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Edvo-Kit # 334

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.

Version 334.240214

SAMPLE LITERATURE
Please refer to included weblink for correct version

PROTOCOL HAS BEEN UPDATED!
Please review before beginning experiment!

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Experiment Components

Component		Storage	Check (✓)
•	PCR EdvoBeads™*	-20°C Freezer	❑
A	Universal DNA Buffer	-20°C Freezer	❑
B	TE Buffer	-20°C Freezer	❑
C	LyphoPrimer™ Mix	-20°C Freezer	❑
D	LyphoControl™ *(Complete PCR Control)*	-20°C Freezer	❑
E	EdvoQuick™ DNA ladder	-20°C Freezer	❑
•	Proteinase K	-20°C Freezer	❑
•	SYBR® Safe Stain	-20°C Freezer	❑

NOTE: Components C and D are supplied in lyophilized form and require rehydration prior to setting up PCR reactions.

**Each PCR EdvoBead™ contains: dNTP Mixture, Taq DNA Polymerase Buffer, Taq DNA Polymerase, and $MgCl_2$.*

This experiment is designed for 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.

NEW Update:
PCR EdvoBeads™ are now conveniently stored at -20°C with the other perishable components.

Reagents & Supplies

Store all components below at room temperature.

Component	Check (✓)
• UltraSpec-Agarose™	❑
• TBE Electrophoresis Buffer Powder	❑
• Snap-top microcentrifuge tubes	❑
• Screw-top microcentrifuge tubes (Use for boiling)	❑
• 0.2 mL PCR tubes	❑
• Disposable plastic cups	❑
• Salt packets	❑
• 15 mL Conical tube	❑

NOTE: Check that your PCR program matches the protocol found in the student and instructor's guides before starting the experiment!

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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Requirements

- Thermal cycler (EDVOTEK® Cat. #540 or #541-542 highly recommended)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- Two Water baths for 55°C and 99°C incubations (EDVOTEK® Cat. #539 highly recommended)
- UV transilluminator or blue light visualization for SYBR® Safe stained gels (EDVOTEK® Cat #557 recommended)
- UV safety goggles (for visualizing SYBR® Safe stained gels)
- Automatic micropipettes (5-50 µL) with tips
- Microwave
- 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

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Background Information

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.

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

STR
2 - 6 bp repeats, repeated three to 100 times.

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.

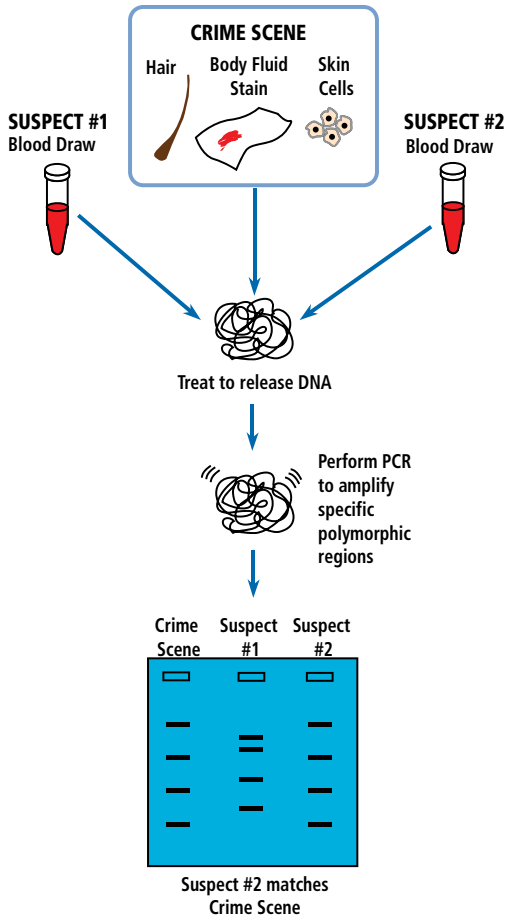

Figure 1:
Extraction and Electrophoresis of DNA Samples.

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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.

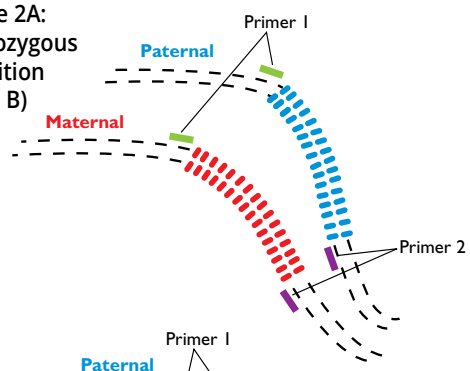
Forensic scientists use PCR to analyze highly polymorphic DNA regions. By examining several different VNTRs or STRs from the same individual, investigators obtain a unique DNA fingerprint for that individual which is unlike that of any other person (except for an identical twin). One VNTR, known as D1S80, 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 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.

