EDVOTEK®

THE BIOTECHNOLOGY EDUCATION COMPANY®

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

REVISED & UPDATED

Edvo-Kit # 333

Edvo-Kit #333

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.

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

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Table of Contents

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

Experiment Components

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Components		Storage	Check (✓)
A	PCR EdvoBeads™	Room Temp.	❑
	Each PCR EdvoBead™ contains:		
	• *dNTP Mixture*		
	• *Taq DNA Polymerase Buffer*		
	• *Taq DNA Polymerase*		
	• *$MgCl_2$*		
	• *Reaction Buffer*		
B	PV92 Primer Mix concentrate	-20° C Freezer	❑
C	EdvoQuick™ DNA ladder	-20° C Freezer	❑
D	Control DNA concentrate	-20° C Freezer	❑
E	TE Buffer	-20° C Freezer	❑
F	Proteinase K	Room Temp., desiccated	❑

This experiment is designed for 25 human DNA typing reactions.

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.

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

REAGENTS & SUPPLIES

Store all components below at room temperature.

Component	Check (✓)
• UltraSpec-Agarose™	❑
• Electrophoresis Buffer (50x)	❑
• 10x Gel Loading Solution	❑
• InstaStain® Ethidium Bromide	❑
• FlashBlue™ Stain	❑
• Snap-top Microcentrifuge Tubes (1.5 ml - use for boiling)	❑
• Screw-top Microcentrifuge Tubes (1.5 ml - use for boiling)	❑
• 0.2 ml PCR tubes	❑
• Disposable plastic cups	❑
• Salt packets	❑
• 15 ml Conical tube	❑
• Wax beads (for thermal cyclers without heated lid)	❑

Requirements

- Thermal cycler (EDVOTEK Cat. # 532 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 (use if staining with InstaStain® Ethidium Bromide)
- UV safety goggles
- White light visualization system (optional - use if staining with FlashBlue™)
- Automatic micropipets (5-50 µl) with tips
- Microwave, 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

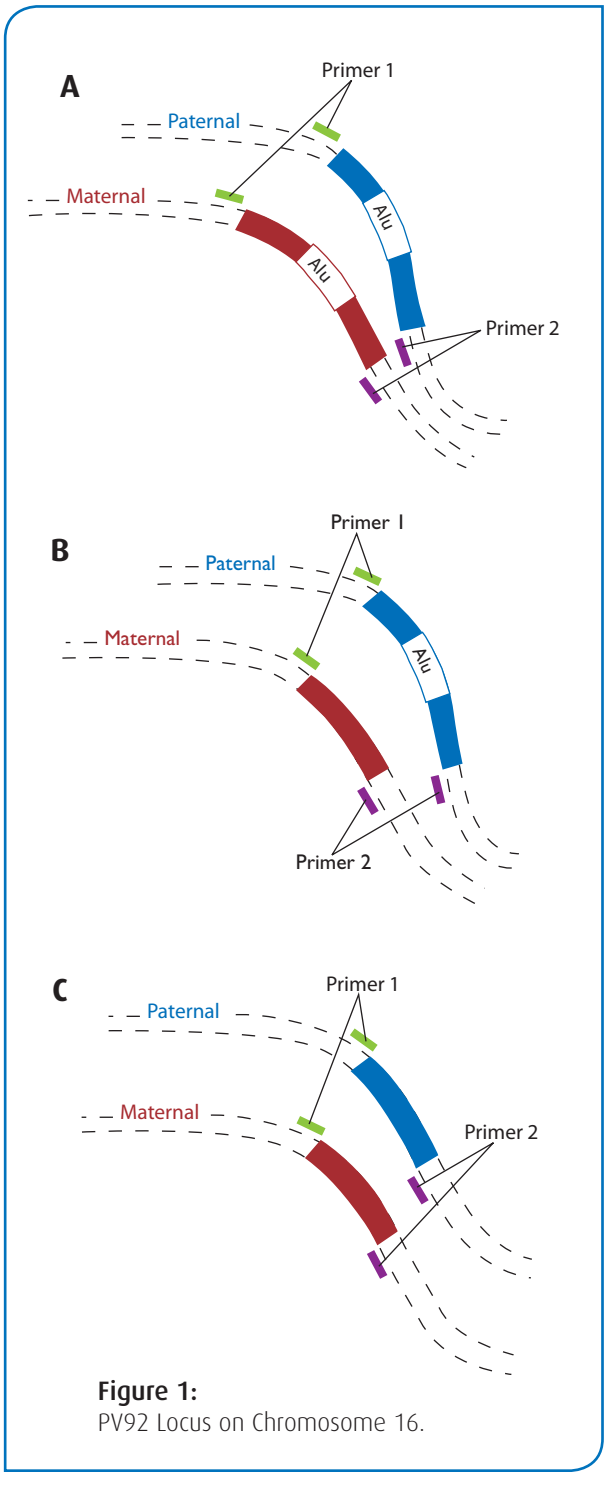


Figure 1:
PV92 Locus on Chromosome 16.

