EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

EDVO-Kit #
334

VNTR Human DNA Typing Using PCR

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

In this experiment, students will use their own genomic DNA. Using Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis, they will identify polymorphisms in the D1S80 region of their chromosome 1.

Table of Contents

Material Safety Data Sheets can be found on our website: www.edvotek.com

Experiment Components

Experiment # 334 contains material for up to 25 human DNA typing reactions.

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

	Component	Storage	Check (✓)
A	Tubes with PCR EdvoBeads™ Each PCR EdvoBead™ contains • dNTP Mixture • *Taq* DNA Polymerase Buffer • *Taq* DNA Polymerase • $MgCl_2$ • Reaction Buffer	Room Temperature	❑
B	D1S80 Primer mix concentrate	-20°C Freezer	❑
C	200 base pair ladder	-20°C Freezer	❑
D	Control DNA concentrate	-20°C Freezer	❑
E	TE buffer	-20°C Freezer	❑
F	Proteinase K	Room temperature	❑

NOTE: Components B and D are now supplied in concentrated form.

Reagents & Supplies

Store all components below at room temperature.

Component	Check (✓)
• UltraSpec-Agarose™	❑
• Electrophoresis Buffer (50x)	❑
• 10x Gel Loading Solution	❑
• InstaStain® Ethidium Bromide	❑
• FlashBlue™ Liquid Stain	❑
• Conical tube (15 ml)	❑
• Microcentrifuge Tubes	❑
• PCR tubes (0.2 ml - for thermal cyclers with 0.2 ml template)	❑
• Disposable plastic cups	❑
• Salt packets	❑
• 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.

Experiment Requirements **(NOT included in this experiment)**

*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. # 541 highly recommended) or three waterbaths*
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- Waterbath (55° C and boiling) (EDVOTEK Cat. # 539 highly recommended)
- UV Transilluminator or UV Photodocumentation system *(use if staining with InstaStain® Ethidium Bromide)*
- UV safety goggles
- White light visualization system *(optional - use if staining with FlashBlue™)*
- Automatic micropipets (5-50 µl) with tips
- Microwave or hot plate
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Disposable laboratory gloves
- Distilled or deionized water
- Spring water
- Ice buckets and ice
- Bleach solution

VNTR Human DNA Typing

In humans, DNA is packaged into 23 pairs of chromosomes that are inherited from an individual's biological parents. Although most of this genetic material is identical in every person, small differences or "polymorphisms" in the DNA sequence occur throughout the genome, making each of us unique. For example, the simplest difference is a Single Nucleotide Polymorphism (or SNP). Short repetitive stretches of DNA at specific locations in the genome can vary in number to produce STRs (Short Tandem Repeats) and longer repetitive segments are called VNTRs (Variable Number of Tandem Repeats). Most polymorphisms occur in non-coding regions of DNA, but those that do not may disrupt a gene and can result in disease. Medical diagnostic tests are used routinely to identify specific polymorphisms associated with disease.

Analyzing several different polymorphisms within a person's genome generates a unique DNA "fingerprint". DNA fingerprints can allow us to distinguish one individual from another. Because polymorphisms are inherited, DNA fingerprints can also be used to determine paternity/maternity (and other familial relationships). The best-known application of DNA fingerprinting is in the field of forensic science. The first step in forensic DNA fingerprinting is the legal collection of biological evidence (often present as a stain) from the crime scene or victim. The sample is treated with a detergent to rupture (lyse) cell membranes, and the cellular DNA is extracted for further analysis (Figure 1). After DNA is extracted from these samples, forensic scientists can develop a DNA fingerprint. The DNA fingerprint from a crime scene can then be compared to the DNA fingerprints of different suspects. A match provides strong evidence that the suspect was present at the crime scene.

The first use of forensic 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 conviction using DNA evidence occurred on November 6, 1987 in Orlando, Florida. Since then, DNA analysis has been used in thousands of convictions. Additionally, hundreds of convicted prison inmates have been exonerated from their crimes, including several death row inmates. The original DNA fingerprinting technology utilized a method called Restriction Fragment Length Polymorphism (RFLP) analysis, which 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. Although RFLP is very precise, it is time-consuming and requires large amounts of DNA. Because of this, the RFLP method is no longer used in forensics; however, it remains in use in certain medical diagnostic tests.

NOTE:
VNTR - 15 - 70 bp repeats, repeated five to 100 times.

STR - 2-6 bp repeats, repeated 3-100 times.