Figure 2A:
Homozygous Condition (Lane B)



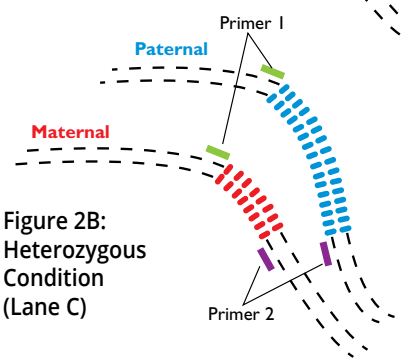
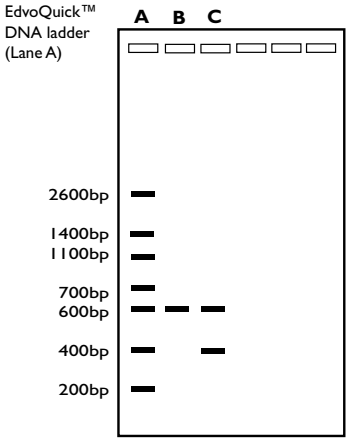


Figure 2B:
Heterozygous Condition (Lane C)



Gel results are not drawn to scale.

Figure 2:
PCR Amplification Products of D1S80

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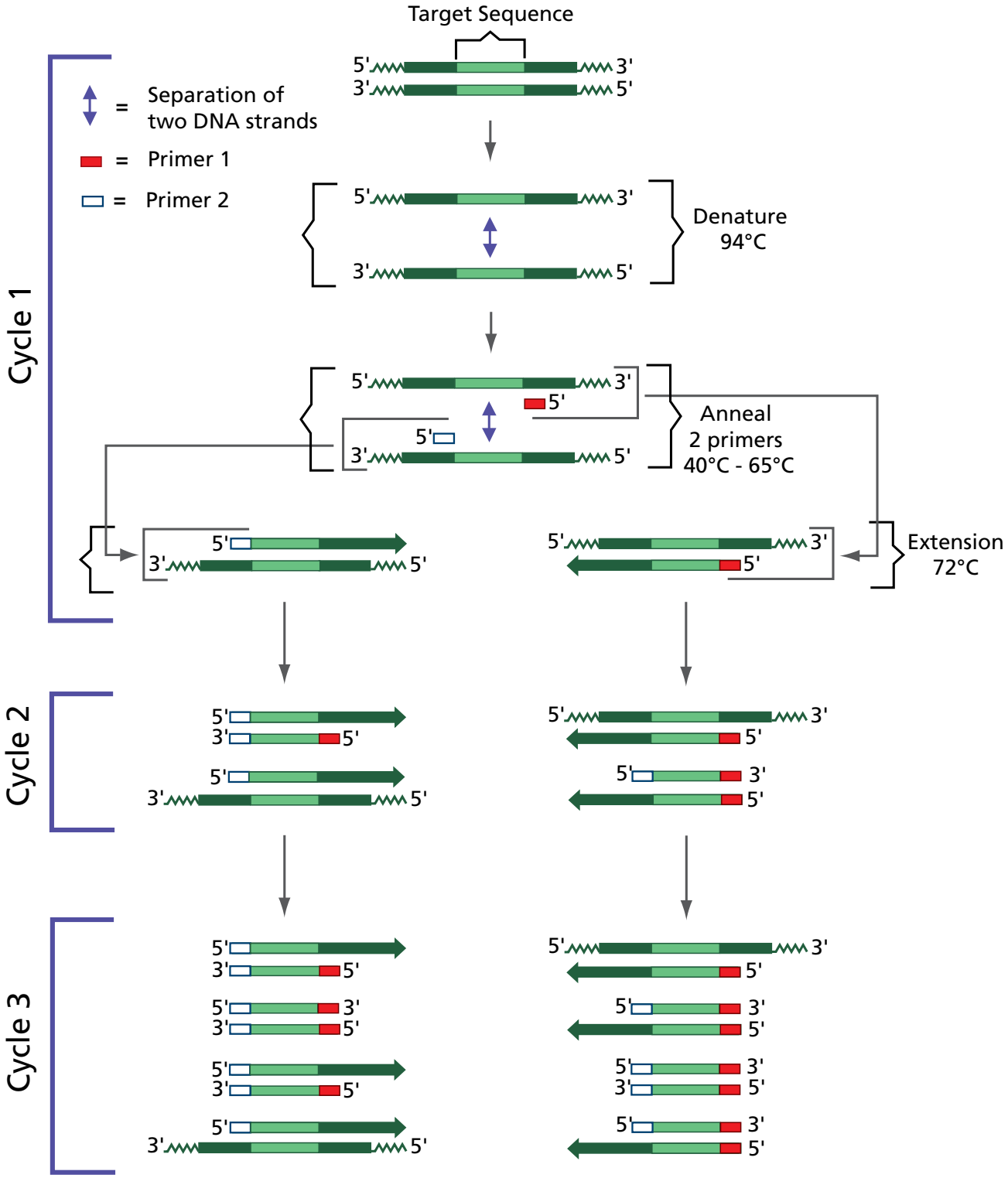

Figure 3:
Polymerase Chain Reaction

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- 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.