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.

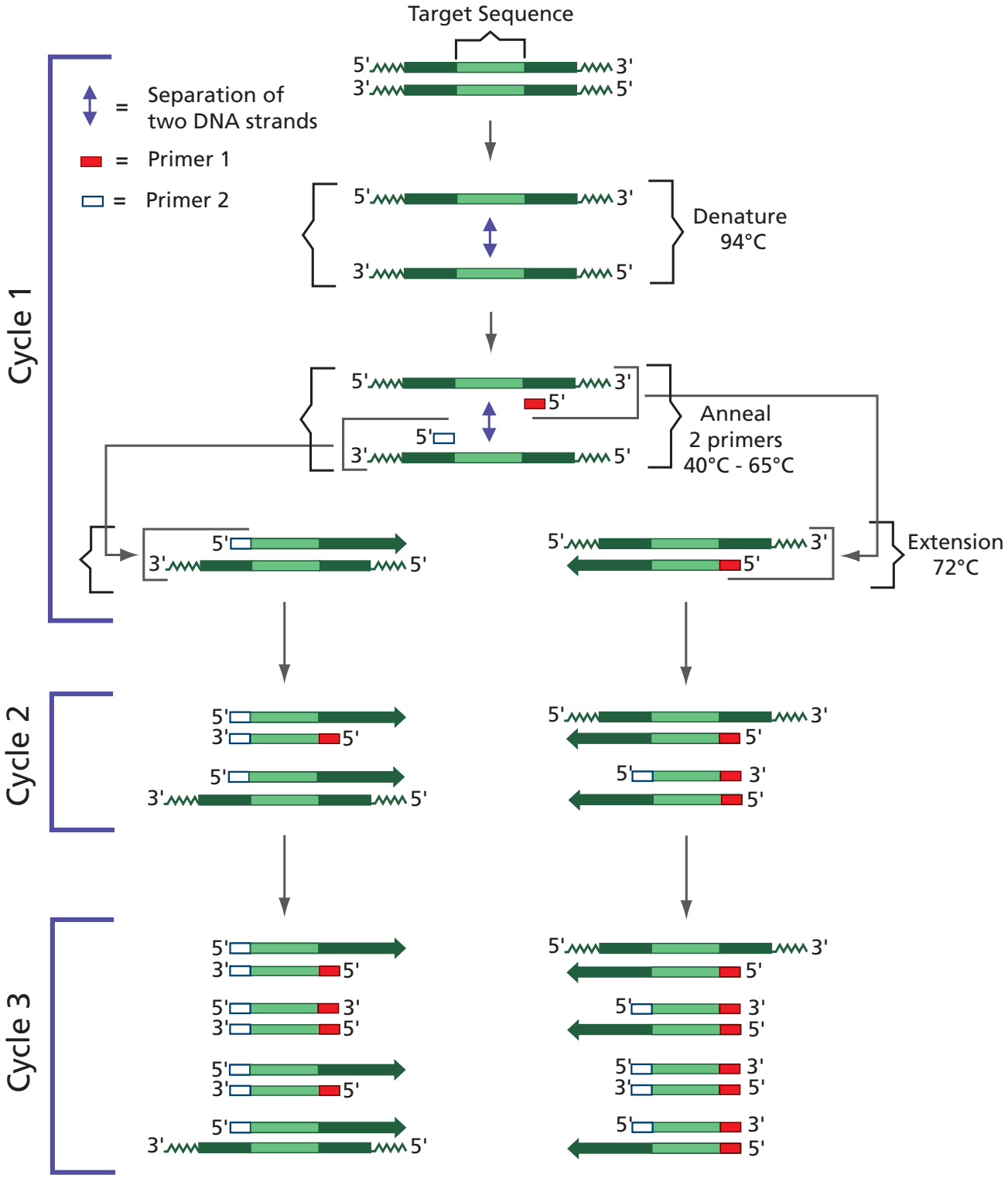


Figure 2:
Polymerase Chain Reaction

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: 5-30 min.
Staining Agarose Gels