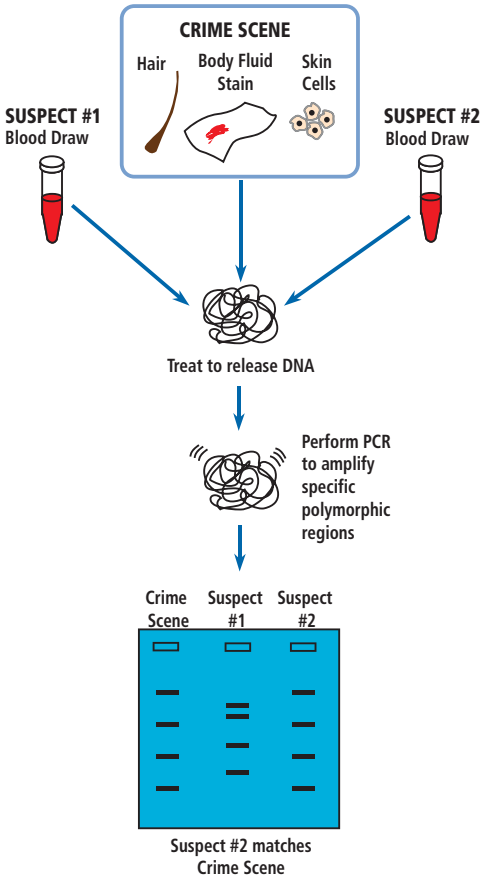


Figure 1:
Extraction and Electrophoresis of DNA Samples

Background Information

Background Information

VNTR Human DNA Typing

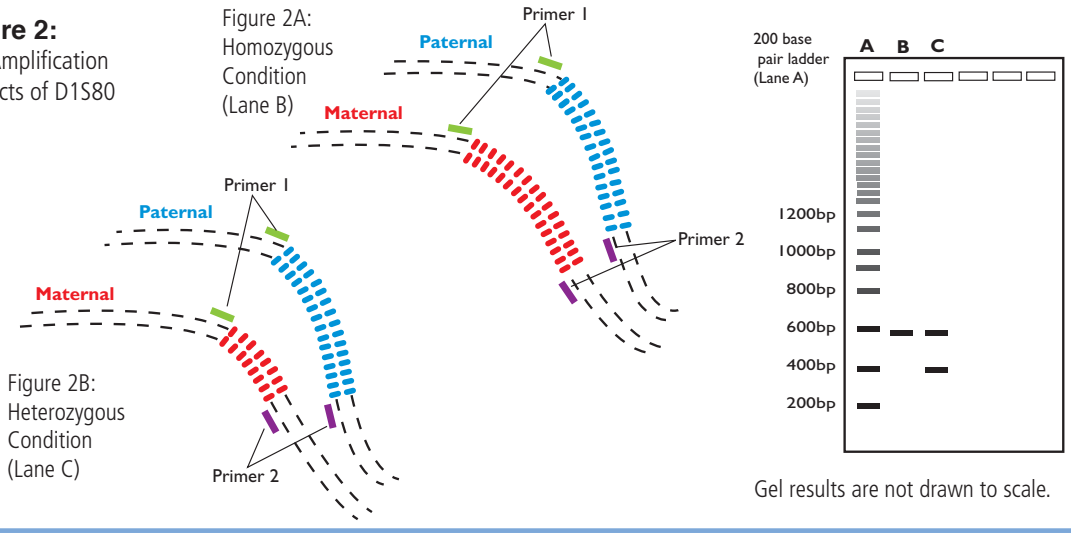
Today, forensic scientists use the Polymerase Chain Reaction (PCR) to produce DNA fingerprints. PCR is a technology that has further revolutionized the science of DNA fingerprinting based on its ease of use and its ability to amplify DNA. This technique allows researchers to quickly create many copies of a specific region of DNA in vitro. PCR requires 500-fold less DNA than traditional RFLP analysis and it can be performed in one afternoon. PCR was invented in 1984 by Dr. Kary Mullis at the Cetus Corporation in California. For this ground breaking technology, Mullis was awarded the Nobel Prize in Chemistry in 1993.

Forensic scientists use PCR to analyze highly polymorphic DNA regions. 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). One VNTR, known as D1S80, is present on human chromosome 1. It comprises a 16-nucleotide sequence that is 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 agarose gel electrophoresis (Figure 2A). More commonly, a person will be heterozygous at this loci, resulting in 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 will analyze STRs, as their smaller size makes them easier to amplify, thus requiring less starting DNA.

Before performing PCR, template DNA is extracted from various biological sources (in forensic cases - blood, tissue, or bodily fluid). Because PCR is very sensitive, only a few copies of the gene are required. Nevertheless, freshly isolated DNA will provide better amplification results than older DNA specimens that may have become degraded. In order to amplify the specific DNA or target sequence, two primers (short & synthetic DNA molecules) are designed to correspond to the ends of the target sequence.

To perform PCR, the template DNA and a molar excess of primers are mixed with the four "free" deoxynucleotides (dATP, dCTP, dGTP, and dTTP), and a thermostable DNA polymerase. The most commonly used DNA polymerase is *Taq* DNA polymerase. This enzyme, originally purified from a bacterium that inhabits hot springs, is stable at very high temperatures. These components (template DNA, primers, the four deoxynucleotides, and *Taq* DNA polymerase) are mixed with a buffer that contains Mg^{+2}, an essential

Figure 2: PCR Amplification Products of D1S80



VNTR Human DNA Typing

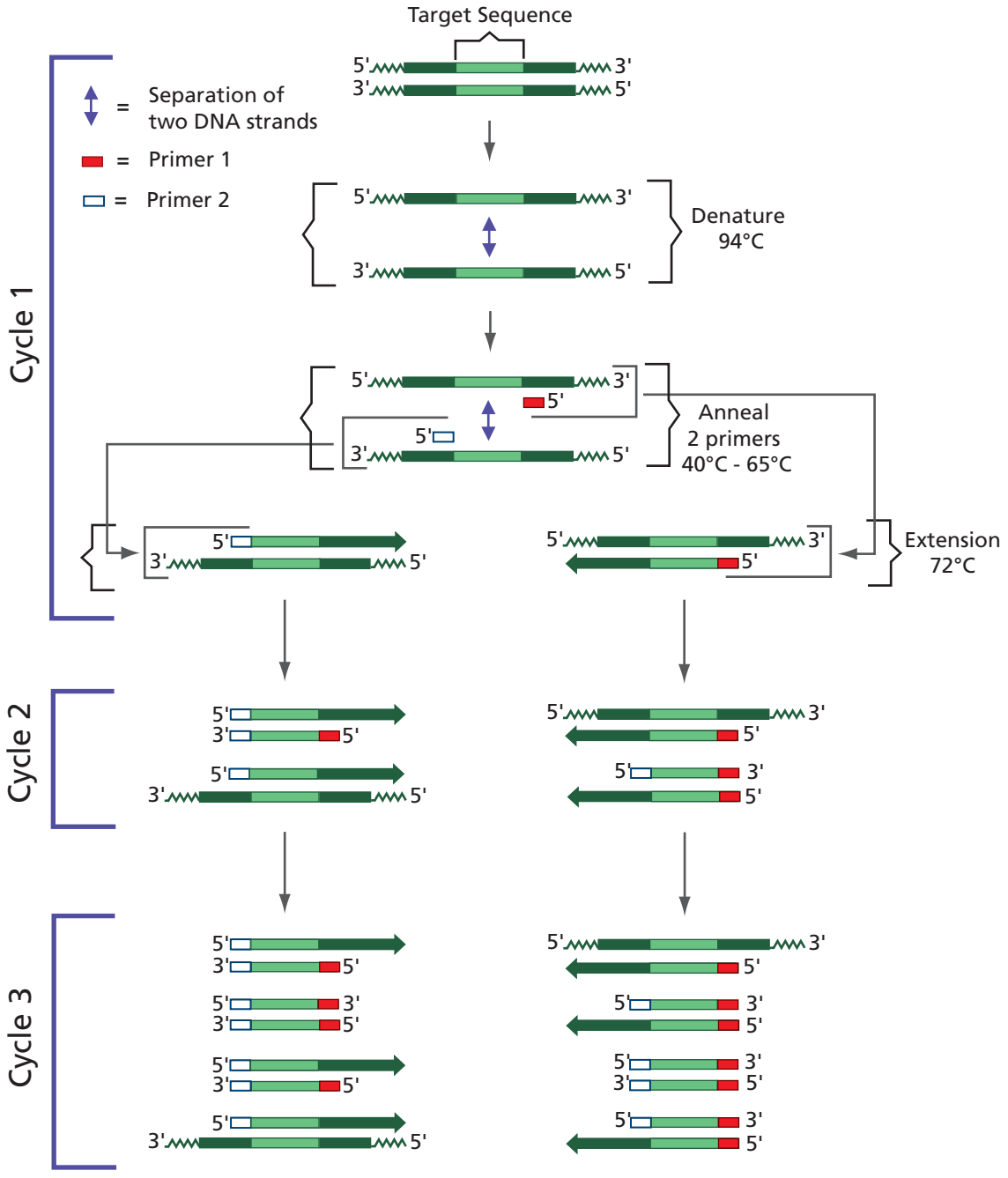


Figure 3: Polymerase Chain Reaction

Background Information

VNTR Human DNA Typing

cofactor for *Taq* polymerase. The PCR reaction mixture is subjected to sequential heating/cooling cycles at three different temperatures in a thermal cycler.

