTION (OPTIONAL):

One control reaction can be prepared for the entire class by a student or the instructor.

7. To prepare the PCR Control reaction, add the following to the pellet:

D1S80 primer solution	20.0 μ l
Control DNA	5.0 μ l

8. Gently mix the control tube and quickly spin it in a microcentrifuge to collect all the sample at the bottom of the tube. Make sure the PCR reaction pellet™ is completely dissolved.
9. If your thermal cycler has a 0.5 ml template, proceed to step 10.
If your thermal cycler has a 0.2 ml template, carefully transfer the entire contents of your PCR reaction tube into a clean 0.2 ml PCR tube before proceeding to step 10.



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

Module II: Amplification of the D1S80 Locus

10. If your thermal cycler is equipped with a heated lid, proceed directly to polymerase chain reaction cycling.

If your thermal cycler does not have a heated lid, or if you are cycling manually with three water baths, add one wax bead to the tube before proceeding to polymerase chain reaction cycling.

POLYMERASE CHAIN REACTION CYCLING

11. For automatic cycling, each student should place his/her PCR tube (and the optional control reaction) in the programmed thermal cycler. Follow the same cycling schedule if you are manually cycling in three water baths.

<u>Initial Denaturation</u>	<u>32 cycles @</u>	<u>Final Extension</u>
94°C for 5 min.	94°C for 30 sec.	72°C for 4 min.
	65°C for 30 sec.	
	72°C for 30 sec.	

12. After the cycles are completed, add 5 μ l of 10x Gel Loading Solution to the sample and store on ice until ready for electrophoresis.



OPTIONAL STOPPING POINT

The samples can be held in the thermal cycler at 4°C or frozen after addition of 5 μ l of 10x Gel Loading Solution until ready for electrophoresis.

Module III: Electrophoresis - Agarose Gel Preparation

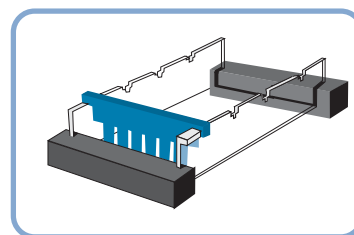
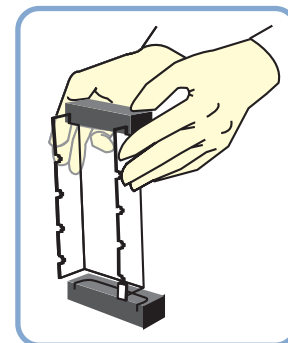


Wear gloves
and safety
goggles

If using EDVOTEK electrophoresis units, a 7 x 14 cm gel is recommended for this experiment to achieve better resolution of the PCR products.

PREPARING THE GEL BED

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
 - A. Using Rubber dams:
 - Place a rubber dam on each end of the bed. Make sure the rubber dam fits firmly in contact with the sides and bottom of the bed.
 - B. Taping with labeling or masking tape:
 - With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
 - Fold the extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.
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.



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/ laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

Module III: Electrophoresis - Agarose Gel Preparation

CASTING AGAROSE GELS

The preparation of the concentrated agarose gel (1.0%) for this experiment requires special attention. Make sure that the agarose solution is completely clear of "clumps". Distortion of electrophoresis DNA band patterns will result if the gel is not correctly prepared.

3. Use a 250 ml flask to prepare the gel solution. Add the following components to the flask as specified for your experiment (refer to Table A).