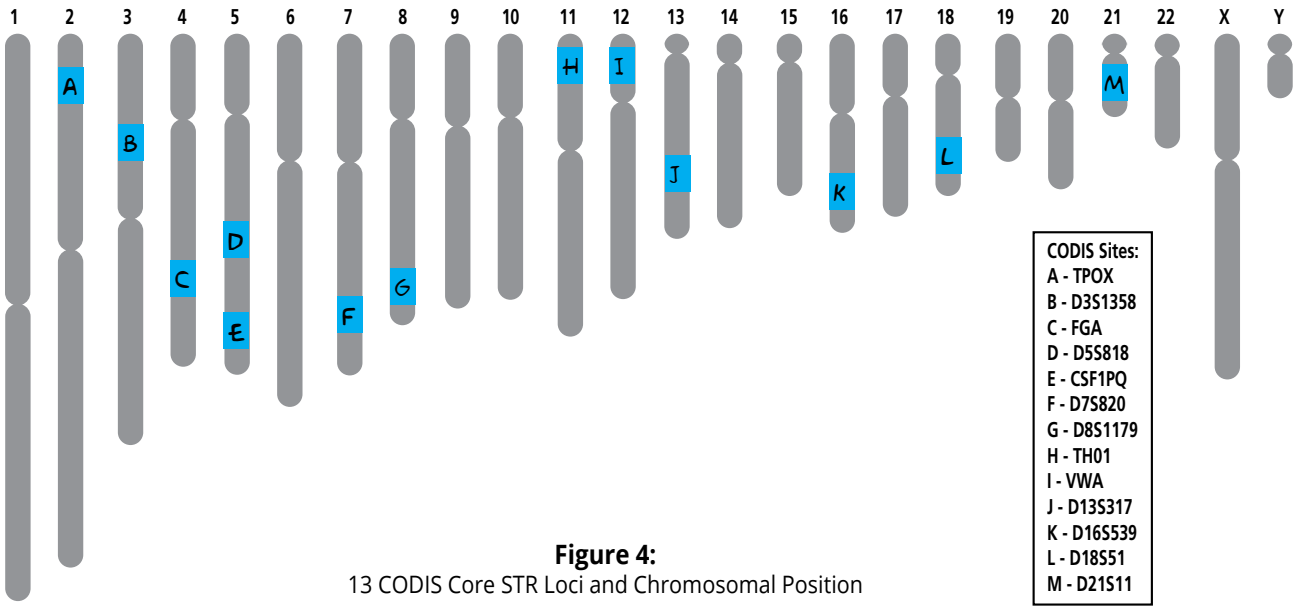

Figure 4:
13 CODIS Core STR Loci and Chromosomal Position

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Experiment Overview

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:

Wear gloves and safety goggles

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, pipette, 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.

Module I: 30 min.
Isolation of DNA from Cheek Cells

↓

Module II: 90 min.
Amplification of the D1S80 Locus

↓

Module III: 50-70 min.
Separation of PCR Products by Electrophoresis

NOTE: Experimental times are approximate.

NOTE: Check that your PCR program matches the protocol found in the student and instructor's guides before starting the experiment!

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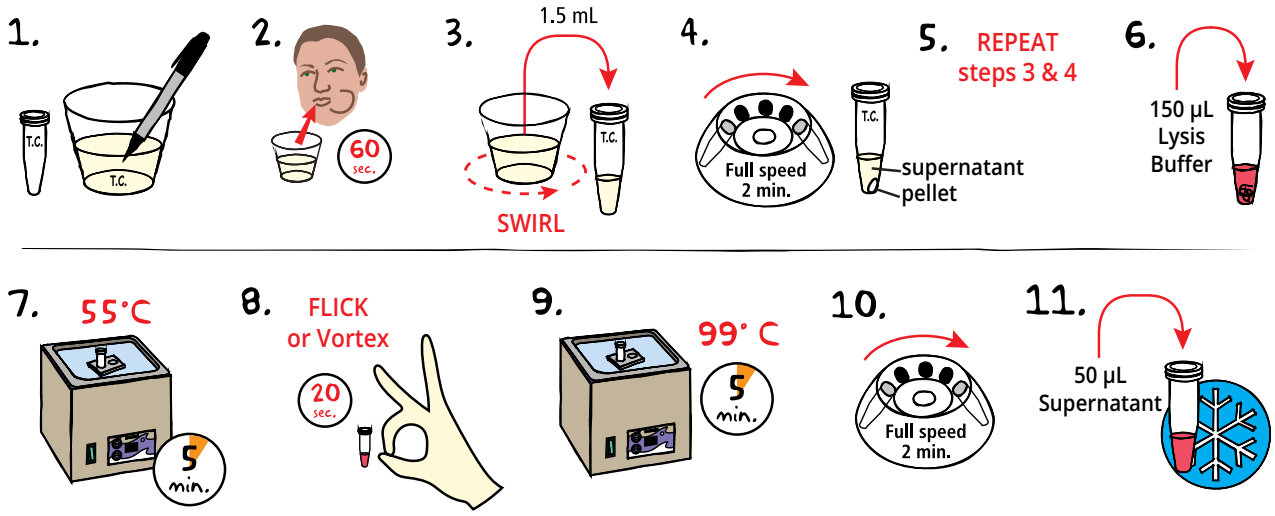
Module I: Isolation of DNA from Human Cheek Cells



