EDVOTEK®

THE **BIOTECHNOLOGY EDUCATION** COMPANY®

REVISED & UPDATED

Edvo-Kit # 333

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Alu-Human DNA Typing Using PCR

Experiment Objective:

In this experiment, students will extract their own genomic DNA. The Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis will be used to analyze polymorphisms between individuals at the Alu insertion in chromosome 16 (PV92).

See page 3 for storage instructions.

LyphoPrimer™

LyphoControl™

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

	Components	Storage	Check (✓)
•	PCR EdvoBeads™ PLUS	Room Temp.	❑
	(Each PCR EdvoBead™ PLUS contains: dNTP Mixture, Taq DNA Polymerase Buffer, Taq DNA Polymerase, and $MgCl_2$)		
A	Universal DNA Buffer	-20° C Freezer	❑
B	TE Buffer	-20° C Freezer	❑
C	PV92 LyphoPrimer™ Mix	-20° C Freezer	❑
D	LyphoControl™	-20° C Freezer	❑
	(Complete PCR Control)		
E	EdvoQuick™ DNA ladder	-20° C Freezer	❑
•	Proteinase K	-20° C Freezer	❑

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

This experiment is designed for 25 human DNA typing reactions.

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

REAGENTS & SUPPLIES

Store all components below at room temperature.

Component	Check (✓)
• UltraSpec-Agarose™	❑
• TBE Electrophoresis Buffer Powder	❑
• SYBR® Safe Stain	❑
• FlashBlue™ Stain	❑
• 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	❑

Requirements

- Thermal cycler (EDVOTEK® Cat. #541 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 (EDVOTEK® Cat. #557 or #558 highly recommended)
- UV safety goggles
- White light visualization system (optional - use if staining with FlashBlue™)
- Automatic micropipettes (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 (if isolating DNA from cheek cells)
- Bleach solution

Background Information

ALU-HUMAN DNA TYPING

The haploid human genome consists of 2.9 billion base pairs of DNA, of which about 5% consists of protein-coding genes. Introns and other noncoding sequences make up the remainder; some of the non-coding sequences comprise gene promoters, ribosomal and transfer RNAs, and microRNAs. However, many of these noncoding sequences appear to be self-replicating and are repeated hundreds or thousands of times throughout the genome. These repetitive sequences have been termed "selfish" or "parasitic" DNA, as they often appear to possess no function except for their own reproduction. These repetitive elements account for more than 20 percent of the human genome.

In 1979, a specific 300 base pair DNA element was identified in many different locations throughout the human genome. Copies of this element contain a recognition site for the restriction enzyme Alu I, and were subsequently named Alu elements. Although Alu elements have been found in exons, most exist in introns and other non-coding regions. However, when Alu elements disrupt specific genes, they can result in human disease or other defects. The details of the Alu insertion process are not well understood. Alu sequences replicate through an RNA intermediate which is copied into a double-stranded DNA segment called a retrotransposon. The retrotransposon then inserts elsewhere in the genome. It is theorized that most Alu sequences are incapable of replication and that only a small number of "master genes" are duplicated to form new elements.

Humans (and other primates) possess hundreds of thousands of Alu elements throughout their genome. Most of them are fixed, meaning that both chromosomes have the same insertion. However, other Alus are dimorphic, meaning that the element may or may not be present at a specific chromosomal location. These differences in DNA sequences between individuals are known as polymorphisms. Individuals can be heterozygous or homozygous for a specific Alu, meaning that the sequence may be present in one or both of the homologous chromosomes.

One example of a dimorphic Alu insertion is found on chromosome 16 at the PV92 locus. The Polymerase Chain Reaction, or PCR, can be used to determine whether a