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:

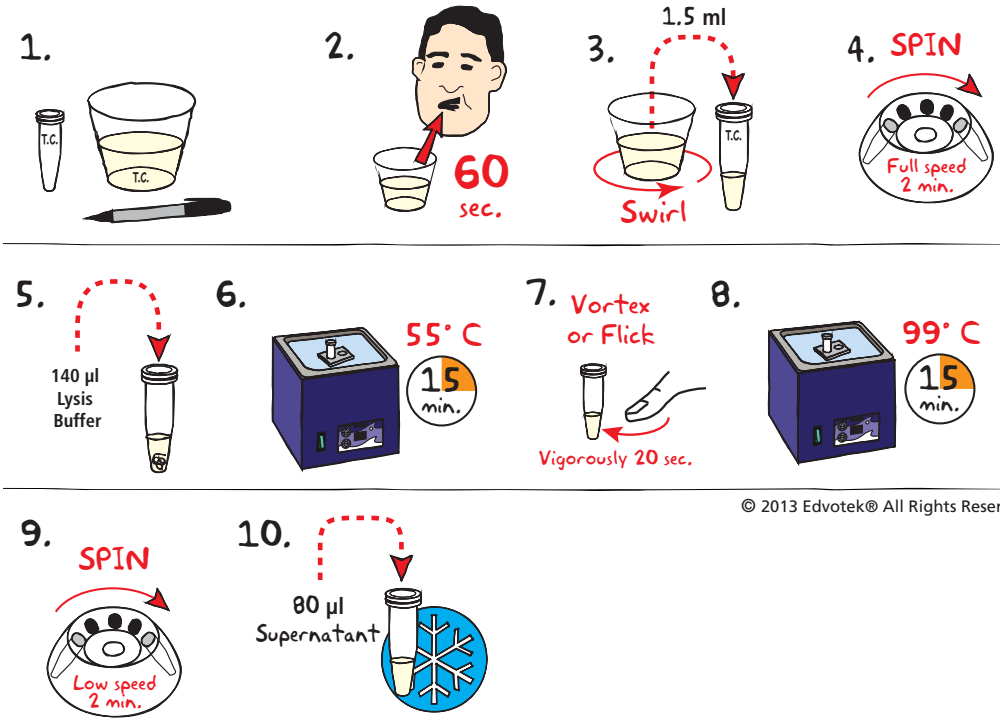
- Record your observations.

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



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

1. **LABEL** a 1.5 ml screw top microcentrifuge tube and a cup with your lab group and/or initials.
2. **RINSE** your mouth vigorously for 60 seconds using 10 ml saline solution. **EXPEL** the solution into cup.
3. **SWIRL** the cup gently to resuspend the cells. **TRANSFER** 1.5 ml of solution into the labeled tube.
4. **CENTRIFUGE** the cell suspension for 2 min. at full speed to pellet the cells. **POUR** off the supernatant, but **DO NOT DISTURB THE CELL PELLET**! Repeat steps 3 and 4 twice more.
5. **RESUSPEND** the cheek cells in 140 µl lysis buffer by pipetting up and down or by vortexing vigorously.
6. **CAP** the tube and **PLACE** in a waterbath float. **INCUBATE** the sample in a 55° C waterbath for 15 min.
7. **MIX** the sample by vortexing or flicking the tube vigorously for 20 seconds.
8. **INCUBATE** the sample in a 99° C waterbath for 15 min. Be sure to use screw-cap tubes when boiling DNA isolation samples.
9. **CENTRIFUGE** the cellular lysate for 2 minutes at low speed (6000 rpm).
10. **TRANSFER** 80 µl of the supernatant to a clean, labeled microcentrifuge tube. **PLACE** tube in ice.
11. **PROCEED** to Module II: Amplification of the PV92 Locus.