- Buffer concentrate
- Distilled water
- Agarose powder

Table A Individual 1.0% UltraSpec-Agarose™ Gel

Size of Gel (cm)	Amt of Agarose (gm)	+ Concentrated Buffer (50x) (ml)	+ Distilled Water (ml)	= Total Volume (ml)
7 x 7	0.25	0.5	24.5	25
7 x 14	0.5	1.0	49.0	50

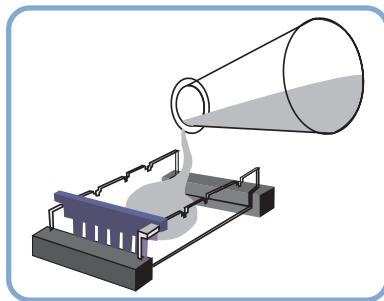
4. Swirl the mixture to disperse clumps of agarose powder.
5. With a marking pen, indicate the level of the solution volume on the outside of the flask.
6. Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any undissolved particles.
 - A. Microwave method:
 - Cover the flask with plastic wrap to minimize evaporation.
 - Heat the mixture on High for 1 minute.
 - Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.
 - B. Hot plate method:
 - Cover the flask with aluminum foil to prevent excess evaporation.
 - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

At high altitudes, it is recommended to use a microwave oven to reach boiling temperatures.



Module III: Electrophoresis - Agarose Gel Preparation

7. Cool the agarose solution to 55°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 as marked on the flask in step 5.

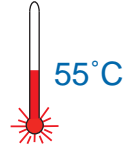


After the gel is cooled to 55°C:

**If you are using rubber dams, go to step 9.
If you are using tape, continue with step 8.**

8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.
- Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
 - Wait approximately 1 minute for the agarose to solidify.
9. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.
10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

Cool the agarose to 55°C



DO NOT POUR BOILING HOT AGAROSE INTO THE GEL BED.

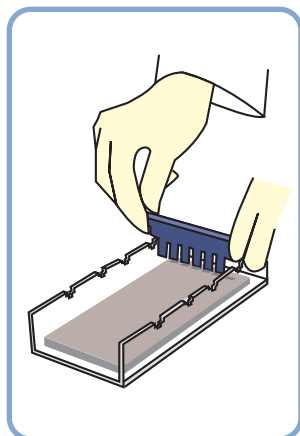
Hot agarose solution may irreversibly warp the bed.



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

Module III: Electrophoresis - Agarose Gel Preparation

PREPARING THE GEL FOR ELECTROPHORESIS



- After the gel is completely solidified, carefully and slowly remove the rubber dams or tape from the gel bed.

Be especially careful not to damage or tear the gel wells when removing the rubber dams. A thin plastic knife, spatula or pipet tip can be inserted between the gel and the dams to break possible surface tension.

- Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.
- Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
- Fill the electrophoresis apparatus chamber with the required volume of diluted buffer for the specific unit you are using (see guidelines in Table B).

For DNA analysis, the same EDVOTEK 50x Electrophoresis Buffer is used for preparing both the agarose gel buffer and the chamber buffer. The formula for diluting EDVOTEK (50x) concentrated buffer is 1 volume of buffer concentrate to every 49 volumes of distilled or deionized water.

The electrophoresis (chamber) buffer recommended is Tris-acetate-EDTA (20 mM tris, 6 mM sodium acetate, 1 mM disodium ethylenediamine tetraacetic acid) pH 7.8. Prepare the buffer as required for your electrophoresis apparatus.

Table B Dilution of Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Concentrated Buffer (50x) (ml)	+ Distilled Water (ml)	= Total Volume (ml)
M6+	6	294	300
M12	8	392	400
M36 (blue)	10	490	500
M36 (clear)	20	980	1000

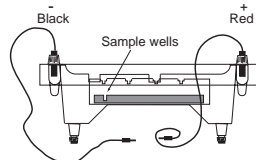
- Make sure the gel is completely covered with buffer.
- Proceed to loading the samples and conducting electrophoresis.



Module III: Electrophoresis - Conducting Agarose Gel Electrophoresis

Reminder:

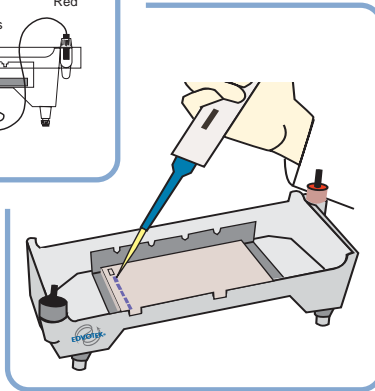
During electrophoresis, the DNA samples migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

**LOADING THE SAMPLES**

This experiment requires a 1.0% agarose gel. Have a water bath or beaker of water warmed to 50°C for heating tubes containing DNA fragments before gel loading.