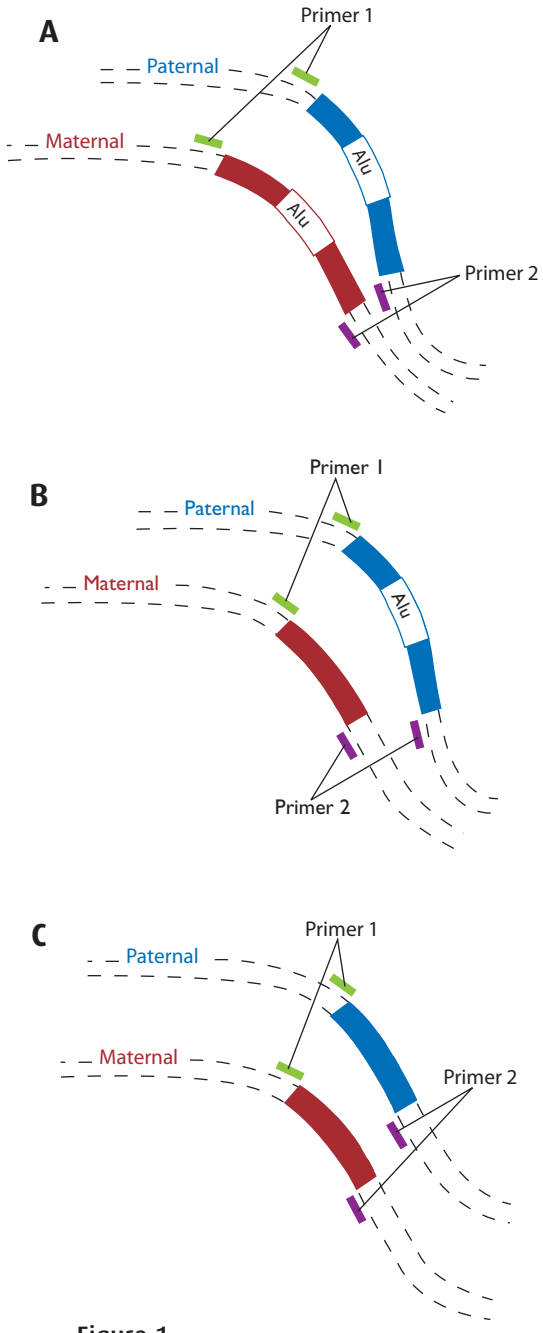


Figure 1:
PV92 Locus on Chromosome 16.

person possesses an Alu insertion at the PV92 locus. If a person is homozygous for the insertion, a gel of the PCR product will result in a single band at 700 base pairs (Figure 1A). If a person is heterozygous, i.e., possesses the insertion on one chromosome 16 homologue but not the other, two bands will be present following PCR. One band will be 700 base pairs and the other will be 400 (Figure 1B). If a person lacks the insertion on either chromosome homologue, that person is said to possess the null genotype and PCR will result in only one band at 400 base pairs (Figure 1C).

To examine the PV92 locus, the Polymerase Chain Reaction (PCR) is usually employed. PCR was invented in 1984 by Dr. Kary Mullis at the Cetus Corporation in California. The enormous utility of the PCR method is based on its ease of use and its ability to allow the amplification of small DNA fragments. For this ground breaking technology, Mullis was awarded the Nobel Prize in Chemistry in 1993.

Before performing PCR, template DNA is extracted from various biological sources. 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 ofactor 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 2). 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.

In this experiment, each student will extract his/her DNA from hair or cheek cells and amplify DNA at the PV92 locus by PCR. As a control, DNA purified from a cultured human cell line may be used. The PCR product(s) will then be examined on agarose gels to determine whether the student is homozygous (+/+), heterozygous (+/-), or null (-/-) for an Alu insertion at the locus. Objectives of this experiment are the isolation of human DNA and the comparison of DNA polymorphisms between individuals by PCR amplification and gel electrophoresis.