- In the first step, known as "denaturation", the mixture is heated to near boiling (94° C - 96° C) to "unzip" (or melt) the target DNA. The high temperature disrupts the hydrogen bonds between the two complementary DNA strands and causes their separation.
- In the second step, known as "annealing", the reaction mixture is cooled to 45° C - 65° C, which allows the primers to base pair with the target DNA sequence.
- In the third step, known as "extension", the temperature is raised to 72° C. This is the optimal temperature at which *Taq* polymerase can add nucleotides to the hybridized primers to synthesize the new complementary strands.

These three steps - denaturation, annealing, and extension - constitute one PCR "cycle" (Figure 3). Each PCR cycle doubles the amount of the target DNA in less than five minutes. In order to produce enough DNA for analysis, twenty to forty cycles may be required. To simplify this process, a specialized machine, called a "thermal cycler" or a "PCR machine", was created to rapidly heat and cool the samples.

A match between the crime scene DNA and a suspect's DNA at a single locus does not prove guilt, nor does it rule out innocence. Therefore, multiple loci are tested. 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. The DNA fingerprints stored in CODIS contain data on thirteen loci (see Figure 4). The odds of a match at all thirteen loci are less than one in a trillion. 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.

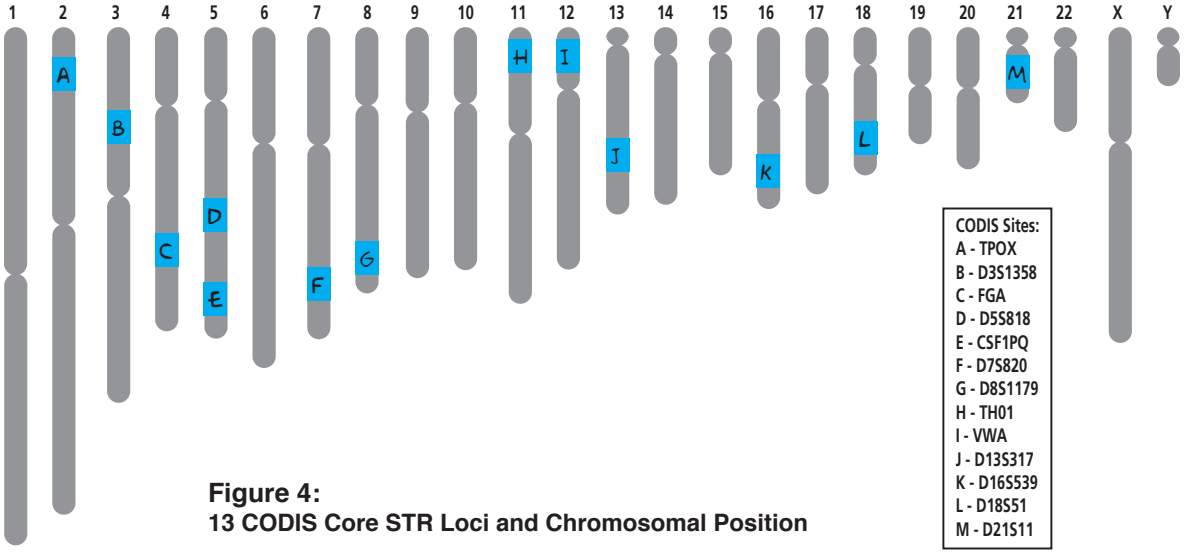


Figure 4:
13 CODIS Core STR Loci and Chromosomal Position

 334.130821

Experiment Overview and General Instructions

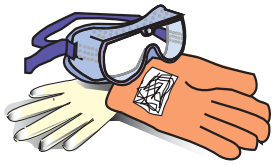
EXPERIMENT OBJECTIVE:

In this experiment, students will use their own genomic DNA. Using Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis, they will identify polymorphisms in the D1S80 region of their chromosome 1.

IMPORTANT

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working in the laboratory.
- Contaminated laboratory waste (saliva solution, cup, pipet, etc.) must be disinfected with 15% bleach solution prior to disposal. Be sure to properly dispose any biological samples according to your institutional guidelines.



Wear gloves and safety goggles

Module I – 50 min.
Isolation of DNA from Cheek Cells or Human Hair

↓

Module II – 70 min.
Amplification of extracted DNA by PCR

↓

Module III – 50-70 min.
Separation of PCR Product by Electrophoresis

↓

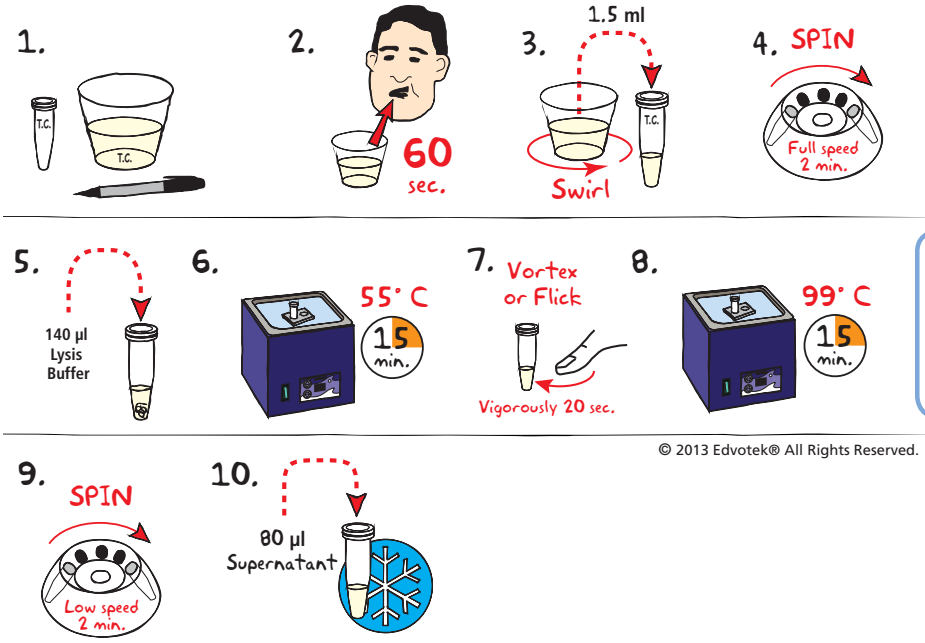
Module IV – 5 min.
Staining Agarose Gels

NOTE: Experimental times are approximate.

