Edvo-Kit #334

VNTR Human DNA Typing Using PCR

Experiment Objective:

In this experiment, students will extract genomic DNA from cheek cells. The Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis will be used to analyze polymorphisms between individuals at the D1S80 region of chromosome 1.

See page 3 for storage instructions.

IMPORTANT NOTE:
The PCR cycling conditions and electrophoresis buffer have changed. Please review the literature before performing the experiment.
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Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets
### Experiment Components

<table>
<thead>
<tr>
<th>Components</th>
<th>Storage</th>
<th>Check (√)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A PCR EdvoBeads™</td>
<td>Room Temp.</td>
<td></td>
</tr>
<tr>
<td>Each PCR EdvoBead™ contains:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• dNTP Mixture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Taq DNA Polymerase Buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Taq DNA Polymerase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• MgCl₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Reaction Buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B D1S80 Primer Mix concentrate</td>
<td>-20°C Freezer</td>
<td></td>
</tr>
<tr>
<td>C EdvoQuick™ DNA ladder</td>
<td>-20°C Freezer</td>
<td></td>
</tr>
<tr>
<td>D Control DNA concentrate</td>
<td>-20°C Freezer</td>
<td></td>
</tr>
<tr>
<td>E TE Buffer</td>
<td>-20°C Freezer</td>
<td></td>
</tr>
<tr>
<td>F Proteinase K</td>
<td>-20°C Freezer</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Components B and D are supplied in concentrated form and require dilution prior to setting up PCR reactions.

### REAGENTS & SUPPLIES

*Store all components below at room temperature.*

<table>
<thead>
<tr>
<th>Component</th>
<th>Check (√)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• UltraSpec-Agarose™</td>
<td></td>
</tr>
<tr>
<td>• TBE Electrophoresis Buffer Powder</td>
<td></td>
</tr>
<tr>
<td>• 10x Gel loading solution</td>
<td></td>
</tr>
<tr>
<td>• InstaStain® Ethidium Bromide</td>
<td></td>
</tr>
<tr>
<td>• Snap-top microcentrifuge tubes</td>
<td></td>
</tr>
<tr>
<td>• Screw-top microcentrifuge tubes (Use for boiling)</td>
<td></td>
</tr>
<tr>
<td>• 0.2 ml PCR tubes</td>
<td></td>
</tr>
<tr>
<td>• Disposable plastic cups</td>
<td></td>
</tr>
<tr>
<td>• Salt packets</td>
<td></td>
</tr>
<tr>
<td>• 15 ml Conical tube</td>
<td></td>
</tr>
<tr>
<td>• Wax beads (for thermal cyclers without heated lid)</td>
<td></td>
</tr>
</tbody>
</table>

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Requirements

- Thermal cycler (EDVOTEK® Cat. # 541 highly recommended)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- Two Waterbaths for 55°C and 99°C incubations (EDVOTEK® Cat. #539 highly recommended)
- UV Transilluminator or UV Photodocumentation system
- UV safety goggles
- Automatic micropipets (5-50 µl) 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
- Drinking Water
- 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 electro-

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.
phoresis, 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.

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
Figure 3: Polymerase Chain Reaction
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 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
EXPERIMENT OBJECTIVE:
In this experiment, students will extract genomic DNA from cheek cells. The Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis will be used to analyze polymorphisms between individuals at the D1S80 region of chromosome 1.

LABORATORY SAFETY:
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.

LABORATORY NOTEBOOKS:
Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you’ll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:
• Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
• Predict the results of your experiment.

During the Experiment:
• Record your observations.

After the Experiment:
• Interpret the results – does your data support or contradict your hypothesis?
• If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

NOTE: Experimental times are approximate.
Module I: Isolation of DNA from Human Cheek Cells

1. **LABEL** a 1.5 ml screw top microcentrifuge tube and a cup with your lab group and/or initials.
   
   **NOTE:** Saline solution **MUST** be used for cheek cell wash. Sports drinks will inhibit amplification of DNA by Polymerase Chain Reaction in Module II.

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 minutes at full speed to pellet the cells. **POUR** off the supernatant, but **DO NOT DISTURB THE CELL PELLET**! **REPEAT** steps 3 and 4 once 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 5 minutes.

7. **MIX** the sample by vortexing or by flicking the tube vigorously for 20 seconds.

8. **INCUBATE** the sample in a 99°C waterbath for 5 minutes. **NOTE:** Students **MUST** use screw-cap tubes **when boiling DNA isolation samples**.

9. **CENTRIFUGE** the cellular lysate for 2 minutes at full speed.

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.