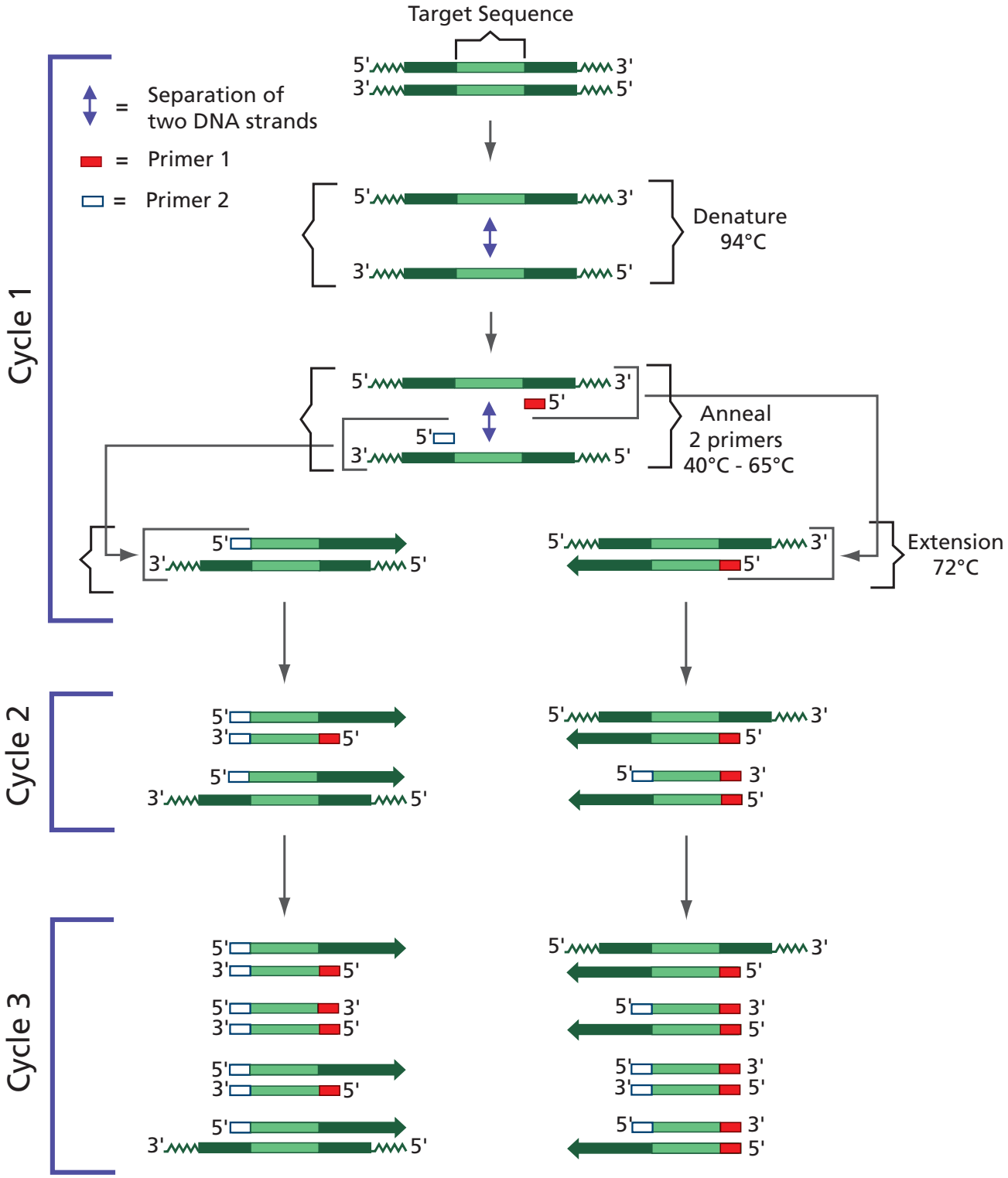


Figure 2:
Polymerase Chain Reaction

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will extract their own genomic DNA. The Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis will be used to analyze polymorphisms between individuals at the Alu insertion in chromosome 16 (PV92).

Wear gloves and safety goggles

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.

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

↓

Module II: 90 min.
Amplification of the PV92 Locus

↓

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

↓

Module IV: 5-20 min.
Staining Agarose Gels with FlashBlue™ (OPTIONAL)

NOTE: Experimental times are approximate.

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:

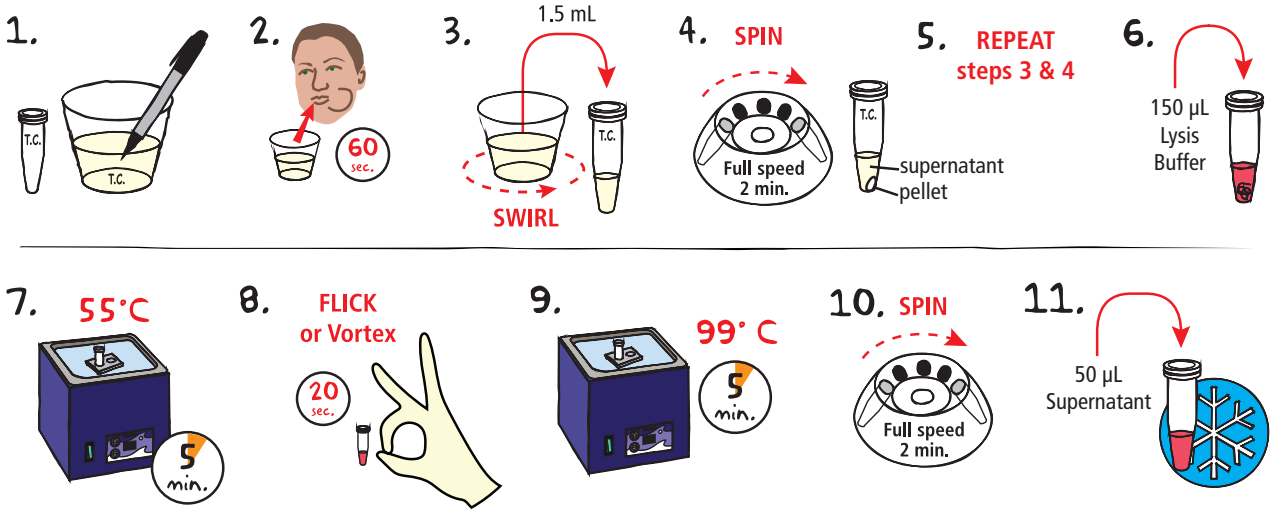
- Record your observations.