The Experiment

Module I-A: Isolation of DNA from Human Cheek Cells

Preferred Method



Warning! Students should use screw-cap tubes when boiling samples.

1. **LABEL** a 1.5 ml screw top microcentrifuge tube and a cup with your lab group and/or initials.
2. **RINSE** your mouth vigorously for 60 seconds using 10 ml saline solution. **EXPEL** the solution into cup.
3. **SWIRL** the cup gently to resuspend the cells. **TRANSFER** 1.5 ml of solution into the labeled tube.
4. **CENTRIFUGE** the cell suspension for 2 min. at full speed to pellet the cells. **POUR** off the supernatant, but **DO NOT DISTURB THE CELL PELLET**! Repeat steps 3 and 4 twice more.
5. **RESUSPEND** the cheek cells in 140 µl lysis buffer by pipetting up and down or by vortexing vigorously.
6. **CAP** the tube and **PLACE** in a waterbath float. **INCUBATE** the sample in a 55° C waterbath for 15 min.
7. **MIX** the sample by vortexing or flicking the tube vigorously for 20 seconds.
8. **INCUBATE** the sample in a 99° C waterbath for 15 min. Be sure to use screw-cap tubes when boiling DNA isolation samples.
9. **CENTRIFUGE** the cellular lysate for 2 minutes at low speed (6000 rpm).
10. **TRANSFER** 80 µl of the supernatant to a clean, labeled microcentrifuge tube. **PLACE** tube in ice.
11. **PROCEED** to Module II: Amplification of the D1S80 Locus.

STEP 4: If cell pellet size is not large enough, repeat steps 3 - 4 until you have a large size pellet. For best results, make sure your cell pellet is at least the size of a match head.

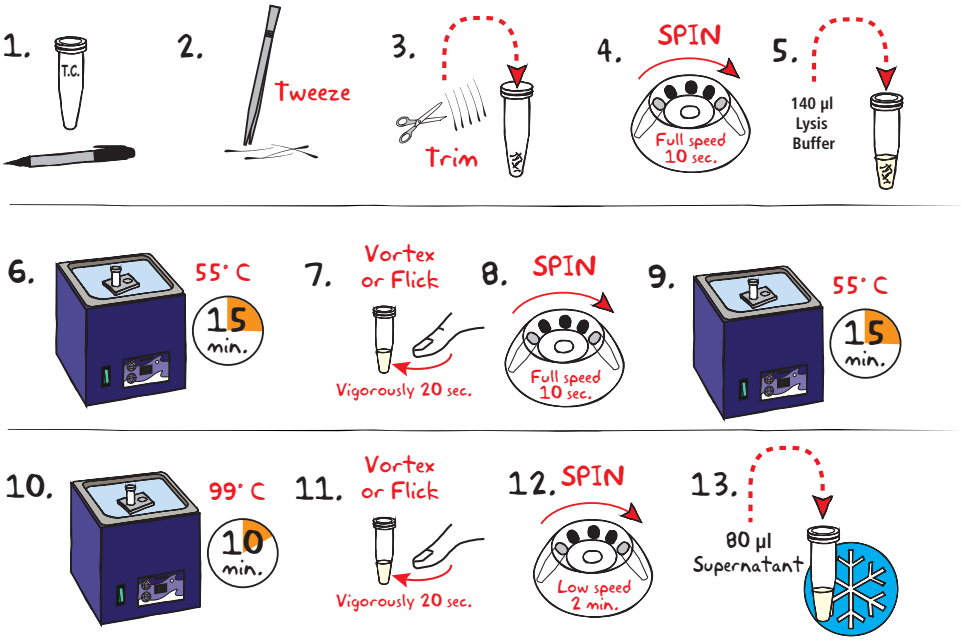
STEP 7: If a vortex is not available, mix samples by flicking the tube vigorously for 20 seconds.

STOP

OPTIONAL STOPPING POINT:

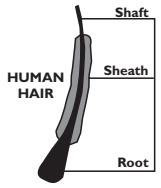
The extracted DNA may be stored at -20°C for amplification at a later time.

Module I-B: Isolation of DNA from Human Hair



Warning!
Students should use screw-cap tubes when boiling samples.

IMPORTANT:
For best results, harvest hairs from the scalp. The root structure from these hairs will be thicker and will yield more DNA than those from the eyebrow.



STEPS 7 & 11
If a vortex is not available, mix samples by flicking the tube vigorously for 20 seconds.

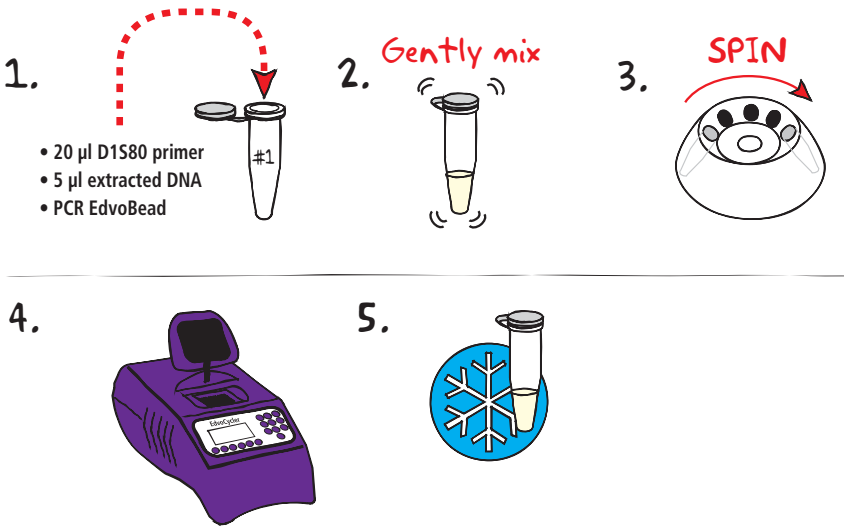
1. **LABEL** a 1.5 ml screw top microcentrifuge tube with your lab group and/or initials.
2. Using tweezers, **GRASP** 2-3 hair shafts at the base and **PULL** quickly. **COLLECT** at least 5 hairs that include the root and the sheath (a sticky barrel-shaped layer of cells that encircles the root end of the hair).
3. Using a clean scalpel or scissors, **TRIM** away any extra hair from the root (leave about 1 cm in length from the root). **TRANSFER** the roots to the labeled tube using forceps.
4. **CAP** the tube and **CENTRIFUGE** the sample for 10 seconds at full speed to collect the roots at the bottom of the tube.
5. **ADD** 140 µL lysis buffer to the tube. For best results, completely **IMMERSE** the follicles in the solution.
6. **CAP** the tube and **PLACE** it in a waterbath float. **INCUBATE** the sample in a 55° C waterbath for 15 min.
7. **MIX** the sample by vortexing or flicking the tube vigorously for 20 seconds.
8. **CENTRIFUGE** the sample for 10 seconds at full speed to collect the roots at the bottom of the tube.
9. **INCUBATE** the sample at 55° C for an additional 15 min.
10. **MOVE** the sample to a 99° C waterbath. **INCUBATE** for 10 min. Be sure to use screw-cap tubes when boiling samples.
11. **MIX** the sample by vortexing or flicking the tube vigorously for 20 seconds.
12. **CENTRIFUGE** the cellular lysate for 2 min. at low speed (6000 rpm).
13. **TRANSFER** 80 µl of the supernatant to a clean, labeled microcentrifuge tube. **PLACE** tube in ice.
14. **PROCEED** to Module II: Amplification of the D1S80 Locus.