1. **LABEL** an empty 1.5 mL screw top microcentrifuge tube and a cup of saline with your lab group and/or initials.
2. **RINSE** your mouth vigorously for 60 seconds using 10 mL saline solution. **EXPEL** the solution back into the same cup.
3. **SWIRL** the cup gently to resuspend the cells. **TRANSFER** 1.5 mL of the cell solution into the tube with your initials.
4. **CENTRIFUGE** the cell suspension for 2 minutes at full speed to pellet the cells. **POUR** off the supernatant, the liquid above the cell pellet, but **DO NOT DISTURB THE CELL PELLET**!
5. **REPEAT** steps 3 and 4 once more.
6. **RESUSPEND** the cheek cell pellet in 150 µL lysis buffer by pipetting up and down or by vortexing vigorously. *NOTE: Ensure that the cell pellet is fully resuspended and that no clumps of cells remain.*
7. **CAP** the tube and **PLACE** it in a water bath float. **INCUBATE** the sample in a 55°C water bath for 5 minutes.
8. **MIX** the sample by vortexing or by flicking the tube vigorously for 20 seconds.
9. **INCUBATE** the sample in a 99°C water bath for 5 minutes. *NOTE: Students MUST use screw-cap tubes when boiling samples.*
10. **CENTRIFUGE** the cellular lysate for 2 minutes at full speed.
11. **TRANSFER** 50 µL of the supernatant to a clean, labeled microcentrifuge tube. **PLACE** the tube in ice.

The extracted DNA is now ready for Module II: Amplification of the D1S80 Locus. Alternatively, the extracted DNA may be stored in the **FREEZER** (-20°C) until needed.

STOP **OPTIONAL STOPPING POINT:**
The extracted DNA may be stored in the freezer (-20°C) until needed.

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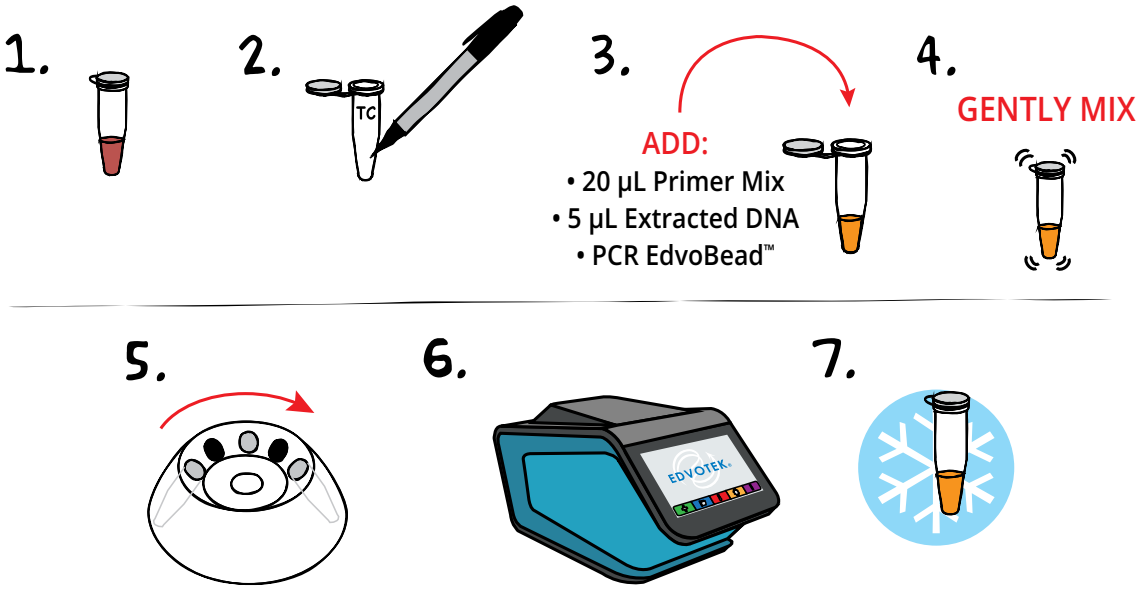
Module II: Amplification of the D1S80 Locus



1. **OBTAIN** the red extracted DNA from Module I.
2. **LABEL** a fresh 0.2 mL PCR tube with your initials.
3. **ADD** 20 µL D1S80 primer mix (yellow), 5 µL extracted DNA (red), and a PCR EdvoBead™.
4. **MIX** the PCR sample. Make sure the PCR EdvoBead™ is completely dissolved. If mixed correctly, the final solution will be light orange.
5. **CENTRIFUGE** the sample for a few seconds to collect the sample at the bottom of the tube.
6. **AMPLIFY** DNA using PCR guidelines listed below.

- Initial denaturation 94°C for 4 minutes.
- 94°C for 30 seconds
- 65°C for 30 seconds
- 72°C for 30 seconds

} 32 cycles

- Final extension at 72°C for 4 minutes.

7. After PCR, **PLACE** the tubes on ice. **PROCEED** to Module III: Separation of PCR Products by Electrophoresis.

NOTE:
The positive control contains primers, template DNA and PCR components, and is ready for PCR amplification.

This kit contains enough Control DNA for 6 reactions. We strongly recommend running all 6 control reactions to ensure the PCR was successful.