**OPTIONAL STOPPING POINT:**
The extracted DNA may be stored at -20°C for amplification at a later time.
Module II: Amplification of the D1S80 Locus

1. **LABEL** a 0.2 ml PCR tube with your initials.

2. **ADD** 20 µl D1S80 primer mix, 5 µl extracted DNA (or control DNA) and one PCR EdvoBead™ to the labeled 0.2 ml tube. At least one control reaction should be performed per class to confirm that PCR was successful.

3. **MIX** the PCR sample. Make sure the PCR EdvoBead™ is completely dissolved.

4. **CENTRIFUGE** the sample for a few seconds to collect the sample at the bottom of the tube.

5. **AMPLIFY** DNA using PCR

   **PCR cycling conditions:**
   - Initial denaturation 94° C for 4 minutes
   - 94° C for 30 seconds
   - 65° C for 30 seconds
   - 72° C for 30 seconds
   - Final Extension 72° C for 4 minutes

   30 cycles

6. After PCR, **ADD** 5 µl of 10x Gel Loading Solution to the sample. **PLACE** tubes on ice. **PROCEED** to Module III: Separation of PCR Products by Electrophoresis.

**NOTE:**
The PCR cycling conditions and electrophoresis buffer have changed. Please review the literature before performing the experiment.

**NOTES AND REMINDERS:**

This kit includes enough DNA for 5 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.

**OPTIONAL STOPPING POINT:**
The PCR samples may be stored at -20° C for electrophoresis at a later time.
Module III: Separation of PCR Products by Electrophoresis

1. MIX agarose powder with 1X TBE Electrophoresis Buffer in a 250 ml flask (see Table A).
2. 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).
3. COOL agarose to 60°C with careful swirling to promote even dissipation of heat.
4. 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.
5. 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.
6. REMOVE end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

IMPORTANT:
For this experiment, 7x7 cm gels are recommended. Each gel can be shared by up to 6 students. Place well-former templates (combs) 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

Table A

<table>
<thead>
<tr>
<th>Individual 2.0% UltraSpec-Agarose™ Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of Gel Casting Tray</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>7x7 cm</td>
</tr>
<tr>
<td>7x14 cm</td>
</tr>
</tbody>
</table>
## Module III: Separation of PCR Products by Electrophoresis

7. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X TBE Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.

8. **LOAD** the entire sample into the well. **RECORD** the position of the samples in Table 1, below.

9. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.

10. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). For best results, the blue tracking dye should migrate at least 4 cm from the wells.

11. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING** the agarose gel.

### Table 1: Sample Table

<table>
<thead>
<tr>
<th>Lane</th>
<th>Recommended</th>
<th>Sample Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EdvoQuick™ DNA Ladder</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control DNA*</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Student #1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Student #2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Student #3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Student #4</td>
<td></td>
</tr>
</tbody>
</table>

* Optional, or additional student sample.

### Table B: 1x TBE Electrophoresis Buffer

<table>
<thead>
<tr>
<th>EDVOTEK Model #</th>
<th>Total Volume Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+</td>
<td>300 ml</td>
</tr>
<tr>
<td>M12</td>
<td>400 ml</td>
</tr>
<tr>
<td>M36</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

### Table C: Time & Voltage Guidelines

<table>
<thead>
<tr>
<th>Volts</th>
<th>Time: 7 x 7 cm gel ~4.0 cm migration</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>75 min.</td>
</tr>
<tr>
<td>125</td>
<td>40 min.</td>
</tr>
<tr>
<td>150</td>
<td>30 min.</td>
</tr>
</tbody>
</table>

**Reminder:** Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.
Module IV: Staining with InstaStain® Ethidium Bromide

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 at least 3-5 minutes. For best results, the gel should be stained for 15 minutes.
6. REMOVE the InstaStain® Ethidium Bromide card(s). VISUALIZE the gel using a mid-range ultraviolet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!
Study Questions

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?
OVERVIEW OF INSTRUCTOR’S PRELAB PREPARATION:

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

<table>
<thead>
<tr>
<th>Preparation For:</th>
<th>What to do:</th>
<th>When:</th>
<th>Time Required:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Module I:</strong> Isolation of DNA from Cheek Cells</td>
<td>Prepare and aliquot various reagents (Saline, Lysis buffer)</td>
<td>Up to one day before performing the experiment. IMPORTANT: Prepare the Lysis buffer no more than one hour before performing the experiment.</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>Equilibrate waterbaths at 55 °C and boiling.</td>
<td>At least 30 min. before performing the experiment.</td>
<td>5 min.</td>
</tr>
<tr>
<td><strong>Module II:</strong> Amplification of the D1S80 Locus</td>
<td>Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.)</td>
<td>One day to 30 min. before performing the experiment.</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>Program Thermal Cycler</td>
<td>Anytime before performing the experiment.</td>
<td>15 min.</td>
</tr>
<tr>
<td><strong>Module III:</strong> Separation of PCR Products by Electrophoresis</td>
<td>Prepare diluted TBE Buffer</td>
<td>Up to one hour before performing the experiment.</td>
<td>45 min.</td>
</tr>
<tr>
<td></td>
<td>Prepare molten agarose and pour gel</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Module IV:</strong> Staining</td>
<td>Prepare staining components</td>
<td>Up to 10 min. before the class period.</td>
<td>5 min.</td>
</tr>
</tbody>
</table>