OPTIONAL STOPPING POINT:

The supernatant may be stored at -20°C for amplification at a later time.

The Experiment

Module II: Amplification of the D1S80 Locus



NOTES AND REMINDERS:

This kit includes enough DNA to set up 3-4 control reactions. At least one control reaction should be performed per class to confirm that PCR was successful.

If your thermal cycler does not have a heated lid, it is necessary to overlay the PCR reaction with wax to prevent evaporation. See Appendix B for guidelines.

1. **ADD** 20 µL D1S80 primer mix, 5 µL extracted DNA (or control DNA) and one PCR EdvoBead to a labeled 0.2 ml or 0.5 ml PCR tube (depending on the Thermal Cycler).
2. **MIX** the PCR sample. Make sure the PCR EdvoBead is completely dissolved.
3. **CENTRIFUGE** to collect the sample at the bottom of the tube.
4. **AMPLIFY** DNA using PCR:

   PCR cycling conditions:
   Initial denaturation 94°C for 4 minutes
   94°C for 30 seconds
   65°C for 30 seconds } 35 cycles
   72°C for 30 seconds
   Final Extension 72°C for 4 minutes

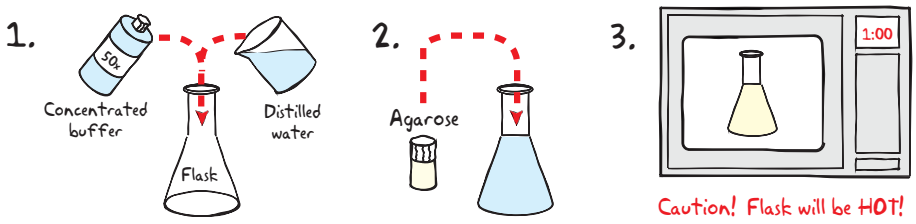
5. **ADD** 5 µL 10x gel loading solution to each tube. **PROCEED** to Module III: Electrophoresis of PCR product.

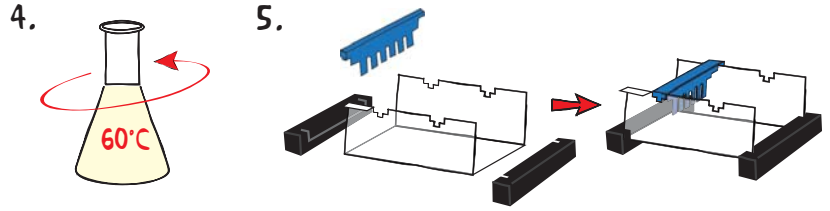
OPTIONAL STOPPING POINT

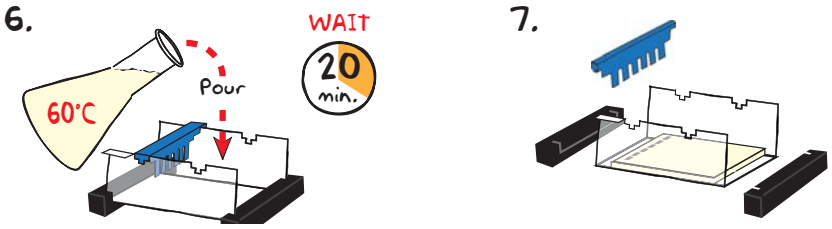
The PCR samples may be stored at -20°C for electrophoresis at a later time.

Module III: Separation of PCR Reaction Products by Electrophoresis

Wear gloves and safety goggles







NOTES:

7 x 14 cm gels are recommended. Each gel can be shared by 4 students. Place well-former template (comb) in the first set of notches.

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

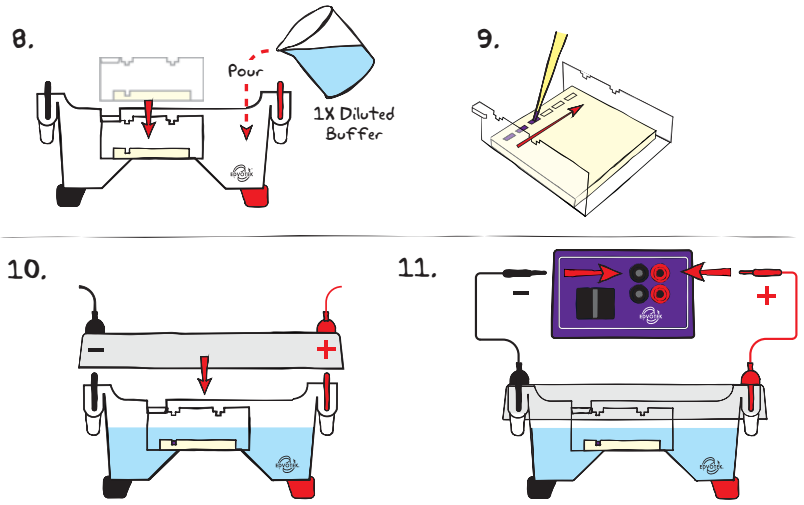
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table B, page 14).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — 1x Electrophoresis Buffer (Chamber Buffer)

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

The Experiment

Module III: Separation of PCR Reaction Products by Electrophoresis



Reminder:

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

Wear gloves and safety goggles

8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **LOAD** the entire sample (30 µL) into the well. **RECORD** the position of the samples in Table 1, below.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING** the agarose gel.