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

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

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

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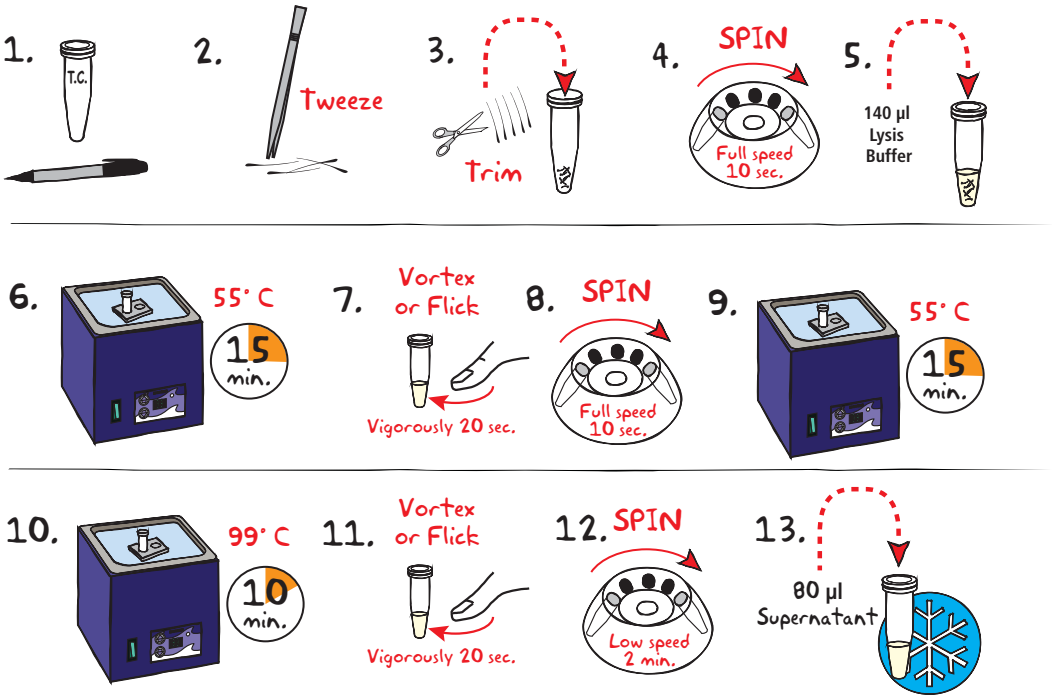
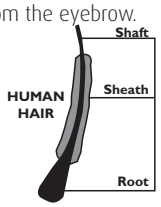
Module I-B: Isolation of DNA from Human Hair



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.

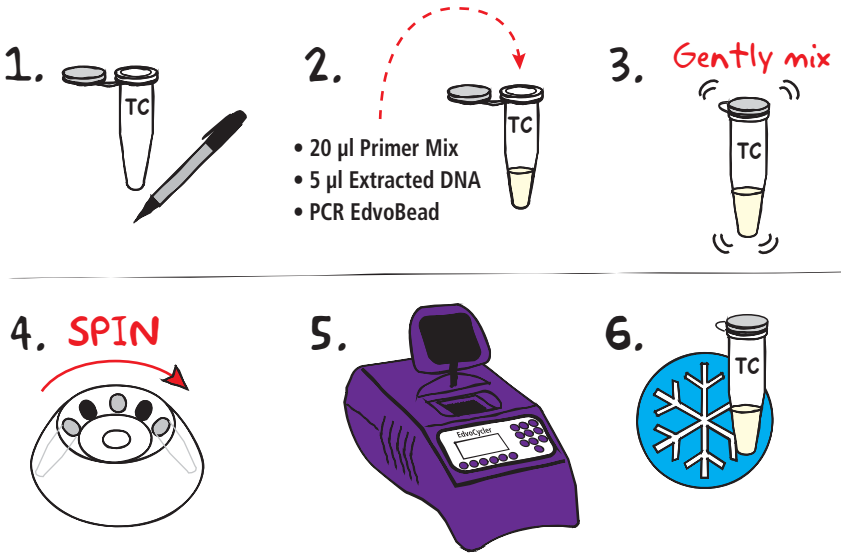


1. **LABEL** a 1.5 ml screw top microcentrifuge tube with your lab group and/or initials.
2. Using tweezers, **GRASP** 2-3 hair shafts at the base and **PULL** quickly. **COLLECT** at least 5 hairs that include the root and the sheath (a sticky barrel-shaped layer of cells that encircles the root end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



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

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

2. **ADD** 20 µl PV92 primer mix, 5 µl extracted DNA (or control DNA) and the 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 5 minutes
   - 94° C for 30 seconds
   - 65° C for 30 seconds } 32 cycles
   - 72° C for 60 seconds
   - Final Extension 72° C for 4 minutes

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.