STOP

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

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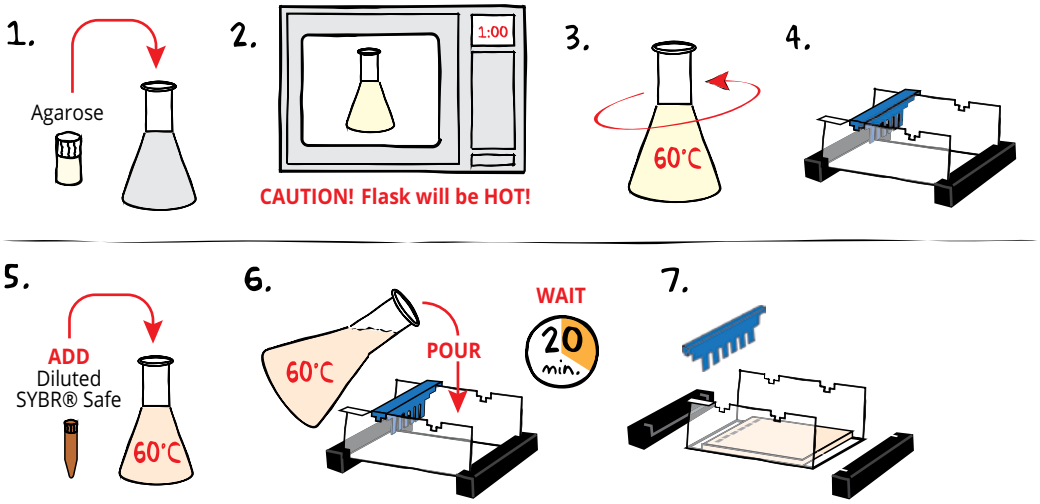
Module III: Separation of PCR Products by Electrophoresis



IMPORTANT:

7 x 7 cm gels are recommended. Each gel can be shared by 4-5 students. Place well-former template (comb) in the first set of notches.

PREPARING THE AGAROSE GEL WITH SYBR® SAFE STAIN

Wear gloves and safety goggles

1. **MIX** the agarose powder with 1X TBE buffer in a 250 mL flask (see Table A).
2. **DISSOLVE** the 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 by carefully swirling the flask 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 comb in the appropriate notch.
5. Before casting the gel, **ADD** diluted SYBR® Safe to the cooled molten agarose and swirl the flask to mix (see Table A).
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** the end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

OPTIONAL STOPPING POINT:
Gels can be stored for up to one week by placing them in a plastic storage bag containing 1 mL of TBE electrophoresis buffer and storing in the refrigerator. DO NOT FREEZE the gels as this will destroy them.

Table A — **Individual 2.0% UltraSpec-Agarose™ with Diluted SYBR® Stain**

Size of Gel Casting tray	1X TBE Buffer	+	Amt of Agarose	=	TOTAL Volume	Diluted SYBR® (Step 5)
7 x 7 cm	25 mL		0.5 g		25 mL	25 µL
10 x 7 cm*	45 mL		0.9 g		45 mL	45 µL
14 x 7 cm	50 mL		1.0 g		50 mL	50 µL

** Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*

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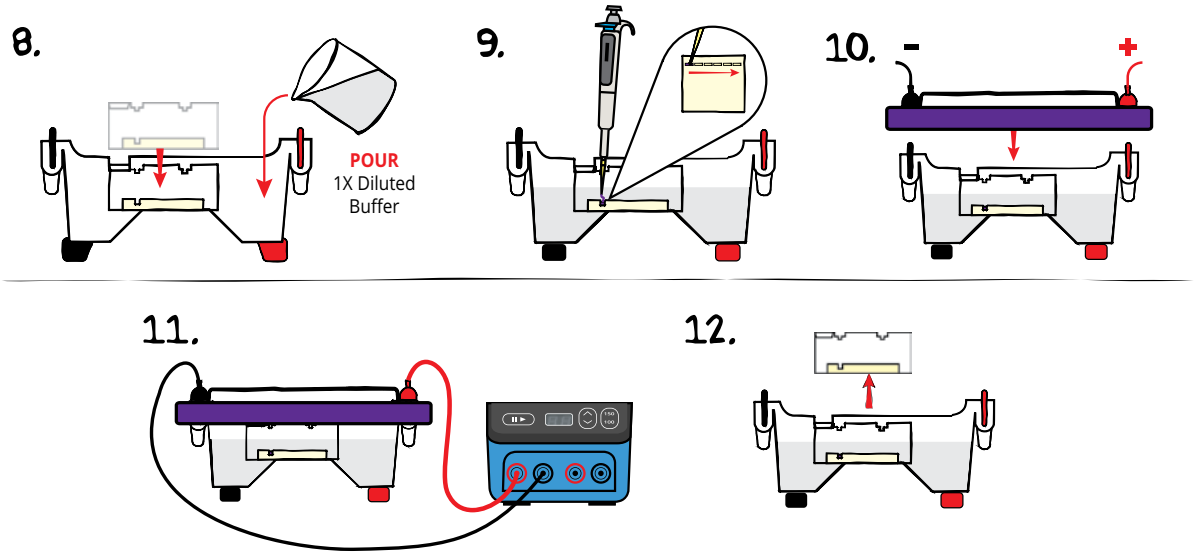
Module III: Separation of PCR Products by Electrophoresis, continued



RUNNING THE GEL

8. **PLACE** the gel (on the tray) into an electrophoresis chamber. **COVER** the gel with 1X TBE electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. Using Table 1 as a guide, **LOAD** the entire sample (25 µL) into the wells in consecutive order.
10. **CHECK** that the gel is properly oriented, then **PLACE** safety cover onto the chamber. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** the 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.