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.

PREFERRED METHOD

Module I-A: 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 PV92 Locus. If you are ready to proceed, turn to page 11. 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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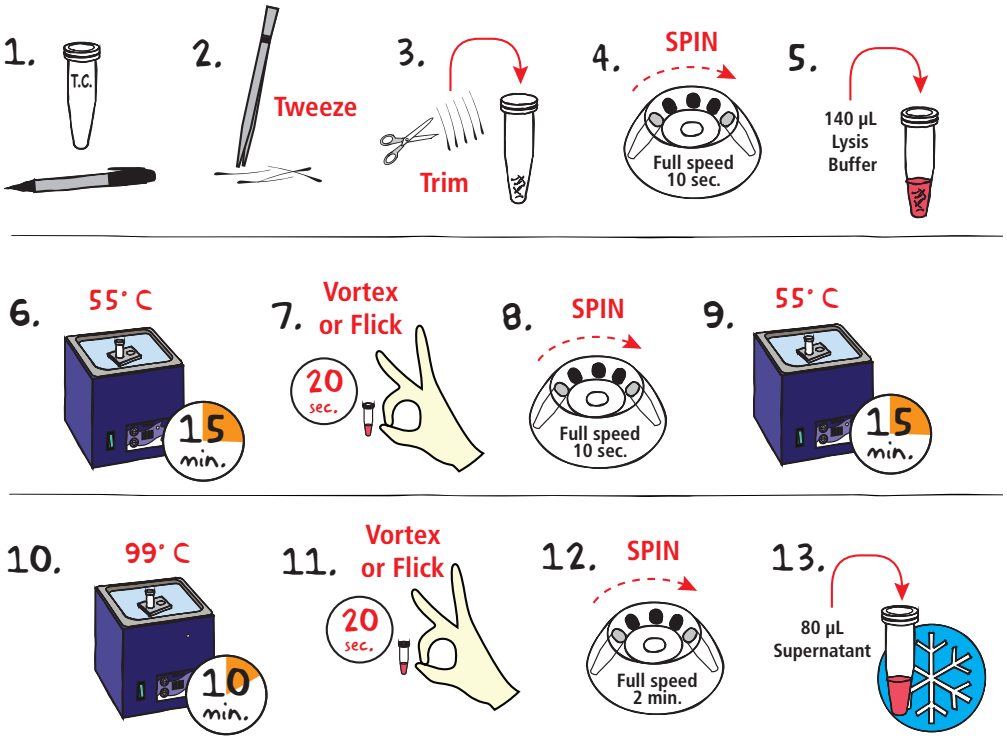
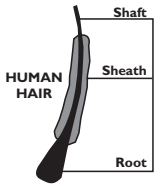
Module I-B: Isolation of DNA from Human Hair



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.