**NOTE:**
The PCR cycling conditions and electrophoresis buffer have changed. Please review the literature before performing the experiment.
Pre-Lab Preparations

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

*NOTE: Saline solution *MUST* be used for cheek cell wash. *Sports drinks will inhibit amplification of DNA by Polymerase Chain Reaction in Module II. If you have used sports drinks for the cheek cell wash, please DISCARD the samples and REPEAT the DNA extraction with saline solution.*

**DISINFECTING LABORATORY MATERIALS:** Contaminated laboratory waste (saliva solution, cup, pipet, etc.) must be disinfected with 15% bleach solution prior to disposal. Be sure to properly dispose of any biological samples according to your institutional guidelines.

**Prepare the Saline Solution:**

1. To prepare the saline solution, dissolve all 8 salt packets (~4 g) 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.

**Prepare the Lysis Buffer:**

*(Prepared no more than 30 min. 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”. At this point, the Lysis Buffer can no longer be stored. It should be used as soon as possible.

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**

Each Group 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.
Pre-Lab Preparations

MODULE II: AMPLIFICATION THE D1S80 LOCUS

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 D1S80 Primer Mix Concentrate. Cap tube and mix.
3. Aliquot 50 µl of the diluted D1S80 Primer Mix into 13 labeled microcentrifuge tubes.
4. Distribute one tube of diluted D1S80 Primer Mix to each student pair.

Preparation of the Control DNA

1. Thaw the tube of Control DNA Concentrate (D) on ice.
2. Add 20 µl of TE Buffer (E) to the tube containing the Control DNA Concentrate. Pipet up and down to mix.
3. Dispense 6 µl of the diluted Control DNA for each control reaction. At least one control reaction should be performed per class to confirm that the PCR was successful.

Additional Materials

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

PCR Amplification

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.

NOTE:
The PCR cycling conditions and electrophoresis buffer have changed. Please review the literature before performing the experiment.
Pre-Lab Preparations

MODULE III: SEPARATION OF PCR PRODUCTS BY ELECTROPHORESIS

Preparation of Agarose Gels:
This experiment requires one 2.0% agarose gel per 4 or 5 students. A 7 x 7 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.

Preparation of TBE Electrophoresis Buffer
For this experiment, we recommend preparing the 1X TBE Electrophoresis Buffer in bulk for sharing by the class. Unused diluted buffer can be used at a later time.

1. Measure 3.7 L of distilled or deionized water and place in a large vessel. \textit{(NOTE: If using purchased water in a gallon jug, remove and discard 80 mL water.)}
2. Add the entire amount of TBE Electrophoresis Buffer powder to the vessel and mix well.
3. Label the vessel as "1X TBE Electrophoresis Buffer".
4. Use within 60 days of preparation.

Individual Gel Preparation
Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module VI in the Student’s Experimental Procedure. Students will need diluted 1x TBE Electrophoresis Buffer 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 stored under buffer in the refrigerator for up to 2 weeks.

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

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

Additional Materials:
- Aliquot 30 µl of the EdvoQuick™ DNA ladder (C) into labeled microcentrifuge tubes and distribute one tube of EdvoQuick™ DNA ladder per gel.
Pre-Lab Preparations

MODULE IV: STAINING

InstaStain® Ethidium Bromide

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 cm² of gel (7 x 7 cm).

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.

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.

SYBR® Safe DNA Staining (Optional)

This fast, easy staining protocol incorporates SYBR® Safe into the molten agarose before the gel is poured into the casting tray. This means that the DNA is staining while the electrophoresis experiment is running!

Results can be visualized immediately post electrophoresis with a mid-range ultraviolet transilluminator (Cat. #558) or a TruBlu™ Blue Light Transilluminator (Cat #557).

Find more information on SYBR® Safe DNA stain at www.edvotek.com/608.
Experiment Results and Analysis

The results photo shows an example of the possible PCR products from different genotypes. Students' PCR products should show one or two bands with lengths between approx. 400 and 785 base pairs. The Control DNA will have bands at approx. 420 bp and 600 bp.