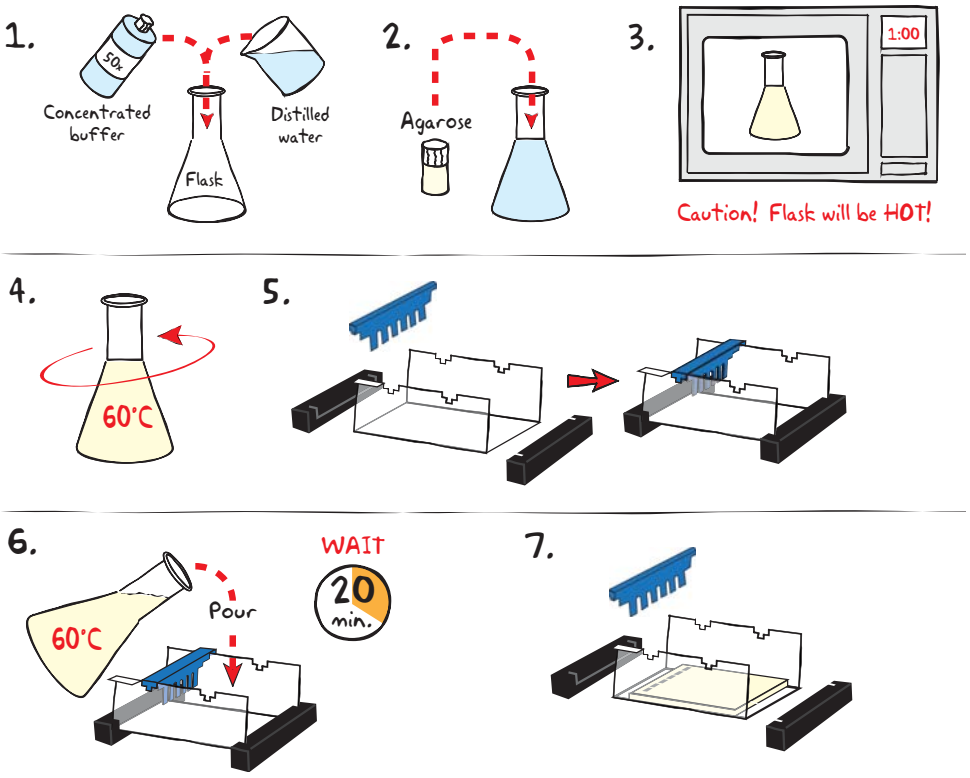
NOTES AND REMINDERS:

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



IMPORTANT:

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

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

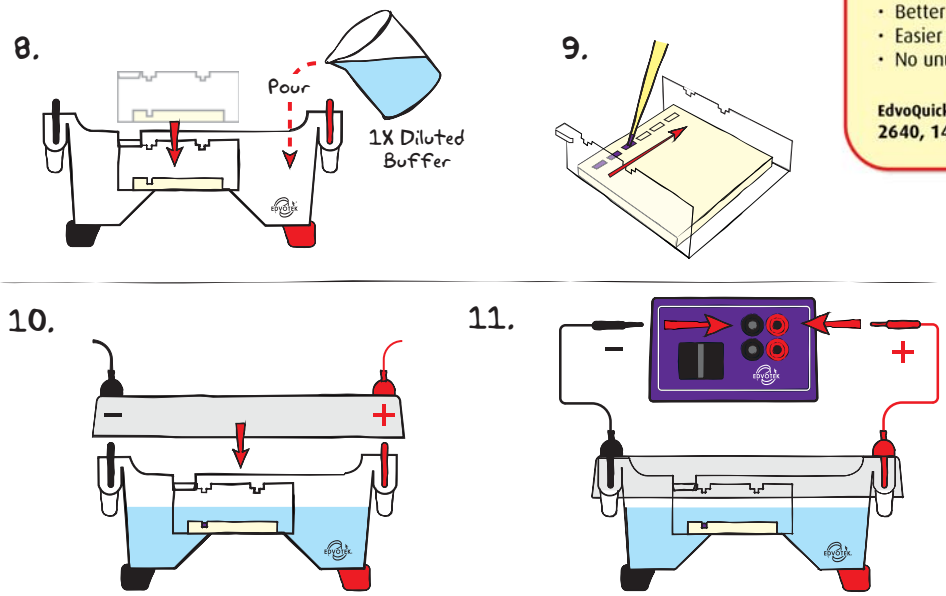
Wear gloves and safety goggles

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

Table A — Individual 1.5% UltraSpec-Agarose™ Gel

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

Module III: Separation of PCR Products by Electrophoresis



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

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

Table 1: 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 Electrophoresis Buffer (Chamber Buffer)