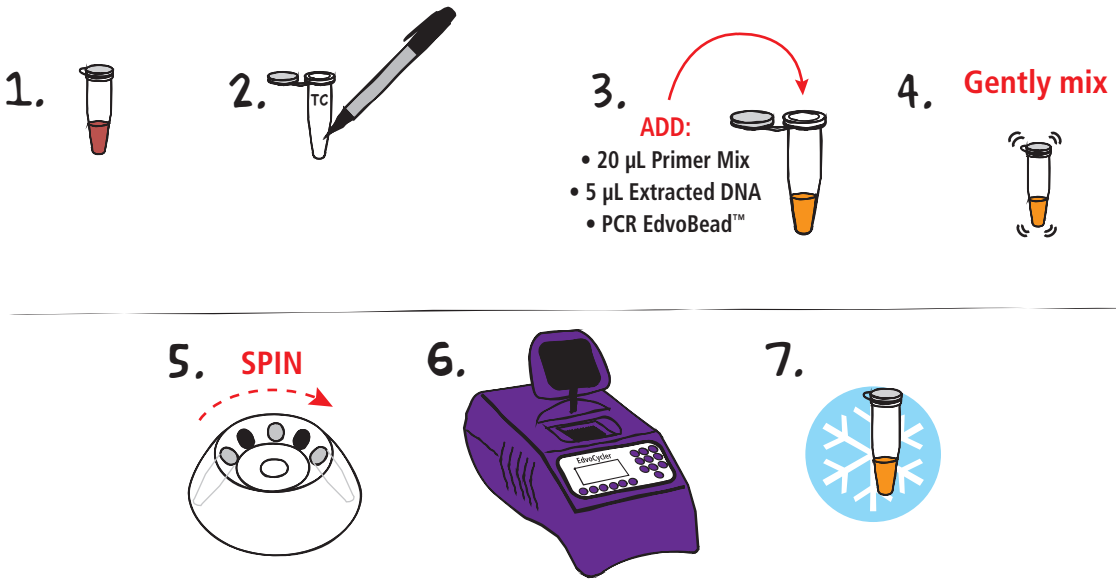


1. **LABEL** a 1.5 mL screw top microcentrifuge tube with your initials.
2. Using tweezers, **GRASP** 2-3 hair shafts at the base and **PULL** quickly. **COLLECT** at least 5 hairs that include the root and the sheath (a sticky barrel-shaped layer of cells that encircles the root end of the hair).
3. 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 water bath float. **INCUBATE** the sample in a 55° C water bath 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 water bath. **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 PV92 Locus.

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

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

Module II: Amplification of the PV92 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 PV92 primer mix (yellow), 5 µL extracted DNA (red), and a PCR EdvoBead™ PLUS.
4. **MIX** the PCR sample. Make sure the PCR EdvoBead™ PLUS 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
   PCR cycling conditions:
   - Initial denaturation 94° C for 5 minutes
   - 94° C for 30 seconds
   - 68° C for 30 seconds } 32 cycles
   - 72° C for 30 seconds
   - Final Extension 72° C for 4 minutes
7. After PCR, **PLACE** the tubes on ice. **PROCEED** to Module III: Separation of PCR Products by Electrophoresis.

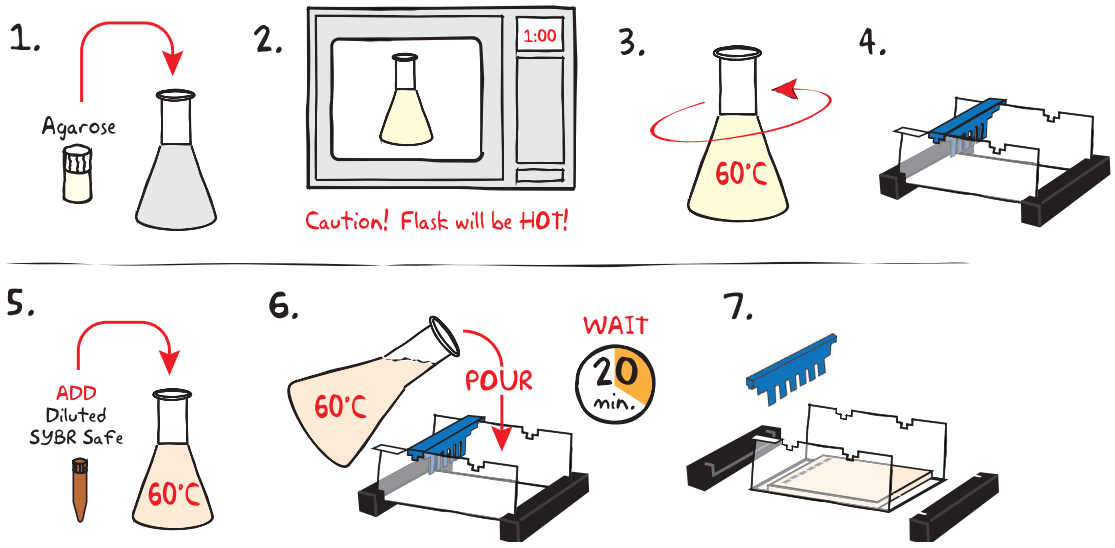
NOTE:
The positive control contains primers, template DNA and PCR components, and is ready for PCR amplification.