LOADING DNA SAMPLES

1. Heat the 200 bp DNA ladder and PCR samples for two minutes at 50°C. Allow the samples to cool for a few minutes.



2. Load Samples

Load entire volume (30 μ l) of the samples in the following sequence.

Lane

1	200 bp DNA ladder
2	Control DNA (optional)
3	Student #1
4	Student #2
5	Student #3
6	Student #4

3. Record the position of your sample in the gel for easy identification after staining.



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

Module III: Electrophoresis - Conducting Agarose Gel Electrophoresis

RUNNING THE GEL

- After the DNA samples are loaded, carefully snap the cover down onto the electrode terminals.

Make sure that the negative and positive color-coded indicators on the cover and apparatus chamber are properly oriented.

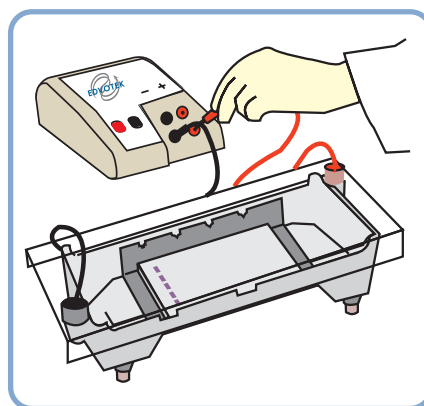
- Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).
- Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor. General guidelines are presented in Table C.

Table C Time and Voltage
(1.0% - 7 x 14 cm gel)

Volts	Recommended Time	
	Minimum	Maximum
125	55 min	1 hr 15 min
70	2 hrs 15 min	3 hrs
50	3 hrs 25 min	5 hrs

- Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.
- Allow the tracking dye to migrate 4.5 cm. (small gel) or 6-7cm. (large gel) from the wells for adequate separation of the DNA bands. Terminate the electrophoresis before the tracking dye moves off the end of the gel.

- After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.
- Remove the gel from the bed for staining with InstaStain® Ethidium Bromide.



Module III: Electrophoresis - Staining with InstaStain® Ethidium Bromide



Wear gloves and safety goggles



Visit our web site for an animated demonstration of InstaStain® EtBr.

STAINING WITH INSTASTAIN® ETBR

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 the clear plastic protective sheet, and place the unprinted side of the InstaStain® EtBr card on the gel.
3. Firmly run your fingers over the entire surface of the InstaStain® EtBr. Do this several times.
4. Place the gel casting tray and a small empty beaker on top to ensure that the InstaStain® card maintains direct contact with the gel surface.

Allow the InstaStain® EtBr card to stain the gel for 10-15 minutes.
5. After 10-15 minutes, remove the InstaStain® EtBr card. Transfer the gel to a ultraviolet (300 nm) transilluminator for viewing. Be sure to wear UV protective goggles.

DISPOSAL OF INSTASTAIN

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

Caution: Ethidium Bromide is a listed mutagen.

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® EtBr. Repeat staining and increase the staining time an additional 10-15 minutes.
- Gels stained alternatively with InstaStain Methylene Blue or liquid methylene blue may fade with time. Re-stain the gel to visualize the DNA bands.
- DNA 200 bp markers should be visible after staining even if the amplified DNA samples are faint or absent. If markers are not visible, troubleshoot for problems with the electrophoretic separation.



1

Moisten the gel.



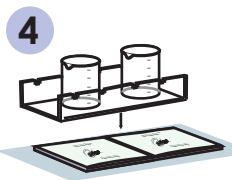
2

Place the InstaStain® card on the gel.



3

Press firmly.



4

Place a small weight to ensure good contact.