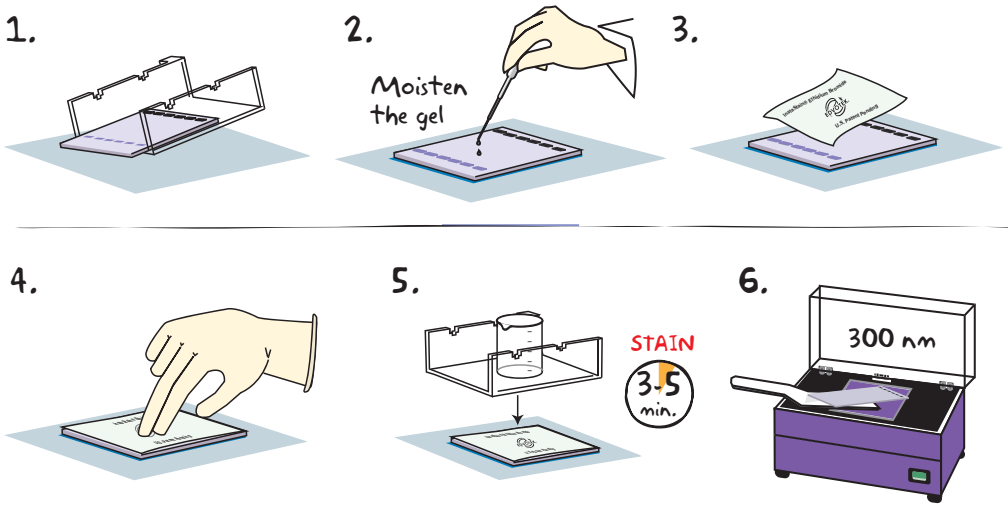
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 ml	6 ml	294 ml
M12	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

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

Volts	Recommended Time: Minimum	Maximum
150	45 min.	60 min.
125	55 min.	1 hour 15 min.
70	2 hours 15 min.	3 hours

Module IV-A: Staining with InstaStain® Ethidium Bromide

PREFERRED METHOD

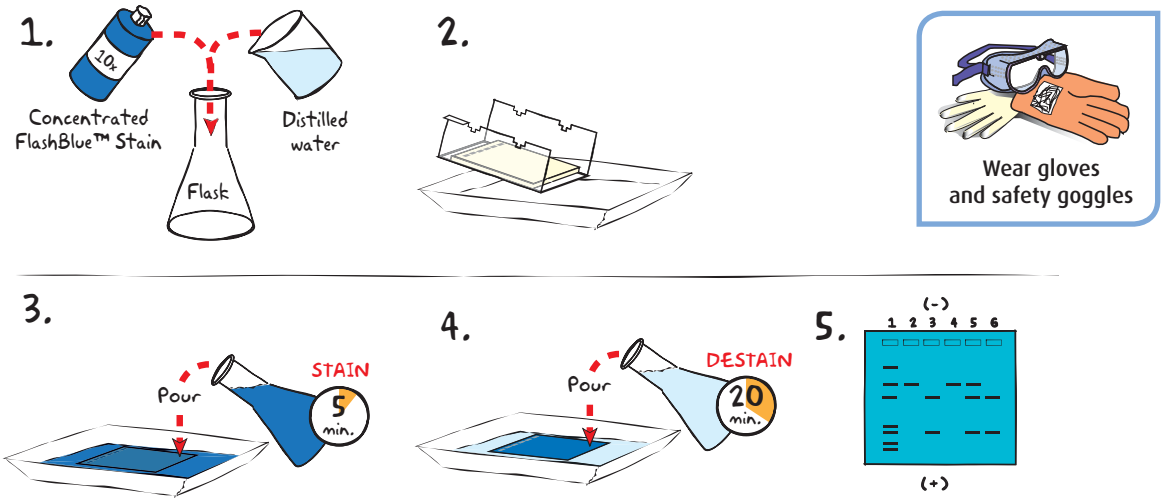


1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface. **DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.**
2. **MOISTEN** the gel with a few drops of electrophoresis buffer.
3. Wearing gloves, **REMOVE** and **DISCARD** the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). **PLACE** the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. You will need 2 cards to stain a 7 x 14 cm gel.
4. With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
5. **PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. **STAIN** the gel for 3-5 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!