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

NOTE:
PCR Cycling Conditions have changed. Please review your PCR program before performing the experiment.

STOP **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



PREPARING THE AGAROSE GEL WITH SYBR® SAFE STAIN

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** 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.

Wear gloves and safety goggles

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.

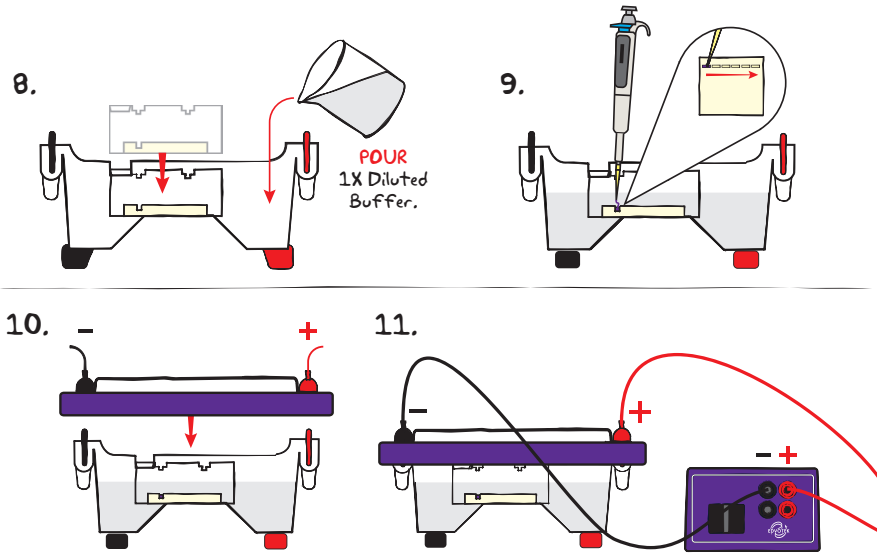
If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

OPTIONAL STOPPING POINT:
Gels can be stored overnight submerged in electrophoresis buffer, in the fridge, and protected from light.

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

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

Module III: Separation of PCR Products by Electrophoresis, continued



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

Wear gloves and safety goggles

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.

OPTIONAL STOPPING POINT:
Gels can be stored for several days. Protect from light, refrigerate, and keep hydrated by storing each gel in a water-tight plastic bag with a small amount of electrophoresis buffer.

Table 1: Sample Table

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.*

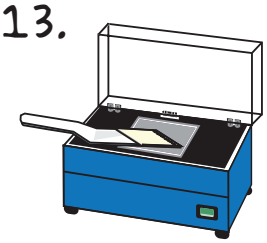
Table B: 1x TBE Electrophoresis Buffer (Chamber Buffer)

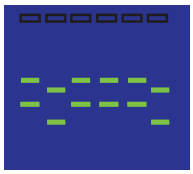
EDVOTEK Model #	Total Volume Required
M6+ & M12 (new)	300 mL
M12 (classic)	400 mL
M36	1000 mL

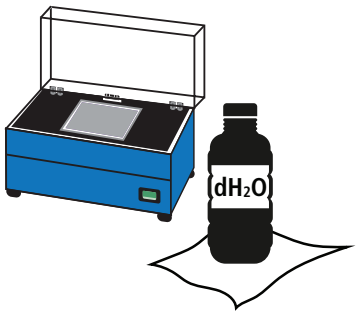
Table C: Time & Voltage Guidelines (2.0% Agarose Gels)

Volts	Time: 7 x 7 cm gel ~4.0 cm migration
75	75 min.
125	40 min.
150	30 min.

Module III: Separation of PCR Products by Electrophoresis, continued

13. 

14. 

15. 