5

View on U.V. (300 nm) transilluminator



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/ laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

Module III: Electrophoresis - Staining with InstaStain® Ethidium Bromide

PHOTODOCUMENTATION OF DNA

There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

The following guidelines are for photographing gels stained with InstaStain® Ethidium Bromide, utilizing the EDVOTEK UV photodocumentation system (Cat. # 555). It is a relatively simple photodocumentation system comprised of a UV transilluminator, a 6 inch safety camera hood, and Polaroid camera fitted with a deep yellow Tiffin 40.5 mm filter. The camera uses Polaroid 667 Black and White film. The recommended settings can be used as a starting point, although optimal conditions for your system may vary.

PHOTOGRAPHY GUIDELINES

1. To assemble the camera, screw the handle into the center hole at the base of the camera.
2. Align the hood onto the camera lens.
3. Carefully and firmly push down the buttons on the inside of the hood on both sides of the lens.
4. Load your Polaroid camera with Polaroid 667 Black and White film.
5. Open the safety cover of the transilluminator and place the gel on the surface of the filter.
6. Cover the gel with the camera hood so that the hood is aligned with the camera mounting plate.
7. Turn on the transilluminator and photograph.
 - Recommended camera setting is f 5.6 for 2 seconds.
 - If the photograph is too light, change the aperture to f 8 and expose for 2 seconds.
 - If too dark, reduce the shutter speed to 1 second at f 5.6.

For additional information, refer to the instructions which accompany your photodocumentation system.


Study Questions

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


1. Compare your D1S80 PCR product with those of the rest of the class. Did any students have genotypes similar to yours? How could you explain such similarities?
2. What is polymorphic DNA? How is it used for identification purposes?
3. What is CODIS? How is it used to solve crimes?
4. What is an STR? A VNTR? Which (STR or VNTR) is predominantly used in law enforcement? Why?




Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2000, 2001, 2003, 2005, 2006 EDVOTEK, Inc., all rights reserved. EVT 003106K

 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) 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) 14676 Rothgeb Drive Rockville, MD 20850																					
Emergency Telephone Number (301) 251-5990 Telephone Number for information (301) 251-5990 Date Prepared 07/01/03 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 <table border="1"> <tr> <td>Boiling Point For 1% solution</td> <td>194 F</td> <td>Specific Gravity (H₂O = 1)</td> <td>No data</td> </tr> <tr> <td>Vapor Pressure (mm Hg.)</td> <td>No data</td> <td>Melting Point</td> <td>No data</td> </tr> <tr> <td>Vapor Density (AIR = 1)</td> <td>No data</td> <td>Evaporation Rate (Butyl Acetate = 1)</td> <td>No data</td> </tr> <tr> <td>Solubility in Water</td> <td colspan="3">Insoluble - cold</td> </tr> <tr> <td>Appearance and Odor</td> <td colspan="3">White powder, no odor</td> </tr> </table>		Boiling Point For 1% solution	194 F	Specific Gravity (H ₂ O = 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	Insoluble - cold			Appearance and Odor	White powder, no odor		
Boiling Point For 1% solution	194 F	Specific Gravity (H ₂ O = 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	Insoluble - cold																				
Appearance and Odor	White powder, no odor																				
Section IV - Physical/Chemical Characteristics N.D. = No data <table border="1"> <tr> <td>Flash Point (Method Used)</td> <td>No data</td> <td>Flammable Limits</td> <td>LEL N.D.</td> <td>UEL N.D.</td> </tr> <tr> <td>Extinguishing Media</td> <td colspan="4">Water spray, dry chemical, carbon dioxide, halon or standard foam</td> </tr> <tr> <td>Special Fire Fighting Procedures</td> <td colspan="4">Possible fire hazard when exposed to heat or flame</td> </tr> <tr> <td>Unusual Fire and Explosion Hazards</td> <td colspan="4">None</td> </tr> </table>		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			
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 Stable X Conditions to Avoid None Incompatibility No data available Hazardous Decomposition or Byproducts									
Hazardous Polymerization May Occur Will Not Occur X Conditions to Avoid None									
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. <table border="1"> <tr> <td>Ventilation</td> <td>Local Exhaust</td> <td>Special</td> </tr> <tr> <td></td> <td>Mechanical (General)Gen. dilution ventilation</td> <td>Other</td> </tr> </table> Protective Gloves Yes Eye Protection Splash proof goggles Other Protective Clothing or Equipment Impervious clothing to prevent skin contact Work/Hygienic Practices None				Ventilation	Local Exhaust	Special		Mechanical (General)Gen. dilution ventilation	Other
Ventilation	Local Exhaust	Special							
	Mechanical (General)Gen. dilution ventilation	Other							