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

OPTIONAL STOPPING POINT:
Gels can be stored for several days. Protect from light, refrigerate, and keep hydrated by storing each gel in a watertight plastic bag with a 1 mL of TBE electrophoresis buffer.

Table 1 : Gel Loading

Lane	Recommended	Sample Name
1	EdvoQuick™ DNA Ladder	
2	Control DNA*	
3	Student #1	
4	Student #2	
5	Student #3	
6	Student #4	

* Optional, or additional student sample.

Table B 1x TBE Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required
EDGE™	150 mL
M12	400 mL

Table C Time and Voltage Guidelines (2.0% Agarose Gel)

	Electrophoresis Model EDGE™ (10 x 7 cm gel)	M12 (14 x 7 cm gel)
Volts	Min/Max (minutes)	Min/Max (minutes)
150	20/30	30/40
100	35/45	45/55
75	NA	60/70

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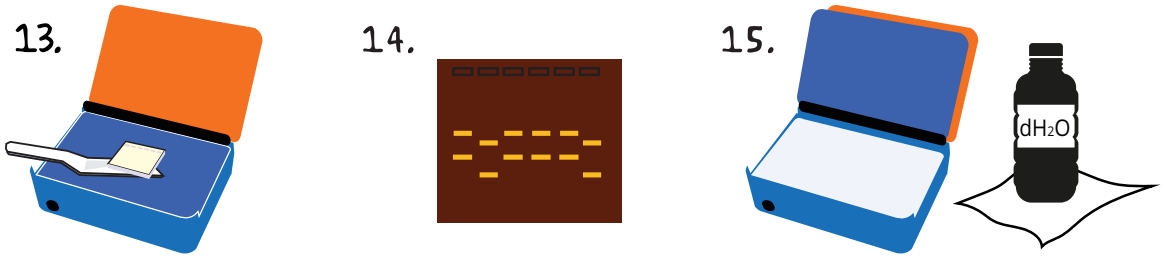
Module III: Separation of PCR Products by Electrophoresis, continued



VISUALIZING THE SYBR® GEL

13. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator.

14. **TURN** the unit on. DNA should appear as bright green bands on a dark background. **PHOTOGRAPH** results.

15. **TURN** the unit off. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.

Be sure to wear UV goggles if using a UV transilluminator.

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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?

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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 Cheek Cells	Prepare and aliquot various reagents (saline).	Up to one day before performing the experiment.	30 min.
	Prepare and aliquot Lysis Buffer.	Prepare on the day the students will be performing the experiment **OR** freeze for up to one week.	15 min.
	Equilibrate water baths at 55° C and boiling.	Anytime before performing the experiment.	5 min.
Module II: Amplification of the D1S80 Locus	Prepare and aliquot various reagents (Primer, control, ladder, etc.).	One day to 30 min. before performing the experiment.	30 min.
	Program Thermal Cycler.	Anytime before performing the experiment.	15 min.
Module III: Separation of PCR Products by Electrophoresis	Prepare TBE buffer and dilute SYBR® Safe Stain.	Up to one week before performing the experiment.	45 min.
	Prepare molten agarose and pour gel.		

Red = Prepare immediately before module. Yellow = Prepare shortly before module. Green = Flexible / prepare up to a week before the module.

IMPORTANT NOTE: Before running the experiment, confirm that your PCR program matches the settings below.

- Initial denaturation 94°C for 4 minutes.
- 94°C for 30 seconds
- 65°C for 30 seconds } 32 cycles
- 72°C for 30 seconds
- Final extension at 72°C for 4 minutes.

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Pre-Lab Preparations: Module I

ISOLATION OF DNA FROM HUMAN CHEEK CELLS

In Module I, the students will isolate DNA from their cheek cells. The easiest, and recommended, method to collect cheek cells involves a brief rinse with a saline solution. The saline rinse will be centrifuged briefly to isolate the cheek cells, which are then mixed with a lysis buffer and incubated at 55˚C and 99˚C. Finally, the students will centrifuge the cell lysate and collect the DNA-containing supernatant. The isolated DNA can be safely stored at -20˚C (freezer) until needed for PCR in Module II.

Before beginning Module I the instructor must prepare the Saline Solution and Lysis Buffer. See below for preparation instructions for both solutions.

Preparation of Saline Solution

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

1. To prepare the saline solution, dissolve all eight 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.

Preparation of Lysis Buffer

NOTE: The Lysis Buffer must be mixed with Proteinase K before performing the experiment. Once prepared, the Lysis should be used the same day or frozen.

1. Add 100 µL of Universal DNA buffer (A) to the tube of Proteinase K 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 Universal DNA buffer (A).