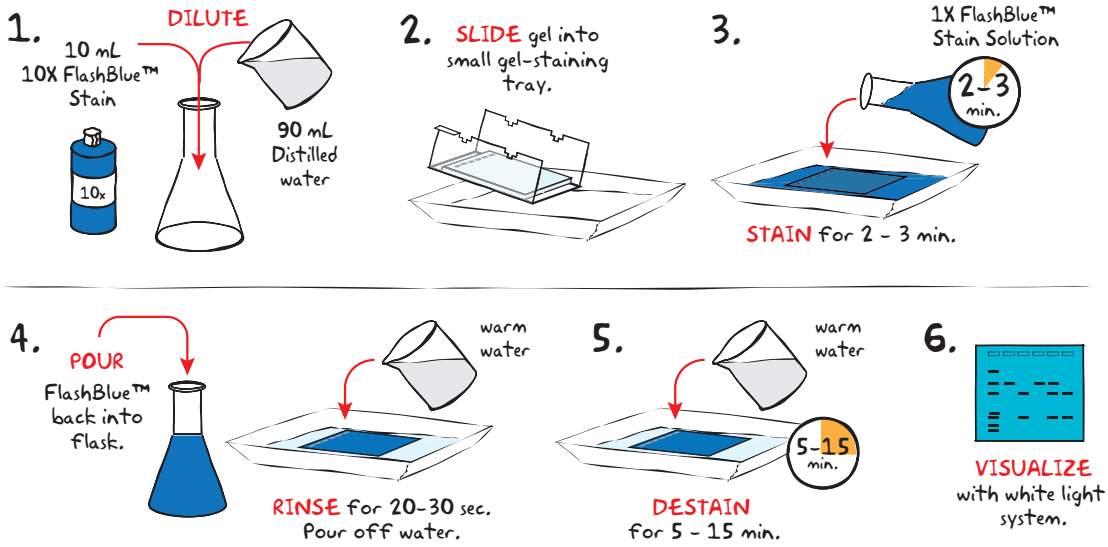
VISUALIZING THE SYBR® GEL

13. **SLIDE** the gel off the casting tray onto the viewing surface of the transilluminator and turn the unit on. **ADJUST** the brightness to the desired level to maximize band visualization. DNA should appear as bright green bands on a dark background.
14. **PHOTOGRAPH** the results.
15. **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.

Module IV: Staining Agarose Gel with FlashBlue™ Stain (OPTIONAL)

FlashBlue™ Stain is a simple and effective visible DNA stain that can be used as an alternative, or in addition to, UV-reactive DNA stains like SYBR® Safe. ***IF staining with both SYBR® Safe and Flash Blue, you must examine and record the SYBR® Safe bands before beginning the FlashBlue™ Staining.***



1. **DILUTE** 10 mL of 10X concentrated FlashBlue™ with 90 mL of distilled water in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1X FlashBlue™ stain solution. **STAIN** the gel for 2-3 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 3 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **POUR** the 1X FlashBlue™ back into the flask (the stain can be reused). **COVER** the gel with warm water (40-45° C). Gently **RINSE** the gel for 20-30 seconds. **POUR** off the water.
5. **COVER** the gel with clean, warm water (40-45° C). **DESTAIN** for 5-15 minutes with gentle shaking (longer periods will yield better results). DNA bands will start to appear after 5 minutes of destaining. Changing the water frequently will accelerate destaining.
6. Carefully **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.


Wear gloves and safety goggles

ALTERNATIVE FLASHBLUE™ STAINING PROTOCOL:

1. **DILUTE** 1 mL of 10X FlashBlue™ stain with 499 mL distilled water.
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.
4. Carefully **REMOVE** the gel from the staining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Study Questions

1. Compare your Alu genotype with those of your classmates. Did anyone else have a similar result? If so, what are some possible explanations?

2. What is "selfish DNA"? How are Alu elements thought to replicate? What is the function(s) of Alu elements?

3. Could dimorphic Alu elements be used for DNA identification (i.e., in criminal investigations)? Why or why not?

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).	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 PV92 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.		
Module IV: Staining Agarose Gels with FlashBlue™ (OPTIONAL)	Prepare staining components.	Up to 10 min. before the class period.	10 min.

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

NOTE:
The PCR cycling conditions may have changed. Before running the experiment, confirm that the program matches the settings below:

- Initial denaturation 94° C for 5 minutes
- 94° C for 30 seconds
- 68° C for 30 seconds } 32 cycles
- 72° C for 30 seconds
- Final Extension 72° C for 4 minutes

Pre-Lab Preparations: Module I

ISOLATION OF DNA

NOTE: For Module I-A, 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, pipette, 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.

FOR MODULE I-A
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

FOR MODULE I-B
Each student receives:
- One screw-cap tube
- One microcentrifuge tube

Reagents to be shared by two students:
- 300 µL Lysis buffer

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.

Preparation of Saline Solution (For Module I-A ONLY):

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.

Preparation of Lysis Buffer (For Module I-A or I-B):

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.