 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) 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) 14676 Rothgeb Drive Rockville, MD 20850																					
Emergency Telephone Number (301) 251-5990 Telephone Number for information (301) 251-5990 Date Prepared 07/01/03 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 <table border="1"> <tr> <td>Boiling Point</td> <td>No data</td> <td>Specific Gravity (H₂O = 1)</td> <td>No data</td> </tr> <tr> <td>Vapor Pressure (mm Hg.)</td> <td>No data</td> <td>Melting Point</td> <td>No data</td> </tr> <tr> <td>Vapor Density (AIR = 1)</td> <td>No data</td> <td>Evaporation Rate (Butyl Acetate = 1)</td> <td>No data</td> </tr> <tr> <td>Solubility in Water</td> <td colspan="3">Appreciable, (greater than 10%)</td> </tr> <tr> <td>Appearance and Odor</td> <td colspan="3">Clear, liquid, slight vinegar odor</td> </tr> </table>		Boiling Point	No data	Specific Gravity (H ₂ O = 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		
Boiling Point	No data	Specific Gravity (H ₂ O = 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 <table border="1"> <tr> <td>Flash Point (Method Used)</td> <td>No data</td> <td>Flammable Limits</td> <td>LEL N.D.</td> <td>UEL N.D.</td> </tr> <tr> <td>Extinguishing Media</td> <td colspan="4">Use extinguishing media appropriate for surrounding fire.</td> </tr> <tr> <td>Special Fire Fighting Procedures</td> <td colspan="4">Wear protective equipment and SCBA with full facepiece operated in positive pressure mode.</td> </tr> <tr> <td>Unusual Fire and Explosion Hazards</td> <td colspan="4">None identified</td> </tr> </table>		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			
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 Stable X Conditions to Avoid None Incompatibility Strong oxidizing agents Hazardous Decomposition or Byproducts Carbon monoxide, Carbon dioxide													
Hazardous Polymerization May Occur Will Not Occur X Conditions to Avoid None													
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 environmental 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) <table border="1"> <tr> <td>Ventilation</td> <td>Local Exhaust</td> <td>Yes</td> <td>Special</td> <td>None</td> </tr> <tr> <td></td> <td>Mechanical (General)</td> <td>Yes</td> <td>Other</td> <td>None</td> </tr> </table> Protective Gloves Yes Eye Protection Safety goggles Other Protective Clothing or Equipment None Work/Hygienic Practices None				Ventilation	Local Exhaust	Yes	Special	None		Mechanical (General)	Yes	Other	None
Ventilation	Local Exhaust	Yes	Special	None									
	Mechanical (General)	Yes	Other	None									

 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) Gel loading solution concentrate, 10x		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) 14676 Rothgeb Drive Rockville, MD 20850		Emergency Telephone Number (301) 251-5990 Telephone Number for information (301) 251-5990 Date Prepared 07/01/03 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 ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water soluble			
Appearance and Odor Blue liquid, no odor			
Section IV - Physical/Chemical Characteristics			
Flash Point (Method Used)	No data	Flammable Limits	LEL No data UEL No data
Extinguishing Media Dry chemical, carbon dioxide, water spray or foam			
Special Fire Fighting Procedures Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.			
Unusual Fire and Explosion Hazards Unknown			


Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility None known			
Hazardous Decomposition or Byproducts Sulfur oxides and bromides			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
Section VI - Health Hazard Data			
Route(s) of Entry: Inhalation? Skin? Ingestion?			
Yes Yes Yes			
Health Hazards (Acute and Chronic) Acute eye contact: May cause irritation No data available for other routes			
Carcinogenicity: None	NTP? No data	IARC Monographs? No data	OSHA Regulation? No data
Signs and Symptoms of Exposure May cause skin or eye irritation			
Medical Conditions Generally Aggravated by Exposure None reported			
Emergency First Aid Procedures Treat symptomatically and supportively Rinse contacted area with copious amounts of water.			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released or Spilled Rinse contacted area with copious amounts of water.			
Waste Disposal Method Observe all federal, state, and local 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) Chemical cartridge respirator with organic vapor cartridge.			
Ventilation	Local Exhaust	Yes	Special Yes
	Mechanical (General)	Yes	Other None
Protective Gloves	yes	Eye Protection	Splash proof goggles
Other Protective Clothing or Equipment None required			
Work/Hygienic Practices Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.			

 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) InstaStain® Ethidium Bromide		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 InstaStain, Inc. P.O. Box 1232 West Bethesda, MD 20827		Emergency Telephone Number (301) 251-5990 Telephone Number for information (301) 251-5990 Date Prepared 07/01/03 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) Ethidium Bromide Data not available (2,7-Diamino-10-Ethyl-9-Phenylphenanthridinium Bromide) CAS# 139-33-3			
Section III - Physical/Chemical Characteristics			
Boiling Point	No data	Specific Gravity (H ₂ O = 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 Chemical bound to paper, 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, carbon dioxide, dry chemical powder, alcohol or polymer foam			
Special Fire Fighting Procedures Wear protective clothing and SCBA to prevent contact with skin & eyes			
Unusual Fire and Explosion Hazards Emits toxic fumes			

Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility Strong oxidizing agents			
Hazardous Decomposition or Byproducts Carbon monoxide, Carbon dioxide, nitrogen oxides, hydrogen bromide gas			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
Section VI - Health Hazard Data			
Route(s) of Entry: Inhalation? Skin? Ingestion?			
Yes Yes Yes			
Health Hazards (Acute and Chronic) Chronic: May alter genetic material Acute: Material irritating to mucous membranes, upper respiratory tract, eyes, skin			
Carcinogenicity: No data available	NTP?	IARC Monographs?	OSHA Regulation?
Signs and Symptoms of Exposure Irritation to mucous membranes and upper respiratory tract			
Medical Conditions Generally Aggravated by Exposure No data			
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 or Spilled Wear SCBA, rubber boots, rubber gloves			
Waste Disposal Method Mix material with combustible solvent and burn in a chemical incinerator equipped afterburner and scrubber			
Precautions to be Taken in Handling and Storing Use in chemical fume hood with proper protective lab gear.			
Other Precautions Mutagen			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) SCBA			
Ventilation	Local Exhaust	Yes	Special Chem. fume hood
	Mechanical (General)	No	Other None
Protective Gloves	Rubber	Eye Protection	Chem. safety goggles
Other Protective Clothing or Equipment Rubber boots			
Work/Hygienic Practices Use in chemical fume hood with proper protective lab gear.			

 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) Chelating Agent			
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) 14676 Rothgeb Drive Rockville, MD 20850			
Emergency Telephone Number (301) 251-5990 Telephone Number for information (301) 251-5990 Date Prepared 07/01/03 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) Iminodiacetic Acid CAS #142-73-4			
Section III - Physical/Chemical Characteristics			
Boiling Point	No data	Specific Gravity (H ₂ O = 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 White fluffy granules (hygroscopic), odorless			
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 Dry chemical, carbon dioxide, water spray or regular foam Special Fire Fighting Procedures Wear NIOSH/MSHA approved SCBA and full protective equipment. Unusual Fire and Explosion Hazards None specified			