Table 1

Lane	Recommended	Sample Name
1	200 bp ladder	
2	Control DNA*	
3	Student #1	
4	Student #2	
5	Student #3	
6	Student #4	

** Optional, or additional student sample.*

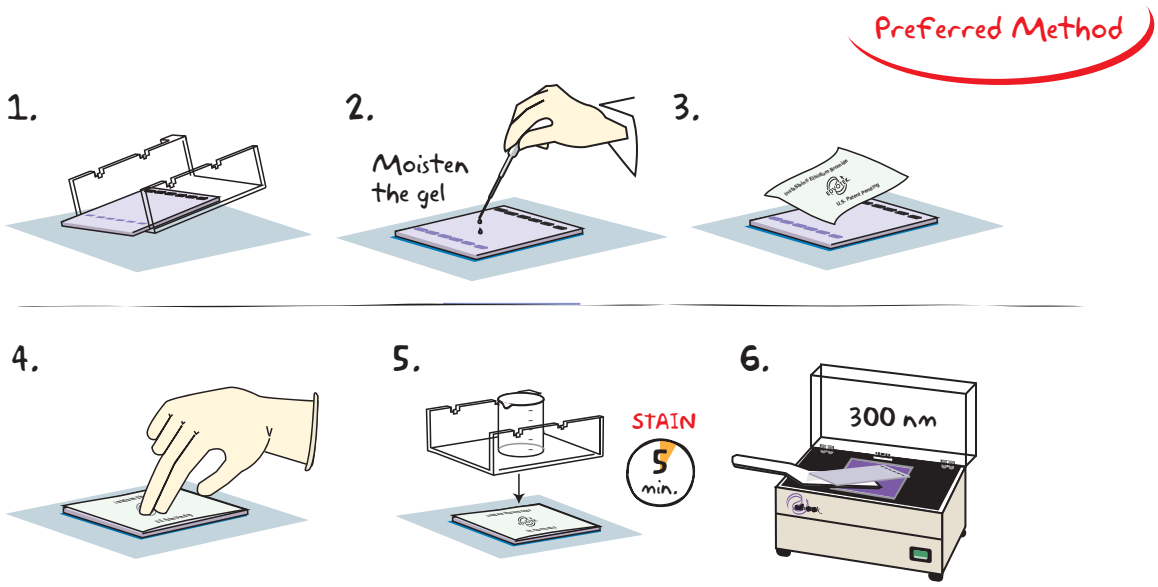
Table B — Individual 1.5% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.5 ml	24.5 ml	0.38 g	25 ml
7 x 14 cm	1.0 ml	49.0 ml	0.75 g	50 ml

Table C — Time and Voltage Guidelines (1.5% - 7 x 14 cm Agarose Gel)

Volts	Recommended Time Minimum	Maximum
125	55 min.	1 hour 15 min.
70	2 hours 15 min.	3 hours
50	3 hours 25 min.	5 hours

Module IV-A: Staining Agarose Gels using InstaStain® Ethidium Bromide



WEAR GLOVES AND GOGGLES WHEN USING THIS PRODUCT.

1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface.

   DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.

2. **MOISTEN** the gel with a few drops of electrophoresis buffer.

3. Wearing gloves, **REMOVE** and **DISCARD** the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). **PLACE** the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. You will need 2 cards to stain a 7 x 14 cm gel.

4. With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.

5. **PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. **STAIN** the gel for 3-5 min. for an 0.8% gel or 8-10 min. for a gel 1.0% or greater.

6. **REMOVE** the InstaStain® Ethidium Bromide card(s). **VISUALIZE** the gel using a long wavelength ultra-violet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

   BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!

The Experiment

Module IV-B: Staining Agarose Gels using FlashBlue™

1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 mL of water in a flask and **MIX** well.

2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.

3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**

4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.

5. **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Alternate Protocol:

1. **DILUTE** one mL of concentrated FlashBlue™ stain with 149 mL dH_2O.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.

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 the difference between a STR and a VNTR? Which (STR or VNTR) is predominantly used in law enforcement? Why?

Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION:

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity.

Preparation For:	What to do:	When:	Time Required:
Module I: Isolation of DNA from Hair or Cheek Cells	Prepare and aliquot various reagents (Saline, Lysis buffer)	Up to one day before performing the experiment. IMPORTANT: Prepare the Lysis buffer no more than one hour before performing the experiment.	30 min.
	Equilibrate waterbaths at 55 ° C and boiling.	One hour before performing the experiment.	5 min.
Module II: Amplification of Extracted DNA	Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.)	One day to 30 min. before performing the experiment.	30 min.
	Program Thermal Cycler	One hour before performing the experiment.	15 min.
Module III: Separation of PCR Product by Electrophoresis	Prepare diluted TAE buffer	Up to one day before performing the experiment.	45 min.
	Prepare molten agarose and pour gel		
Module IV: Staining	Prepare staining components	The class period or overnight after the class period.	10 min.

Pre-Lab Preparations: Module I

FOR MODULE I-A
Each Student should receive:
- One cup containing 10 ml of saline solution
- One screw-cap tube
- One microcentrifuge tube

Reagents to be Shared by Two Students:
- 300 µl Lysis buffer
- 15% bleach solution

Warning !!

Remind students to only use screw-cap tubes when boiling their DNA samples. The snap-top tubes can potentially pop open and cause injury.

MODULE I-A: ISOLATION OF DNA FROM HUMAN CHEEK CELLS

Preparation of Saline Solution

1. To prepare the saline solution, dissolve all 8 salt packets in 500 ml of drinking water. Cap and invert bottle to mix.
2. Aliquot 10 ml of saline solution per cup. Distribute one cup per student.

Preparation of Lysis Buffer
(Prepared no more than one hour before starting the experiment.)

1. Add 100 µl of TE buffer (E) to the tube of Proteinase K (F) and allow the sample to hydrate for several minutes. After the sample is hydrated, pipet up and down several times to thoroughly mix the material.
2. Transfer the entire amount of the rehydrated Proteinase K solution to a 15 ml conical tube containing an additional 4 ml of TE buffer (E).
3. Invert the tube several times to mix. Label this tube "Lysis Buffer".
4. Aliquot 300 µl of Lysis Buffer into 13 labeled microcentrifuge tubes.
5. Distribute one tube of "Lysis Buffer" to each student pair.