Pre-Lab Preparations: Module II

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

Reagents to be shared by two students:
- 50 µL PV92 Primer

AMPLIFICATION THE PV92 LOCUS

The PCR primers are provided as a lyophilized mixture that must be rehydrated by the instructor before performing the experiment. The PCR EdvoBeads™ PLUS can be distributed prior to setting up the PCR – students or instructors can gently transfer the PCR EdvoBeads™ PLUS 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.
NOTE: The PCR EdvoBeads™ PLUS are fragile, use care to not crush the beads while transferring to a PCR tube.

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 PV92 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 PV92 Primer Mix. Cap tube and mix.
4. Aliquot 50 µL of the diluted PV92 Primer Mix into 13 labeled microcentrifuge tubes.
5. Distribute one tube of diluted PV92 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. ***NOTE: The LyphoControl™ already contains all necessary PCR components and does not need a PCR Edvobead™ PLUS. 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 Lypho-Control™ reaction should be run on every student gel to ensure the PCR was successful.***

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 5-10 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. Visit **www.edvotek.com** for instructions.

NOTE:
PCR Cycling Conditions have changed. Please review your PCR program before performing the experiment.

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. **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.

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.

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

NOTE:
Accurate pipetting is critical for good experiment results. This experiment is designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students do not know how to use micropipettes, 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 group receives:
- 1X TBE Buffer
- UltraSpec-Agarose™ Powder
- Tube of SYBR® Safe (25 µL)
- EdvoQuick™ DNA Ladder (30 µL)

NOTE:
QuickGuide instructions and guidelines for casting various agarose gels can be found our website.
www.edvotek.com/quick-guides

Pre-Lab Preparations: Module IV (OPTIONAL)

STAINING AGAROSE GELS WITH FLASHBLUE™ (OPTIONAL)

FlashBlue™ can be used as an alternative or in addition to SYBR® Safe in this experiment. If only staining with FlashBlue™, you can omit SYBR® Safe from the gel preparation. However, FlashBlue™ is less sensitive than SYBR® Safe and will take a longer time to obtain results. Alternatively, gels can be visualized first with SYBR® Safe and then with FlashBlue™.

Wear gloves and safety goggles

FOR MODULE IV
Each group receives:
- 10 mL 10X concentrated FlashBlue OR 100 mL 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

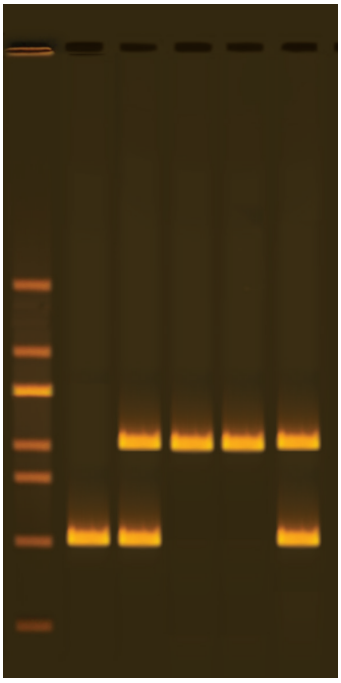
Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to develop in the destaining solution, resulting in dark blue DNA bands that contrast with a uniformly light blue 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 if they are refrigerated, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels should be discarded in the garbage and destaining solutions should be disposed of down the drain.

Photodocumentation of DNA (Optional)

Once the gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that interface directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

Experiment Results and Analysis



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

The results photo shows an example of the possible PCR products from different genotypes.

Lane	Recommended	Molecular Weight	Result
1	EdvoQuick™ DNA Ladder	---	---
2	Control DNA*	400 bp	Null for Alu insertion (-/-)
3	Student #1	700, 400 bp	Heterozygous for Alu insertion (+/-)
4	Student #2	700 bp	Homozygous for Alu insertion (+/+)
5	Student #3	700 bp	Homozygous for Alu insertion (+/+)
6	Student #4	700, 400 bp	Heterozygous for Alu insertion (+/-)

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 (not observed in photo shown). 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.

This experiment has been revised and updated. The results will differ slightly from previous versions of this kit.

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

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
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 or mineral oil.
		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 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.
After staining with FlashBlue™, the gel background is very dark.	The gel needs to be destained longer.	Submerge the gel in distilled or deionized water. Allow the gel to soak for 5 minutes.
After staining with FlashBlue™, 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.
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
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.

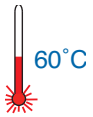
BATCH AGAROSE GELS (2.0%)

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

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

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

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.



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.

NOTE:
QuickGuide instructions and guidelines for casting various agarose gels can be found our website. www.edvotek.com/quick-guides