3. Invert the tube several times to mix. Label this tube "Lysis Buffer".

NOTE: The Lysis Buffer should be red and free of any undissolved clumps.

4. Aliquot 300 µL of Lysis Buffer into 13 labeled microcentrifuge tubes to be shared by pairs of students.

NOTE: At this point, the Lysis Buffer should be stored on ice for use within the same day (up to 6 hours) or frozen.

5. Distribute one tube of "Lysis Buffer" to each student pair. If frozen, the Lysis Buffer can be quickly thawed in a 37°C water bath or by students warming the tube in their hands.

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 any biological samples according to your institutional guidelines.

FOR MODULE I
Each student receives:
- 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.

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Pre-Lab Preparations: Module II

AMPLIFICATION THE D1S80 LOCUS

The PCR primers are provided as a lyophilized mixture that must be rehydrated by the instructor before performing the experiment. The PCR EdvoBeads™ can be distributed prior to setting up the PCR – students or instructors can gently transfer the PCR EdvoBeads™ using gloved hands. Alternatively, beads can be gently "poured" from the vial into individual PCR tubes. After distributing the beads, it is important to close the PCR tubes securely to prevent the beads from absorbing moisture and becoming hard to resuspend prior to the experiment.

IMPORTANT:
The PCR EdvoBeads™ absorb moisture if left open, even for a short period of time. Open the vial only when you are ready to use the beads. If you are not using all of the beads in one session, quickly remove the required number and promptly secure the gray stopper back into the vial. Store the vial of unused beads at -20°C until needed.

This kit features LyphoControl™ and LyphoPrimer™ samples. The reagents are color-coded so that a correctly assembled reaction should appear orange in color.

Preparation of the D1S80 Primer Mix

1. Thaw the TE Buffer (B) and mix well.
2. Ensure that the lyophilized solid is at the bottom of the LyphoPrimer™ tube (C). If not, centrifuge the tube at max speed for 10 seconds.
3. Add 1 mL of TE Buffer (B) to the tube of D1S80 Primer Mix. Cap tube and mix.
4. Aliquot 50 µL of the diluted D1S80 Primer Mix into 13 labeled microcentrifuge tubes.
5. Distribute one tube of diluted D1S80 Primer Mix to each student pair. The tubes can be placed on ice or in a 4°C refrigerator until needed.

Preparation of the PCR Control Mix

NOTE: This kit contains enough Control DNA for 6 reactions. We strongly recommend running all 6 control reactions to ensure the PCR was successful.

1. Ensure that the lyophilized solid is at the bottom of the LyphoControl™ tube (D). If not, centrifuge the tube at max speed for 10 seconds.
2. Add 160 µL of TE Buffer (B) to the tube containing the LyphoControl™ (D). Pipet up and down to mix.
3. Dispense 25 µL of the diluted Control reaction for each control reaction.

NOTES:

- *The LyphoControl™ already contains all necessary PCR components and does not need a PCR Edvobead™. Once diluted, the LyphoControl™ is ready to be amplified by PCR alongside student samples, if there is room in the thermal cycler, or can be run prior to the student experiment and stored at -20°C until needed.*

- *One 25 µL LyphoControl™ reaction should be run on every student gel to ensure the PCR was successful.*

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Pre-Lab Preparations: Module II

IMPORTANT:
The PCR EdvoBeads™ absorb moisture if left open, even for a short period of time. Only open the vial when you are ready to use the beads. If you are not using all of the beads in one session, quickly remove the required number and promptly secure the gray stopper back into the vial. Store the vial of unused beads at -20°C (desiccated) until needed.

PCR EdvoBeads™

NOTE: The beads are vacuum-sealed and may move around once the stopper is removed. Only open the vial when you are ready to use the beads and quickly recap the vial.

Dispense the PCR EdvoBeads™ just prior to the students performing their PCR reactions. Alternatively, student pairs can dispense PCR EdvoBeads™ during Module II. Each student will require 1 bead and 1 tube.

1. Open the vial of PCR EdvoBeads™ (carefully remove metal crimp) and gently/slowly remove the gray rubber stopper.
2. With gloved hand, forceps or sterile loop, remove one single bead and transfer to a PCR tube. Do this for the number of reactions you wish to perform. Quickly cap each of the tubes and distribute to the student groups.

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 or mineral oil above the PCR reactions in the microcentrifuge tubes to prevent evaporation.

IMPORTANT NOTE: Before running the experiment, confirm that your PCR program matches the settings below.

- Initial denaturation 94°C for 4 minutes.
- 94°C for 30 seconds
- 65°C for 30 seconds } 32 cycles
- 72°C for 30 seconds
- Final extension at 72°C for 4 minutes.

FOR MODULE II
Each student receives:
- One PCR tube and PCR EdvoBead™

Reagents to be shared by two students:
- 50 µL D1S80 Primer

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Pre-Lab Preparations: Module III

SEPARATION OF PCR PRODUCTS BY ELECTROPHORESIS

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. See Appendix B for instructions.

SYBR® Safe Stain Preparation:
Prepare **diluted** SYBR® Safe by adding 250 µL of 1X TBE buffer to the tube of SYBR® Safe and tapping the tube several times to mix. Diluted SYBR® Safe will be used during agarose gel preparation.