Section V - Reactivity Data Stability Unstable X Conditions to Avoid None specified Stable X Incompatibility Strong oxidizing agents Hazardous Decomposition or Byproducts Toxic fumes of carbon monoxide, carbon dioxide, nitrogen oxides			
Hazardous Polymerization May Occur X Conditions to Avoid Incompatibles Will Not Occur X			
Section VI - Health Hazard Data Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes Health Hazards (Acute and Chronic) Irritating to mucous membranes Carcinogenicity: No data NTP? IARC Monographs? OSHA Regulation?			
Signs and Symptoms of Exposure None specified			
Medical Conditions Generally Aggravated by Exposure No data			
Emergency First Aid Procedures Skin/Eyes: Immediately flush with copious amounts of water for 15 min. Inhalation: Remove to fresh air, if not breathing give artificial respiration, if difficulty breathing give oxygen Ingestion: Wash out mouth with water. Call physician.			
Section VII - Precautions for Safe Handling and Use Steps to be Taken in case Material is Released for Spilled Wear suitable protective clothing. Sweep up and place in suitable container for later disposal. Waste Disposal Method Observe all federal, state, and local regulations Precautions to be Taken in Handling and Storing Keep tightly closed in a cool, dry place Other Precautions Avoid contact			
Section VIII - Control Measures Respiratory Protection (Specify Type)			
Ventilation	Local Exhaust Yes	Special None	
	Mechanical (General) No	Other None	
Protective Gloves Yes		Eye Protection Chem proof goggles	
Other Protective Clothing or Equipment Eye wash			
Work/Hygienic Practices Wear protective clothing and equipment to prevent contact.			

 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) PBS			
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) 14676 Rothgeb Drive Rockville, MD 20850			
Emergency Telephone Number (301) 251-5990 Telephone Number for information (301) 251-5990 Date Prepared 07/01/03 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)			
Section III - Physical/Chemical Characteristics			
Boiling Point	100C	Specific Gravity (H ₂ O = 1)	1.017
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 solid			
Section IV - Physical/Chemical Characteristics Flash Point (Method Used) Noncombustible Flammable Limits LEL UEL Extinguishing Media Use extinguishing media appropriate to surrounding fire Special Fire Fighting Procedures Wear SCBA and protective clothing to prevent contact with skin and eyes Unusual Fire and Explosion Hazards Emits toxic fumes under fire conditions			

Section V - Reactivity Data Stability Unstable Conditions to Avoid Stable			
Incompatibility Strong acids Hazardous Decomposition or Byproducts Nature of decomposition products not known			
Hazardous Polymerization May Occur Conditions to Avoid Will Not Occur			
Section VI - Health Hazard Data Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes Health Hazards (Acute and Chronic) Cause eye & skin irritation, material is irritating to mucous membranes and upper respiratory tract. The toxicological properties have not been thoroughly investigated. Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?			
Signs and Symptoms of Exposure			
Medical Conditions Generally Aggravated by Exposure			
Emergency First Aid Procedures Swallowed - wash out mouth with water provided person is conscious. Skin/eye contact - flush with water Inhalation - remove to fresh air			
Section VII - Precautions for Safe Handling and Use Steps to be Taken in case Material is Released for Spilled Wear respirator, chemical safety goggles, rubber boots and heavy rubber gloves, sweep up, place in a bag and hold for waste disposal. Waste Disposal Method For small quantities - cautiously add to a large stirred excess of water. Adjust pH to neutral Precautions to be Taken in Handling and Storing Wear appropriate NIOSH/MSHA approved respirator, chemical resistant gloves, safety goggles safety shower and eye bath. Other Precautions			
Section VIII - Control Measures Respiratory Protection (Specify Type) NIOSH/MSHA approved respirator			
Ventilation	Local Exhaust N/A	Special N/A	
	Mechanical (General) N/A	Other N/A	
Protective Gloves Yes		Eye Protection Yes	
Other Protective Clothing or Equipment			
Work/Hygienic Practices Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.			