FOR MODULE I-B
Each Student should receive:
- One screw-cap tube
- One microcentrifuge tube

Reagents to be Shared by Two Students:
- 300 µl Lysis buffer

MODULE I-B: ISOLATION OF DNA FROM HUMAN HAIR

Preparation of Lysis Buffer
(Prepared no more than one hour before starting the experiment)

1. Add 100 µl of TE buffer (E) to the tube of Proteinase K (F) and allow the sample to hydrate for several minutes. After the sample is hydrated, pipet up and down several times to thoroughly mix the material.
2. Transfer the entire amount of the rehydrated Proteinase K solution to a 15 ml conical tube containing an additional 4 ml of TE buffer (E).
3. Invert the tube several times to mix. Label this tube "Lysis Buffer".
4. Aliquot 300 µl of Lysis Buffer into 13 labeled microcentrifuge tubes.
5. Distribute one tube of "Lysis Buffer" to each student pair.

Pre-Lab Preparations - Module II: Amplification of the Extracted DNA

FOR MODULE II
Each Student should receive:
- One PCR tube and PCR EdvoBead™
- 20 µl Gel Loading Solution

Reagents to be Shared by Two Students:
- 50 µl D1S80 Primer Solution

Preparation of the D1S80 Primer

1. Thaw the D1S80 Primer Mix Concentrate (B) on ice.
2. Add 1 ml of TE Buffer (E) to the tube of Primer Mix Concentrate (B). Cap tube and mix.
3. Aliquot 50 µl of the diluted Primer Mix into 13 labeled microcentrifuge tubes.
4. Distribute one tube of diluted D1S80 Primer to each student pair.

Preparation of the Control DNA

1. This kit includes enough DNA to set up 4 control reactions. At least one control reaction should be performed per class to confirm that PCR was successful.
2. Thaw the tube of Control DNA Concentrate (D) on ice.
3. Add 20 µl of TE buffer (E) to the tube containing Control DNA Concentrate. Pipet up and down to mix.
4. Dispense 8 µl of the diluted control DNA for each control reaction.

Additional Materials

- Dispense 20 µl of 10x Gel Loading Solution to each student pair.

Programming the Thermal Cycler

The Thermal cycler should be programmed as outlined in Module II in the Student's Experimental Procedure.

- Accurate temperatures and cycle times are critical. A pre-run for one cycle (takes approximately 3 to 5 min.) is recommended to check that the thermal cycler is properly programmed.
- For thermal cyclers that do not have a heated lid, it is necessary to place a layer of wax above the PCR reactions in the microcentrifuge tubes to prevent evaporation. See Appendix B for instructions.

Pre-Lab Preparations - Module III: Separation of PCR Product by Electrophoresis

NOTE:
Accurate pipetting is critical for maximizing successful experiment results. EDVOTEK Series 300 experiments are designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students are unfamiliar with using micropipets, we recommended performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this advanced level experiment.

FOR MODULE III
Each Student Group should receive:
- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™ Powder
- Tube of 200 bp ladder (30 µl)
- Control PCR reaction (optional)

PREPARATION OF AGAROSE GELS

This experiment requires one 1.5% agarose gel per student group. A 7 x 14 cm gel is recommended. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Individual Gel Preparation:
Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module III in the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

Batch Gel Preparation:
To save time, a larger quantity of agarose solution can be prepared for sharing by the class. See Appendix C.

Preparing Gels in Advance:

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

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

Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

Additional Materials:
Each 1.5% gel should be loaded with the 200 base pair ladder and samples from 4 or 5 students. The control PCR reaction can also be loaded in one of the wells.

- Aliquot 30 µl of the 200 base-pair ladder (C) into labeled microcentrifuge tubes and distribute one tube of ladder per gel.

Pre-Lab Preparations - Module IV: Staining

Wear gloves and safety goggles

FOR MODULE IV
Each Student Group should receive:
- 2 InstaStain® cards per 7 x 14 cm gel

STAINING WITH INSTASTAIN® ETHIDIUM BROMIDE

Preferred Method

InstaStain® Ethidium Bromide provides the sensitivity of ethidium bromide while minimizing the volume of liquid waste generated by staining and destaining a gel. An agarose gel stained with InstaStain® Ethidium Bromide is ready for visualization in as little as 3 minutes! Each InstaStain® card will stain 49 cm^2 of gel (7 x 7 cm). You will need 2 cards to stain a 7 x 14 cm gel.

Use a mid-range ultraviolet transilluminator (Cat. #558) to visualize gels stained with InstaStain® Ethidium Bromide. **BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!**

- Standard DNA markers should be visible after staining even if other DNA samples are faint or absent. If bands appear faint, repeat staining with a fresh InstaStain card for an additional 3-5 min. If markers are not visible, troubleshoot for problems with electrophoretic separation.
- Ethidium bromide is a listed mutagen. Wear gloves and protective eyewear when using this product. UV protective eyewear is required for visualization with a UV transilluminator.
- InstaStain® Ethidium Bromide cards and stained gels should be discarded using institutional guidelines for solid chemical waste.

STAINING WITH FLASHBLUE™

FlashBlue™ can be used as an alternative to Ethidium Bromide in this experiment. However, FlashBlue™ is less sensitive than InstaStain® Ethidium Bromide and will take a longer time to obtain results.

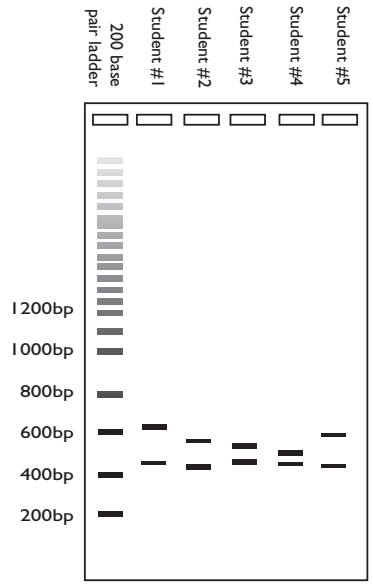
FlashBlue™ stain, however, is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to "equilibrate" in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

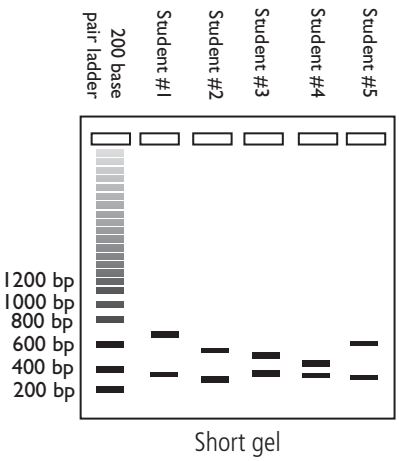
PHOTODOCUMENTATION OF DNA (OPTIONAL)

Once gels are stained, you may wish to photograph your results. 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.