Preparation of Agarose Gels:

This experiment requires one 2.0% agarose gel per 4 students. **7 x 7 cm gels are 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 its own individual gel prior to conducting the experiment (see Module III in the Student's Experimental Procedure). Each 7 x 7 cm gel will require 25 mL of 1X TBE buffer, 0.5 g of agarose powder, and 25 µL of **diluted** SYBR® Safe Stain.

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

Preparing Gels in Advance:
Gels may be prepared ahead and stored for later use. Solidified gels can be stored for up to 1 week in the refrigerator in water-tight bags with a small amount of buffer to prevent drying. We recommend adding 2 mL of buffer to the bag; excess buffer can lead to diffusion of SYBR® Safe out of the gels.

Do not store gels at -20° C as freezing will destroy them.

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 2.0% gel should be loaded with the EdvoQuick™ DNA ladder, a Control DNA reaction, and PCR reactions from 4 students.

- Aliquot 30 µL of the EdvoQuick™ DNA ladder (E) into labeled microcentrifuge tubes and distribute one tube per gel/student group.

FOR MODULE III
Each Group should receive:
- 1X TBE Buffer
- UltraSpec-Agarose™ Powder
- Tube of Diluted SYBR® Safe (25 µL)
- EdvoQuick™ DNA Ladder (30 µL)

VISUALIZING SYBR® SAFE-STAINED GELS

SYBR® Safe is a DNA stain that fluoresces when bound to double-stranded DNA, allowing us to visualize our samples. This DNA stain is compatible with both UV and blue-light transilluminators. For best results, we recommend the TruBlu™ 2 Blue Light Transilluminator (**Cat #557**).

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.

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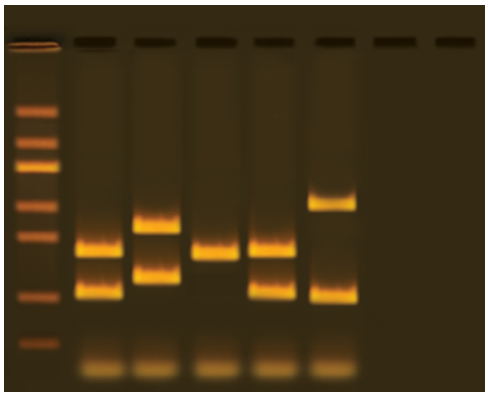
Experiment Results and Analysis



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.

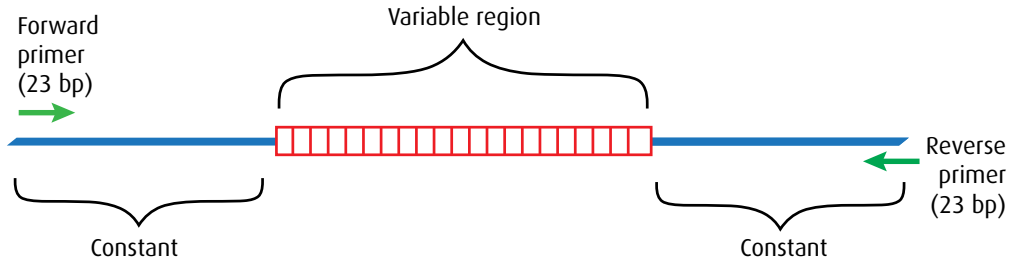
Includes EDVOTEK's
EdvoQuick™ DNA Ladder

- Better separation
- Easier band measurements
- No unused bands

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

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.

D1S80 LOCUS



The PCR products generated by this experiment will range in size from 400 bp to 785 bp.

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

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

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Electrophoresis Buffer and Agarose Gels

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

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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.
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	Repeat cheek cell extraction.
	Sports drink was used for DNA extraction.	Repeat DNA extraction with saline solution.
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.

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Appendix A
EDVOTEK® Troubleshooting Guides

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.
		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.	Ensure that SYBR® Safe was added to the gel before casting. If staining with FlashBlue™, either re-stain with FlashBlue™ solution or destain until bands are seen.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
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 micropipettes.
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.

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Appendix B
Bulk Preparation of Electrophoresis Buffer and Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities that the whole class can share. Leftover diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

BULK 1X TBE ELECTROPHORESIS BUFFER

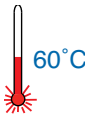
For this experiment, we recommend preparing the 1X TBE Electrophoresis Buffer in bulk for sharing by the class. Unused diluted buffer can be used at a later time.

1. Measure 3.7 L of distilled or deionized water and place in a large vessel. ***(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 D.

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. If staining with SYBR® Safe, add the entire volume of diluted SYBR® Safe from page 20 to the cooled agarose and mix well.
7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 25 mL for each 7 x 7 cm gel, 50 mL for each 7 x 14 cm gel. ***For this experiment, 7 x 7 cm gels are recommended.***
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis. Alternatively, gels can be stored in water-tight plastic bags with 2 mL of 1X electrophoresis buffer for up to 1 week in the refrigerator.

Table D — Batch Prep of 2.0% UltraSpec-Agarose™

Amt of Agarose	+	1x TBE Electrophoresis Buffer
5.0 g		250 mL

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

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