**D1S80 LOCUS**

145 bp are constant between samples: 23 bp (Forward primer) 23 bp (Reverse primer) 99 bp (Flanks VNTR)

256 bp - 640bp are variable between samples, depending on VNTR number: 16 bp per repeat 16 to 40 repeats present in the variable region 16 repeats times 16 bp per repeat equals 256 bp 40 repeats times 16 bp per repeat equals 640 bp

**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.

Includes EDVOTEK’s All-NEW EdvoQuick™ DNA Ladder

- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes: 2640, 1400, 1100, 700, 600, 400, 200
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

Safety Data Sheets can be found on our website:

www.edvotek.com/safety-data-sheets
# Appendix A

**EDVOTEK® Troubleshooting Guides**

## DNA EXTRACTION

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>There is no cell pellet after centrifuging the cheek cell suspension.</td>
<td>Not enough cheek cells in suspension</td>
<td>Mouth must be vigorously rinsed for at least 60 sec. to harvest loose cheek cells.</td>
</tr>
<tr>
<td></td>
<td>Sample not centrifuged fast enough</td>
<td>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.</td>
</tr>
<tr>
<td>Poor DNA Extraction</td>
<td>Samples not mixed well enough during extraction</td>
<td>In addition to flicking the tube, vortex or pipet up and down to mix the sample.</td>
</tr>
<tr>
<td></td>
<td>Proteinase K inactive because it was prepared too far in advance.</td>
<td>Prepare Proteinase K within one hour of use.</td>
</tr>
<tr>
<td></td>
<td>Water baths not at proper temperature</td>
<td>Use a thermometer to confirm water bath set point.</td>
</tr>
<tr>
<td></td>
<td>Not enough DNA</td>
<td>Repeat cheek cell extraction.</td>
</tr>
<tr>
<td></td>
<td>Sports drink was used for DNA extraction.</td>
<td>Repeat DNA extraction with saline solution.</td>
</tr>
<tr>
<td>The extracted DNA is very cloudy.</td>
<td>Cellular debris from pellet transferred to tube</td>
<td>Centrifuge sample again and move supernatant to a fresh tube. Take care to avoid pellet.</td>
</tr>
<tr>
<td></td>
<td>Cellular debris not separated from supernatant</td>
<td>Centrifuge sample again. If possible, centrifuge at a higher speed. Move cleared supernatant to a fresh tube.</td>
</tr>
</tbody>
</table>
## Appendix A
### EDVOTEK® Troubleshooting Guides

#### PCR AND ELECTROPHORESIS

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
</table>
| 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 proper buffer was not used for gel preparation. Make sure to use 1x TBE Electrophoresis Buffer. |
| 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. |
| The gel needs to be destained. | | Submerge the gel in distilled or deionized water. Allow the gel to soak for 5 minutes. |
| After staining, the ladder and control PCR products are visible on the gel but some student samples are not present. | Student DNA sample was not concentrated enough. | Poor DNA extraction. Repeat Module I (Isolation of DNA from Human Cheek Cells).  
Student DNA sample was degraded. | If DNA is not used right after extraction, store sample at -20°C. |
| Wrong volumes of DNA and primer added to PCR reaction. | | Practice using micropipets |
| Some student samples have more/less amplification than others. | Concentration of DNA varies by sample. | There is an inherent variability in the extraction process. |
| Low molecular weight band in PCR samples | Primer dimer | Low concentration of extracted DNA in PCR reaction. |
| DNA bands were not resolved. | To ensure adequate separation, make sure the tracking dye migrates at least 3.5 cm on 7 x 7 cm gels and 6 cm on 7 x 14 cm gels. | Be sure to run the gel the appropriate distance before staining and visualizing the DNA. |
Appendix B
Preparation and Handling of PCR Samples with Wax

ONLY For Thermal Cylcers 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

Bulk Preparation of Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

BULK ELECTROPHORESIS BUFFER

For this experiment, we recommend preparing the 1X TBE Electrophoresis Buffer in bulk for sharing by the class. Unused diluted buffer can be used at a later time.

1. Measure 3.7 L of distilled or deionized water and place in a large vessel. (NOTE: If using purchased water in a gallon jug, remove and discard 80 mL water.)

2. Add the entire amount of TBE Electrophoresis Buffer powder to the vessel and mix well.

3. Label the vessel as "1X TBE Electrophoresis Buffer".

4. Use within 60 days of preparation.

BATCH AGAROSE GELS (2.0%)

Bulk preparation of 2.0% agarose gel is outlined in Table E.

1. Measure 250 ml of 1x TBE Electrophoresis Buffer and pour into a 500 ml flask.

2. Pour 5.0 g 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 IV) or store the gels at 4°C under buffer.