Experiment Results and Analysis



Long Gel



Short gel

Idealized Schematics

The idealized schematics show a few of the possible PCR products from different genotypes.

Students' PCR products should show one or two bands with lengths between 360 and 800 base pairs.

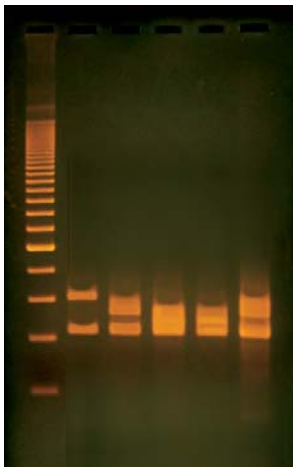


Photo of Gel Results

Note:
Depending on the PCR conditions used, a diffuse, small-molecular weight band, known as a "primer dimer", may be present below the 200 bp marker. This is a PCR artifact and can be ignored. Other minor bands may also appear due to nonspecific primer binding and the subsequent amplification of these sequences.

Instructor's Guide

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

Appendices

A EDVOTEK® Troubleshooting Guide

B Preparation and Handling of PCR Samples With Wax

C Bulk Preparation of Agarose Gels

Material Safety Data Sheets:

Now available for your convenient download on www.edvotek.com.

EDVO-Kit # 334

Appendix A

EDVOTEK® Troubleshooting Guides

DNA EXTRACTION

PROBLEM:	CAUSE:	ANSWER:
There is no cell pellet after centrifuging the cheek cell suspension.	Not enough cheek cells in suspension	Mouth must be vigorously rinsed for at least 60 sec. to harvest loose cheek cells.
	Sample not centrifuged fast enough	Spin cells at maximum speed (17,000 x g) for 2 min. If your centrifuge does not reach this speed, spin at highest available speed for 4 min.
I was not able to extract DNA from hair.	Not enough hairs used for extraction	Use at least five hairs for the DNA extraction.
	No follicle was present on hair shaft	The best place to collect hairs for this experiment is the head. Pick hair follicles which have a bulbous base (sheath cells).
Poor DNA Extraction	Samples not mixed well enough during extraction	In addition to flicking the tube, vortex or pipet up and down to mix the sample.
	Proteinase K inactive because it was prepared too far in advance.	Prepare Proteinase K within one hour of use.
	Water baths not at proper temperature	Use a thermometer to confirm water bath set point.
	Not enough DNA	Try cheek cell extraction. Final DNA concentrations are usually higher.
The extracted DNA is very cloudy.	Cellular debris from pellet transferred to tube	Centrifuge sample again and move supernatant to a fresh tube. Take care to avoid pellet.
	Cellular debris not separated from supernatant	Centrifuge sample again. If possible, centrifuge at a higher speed. Move cleared supernatant to a fresh tube.

Appendix A

EDVO-Kit # 334

PCR AND ELECTROPHORESIS

PROBLEM:	CAUSE:	ANSWER:
There is very little liquid left in tube after PCR	Sample has evaporated	Make sure the heated lid reaches the appropriate temperature.
		If your thermal cycler does not have a heated lid, overlay the PCR reaction with wax (see Appendix B for details)
		Make sure students close the lid of the PCR tube properly.
	Pipetting error	Make sure students pipet 20 µL primer mix and 5 µL extracted DNA into the 0.2 mL tube.
The ladder, control DNA, and student PCR products are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
		Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels.
	The gel was not stained properly.	Repeat staining.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
After staining, the ladder is visible but no PCR products are present.	PCR amplification was unsuccessful.	Repeat PCR with fresh PCR EdvoBeads™ and primers.
		Ensure that the thermal cycler has been properly programmed. See Module II for guidelines
After staining, the ladder and control PCR products are visible on gel, but some student samples are not present.	Student DNA sample was not concentrated enough.	Poor DNA extraction. Extract new DNA. Cheek cell extraction usually results in higher DNA yield.
	Student DNA sample was degraded	If DNA is not used immediately following extraction, store sample at -20°C.
	Wrong volumes of DNA and primer added to PCR reaction	Practice using pipettes
Some students have more or less amplification than others.	Concentration of DNA varies by sample.	There is an inherent variability in the extraction process. For best results, use cheek cell extraction.
Low molecular weight band in PCR samples	Primer dimer	Low concentration of extracted DNA in PCR reaction.
DNA bands were not resolved.	Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time	Re-stain the gel with FlashBlue™

Appendix B

Preparation and Handling of PCR Samples With Wax

ONLY For Thermal Cyclers WITHOUT Heated Lids, or Manual PCR Using Three Waterbaths

Using a wax overlay on reaction components prevents evaporation during the PCR process.

HOW TO PREPARE A WAX OVERLAY

1. Add PCR components to the 0.2 ml PCR Tube as outlined in Module II.
2. Centrifuge at full speed for five seconds to collect sample at bottom of the tube.
3. Using clean forceps, add one wax bead to the PCR tube.
4. Place samples in PCR machine and proceed with Module II.

PREPARING PCR SAMPLES FOR ELECTROPHORESIS

1. After PCR is completed, melt the wax overlay by heating the sample at 94° C for three minutes or until the wax melts.
2. Using a clean pipette, remove as much overlay wax as possible.
3. Allow the remaining wax to solidify.
4. Use a pipette tip to puncture the thin layer of remaining wax. Using a fresh pipette tip, remove the PCR product and transfer to a new tube.
5. Add 5 µL of 10x Gel Loading Buffer to the sample. Proceed to Module III to perform electrophoresis.

Appendix C

EDVO-Kit # 334

Bulk Preparation of Agarose Gels

To save time, 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 can be remelted.

BULK ELECTROPHORESIS BUFFER

Bulk preparation of 1X electrophoresis buffer is outlined in Table D.

Table D: Bulk Preparation of Electrophoresis Buffer

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

BATCH AGAROSE GELS (1.5%)

Bulk preparation of 1.5% agarose gel is outlined in Table E.

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour the appropriate amount 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.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Proceed with electrophoresis (Module II) or store the gels at 4° C under buffer.

Note:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

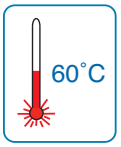


Table E: Batch Preparation of 1.5% UltraSpec-Agarose™

Amt of Agarose	+	50x Conc. Buffer	+	Distilled Water	=	Diluted Buffer (1x)
4.5 g		6.0 ml		294 ml		300 ml
6.0 g		8.0 ml		392 ml		400 ml