Wear gloves and UV safety goggles

Module IV-B: Staining with FlashBlue™



1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
5. 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.

Alternate Protocol:

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

Study Questions

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

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

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION:

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

Preparation For:	What to do:	When:	Time Required:
Module I: Isolation of DNA from Hair or Cheek Cells	Prepare and aliquot various reagents (Saline, Lysis buffer)	Up to one day before performing the experiment. IMPORTANT: Prepare the Lysis buffer no more than 30 minutes before performing the experiment.	30 min.
	Equilibrate waterbaths at 55 ° C and boiling.	At least 30 min. before performing the experiment.	15 min.
Module II: Amplification of the PV92 Locus	Prepare and aliquot various reagents (Primer, DNA template, 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 diluted TAE buffer	Up to one hour before performing the experiment.	45 min.
	Prepare molten agarose and pour gel		
Module IV: Staining	Prepare staining components	Up to 10 min. before the class period.	10 min.

Pre-Lab Preparations

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

Prepare the Saline Solution:

1. To prepare the saline solution, dissolve all 8 salt packets in 500 ml of drinking water. Cap and invert bottle to mix.

2. Aliquot 10 ml of saline solution per cup. Distribute one cup per student.

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-A
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.

MODULE I-B: ISOLATION OF DNA FROM HUMAN HAIR

Preparation of 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-B
Each Group should receive:
- One screw-cap tube
- One microcentrifuge tube

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

Pre-Lab Preparations

MODULE II: AMPLIFICATION THE PV92 LOCUS

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

Reagents to be Shared by Two Students:
- 50 µl PV92 Primer

Preparation of the PV92 Primer

1. Thaw the PV92 Primer Mix Concentrate (B) on ice.
2. Add 1 ml of TE Buffer (E) to the tube of PV92 Primer Mix Concentrate. Cap tube and mix.
3. Aliquot 50 µl of the diluted PV92 Primer Mix into 13 labeled microcentrifuge tubes.
4. Distribute one tube of diluted PV92 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:
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 Agarose Gels

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

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

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

Preparing Gels in Advance:

Gels may be prepared ahead and stored for later use. Solidified gels can be 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:
Each 1.5% gel should be loaded with the EdvoQuick™ DNA ladder and PCR reactions from 4 or 5 students.

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

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

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

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

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: STAINING

InstaStain® Ethidium Bromide *(PREFERRED METHOD)*

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

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

Wear gloves and safety goggles

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.

FlashBlue™

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

FOR MODULE IV-B
Each Group should receive:
- 10 ml 10X concentrated FlashBlue OR 100 ml 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

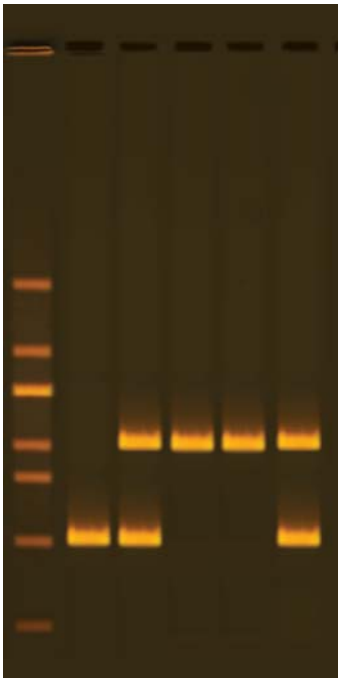
FlashBlue™ stain, however, is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to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

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

Photodocumentation of DNA (Optional)

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

Experiment Results and Analysis



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 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

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 (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 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 the gel, 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, 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.
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time	Re-stain the gel with FlashBlue™

Appendix B
Preparation and Handling of PCR Samples with Wax

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

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

How to Prepare a Wax overlay

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

Preparing PCR Samples for Electrophoresis

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

Appendix C
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

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in **Table D**.

Table D — Bulk Preparation of Electrophoresis Buffer

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

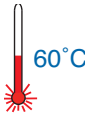
BATCH AGAROSE GELS (1.5%)

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

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

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

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour the appropriate amount of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Proceed with electrophoresis (Module II) or store the gels at 4° C under buffer.

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

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