



The Biotechnology Education Company ®

Revised
and
Updated



NOTE:

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

EDVO-Kit #

333

PCR-based Alu-Human DNA Typing

Storage: See Page 3 for
specific storage instructions

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).

EDVOTEK, Inc. • 1-800-EDVOTEK • www.edvotek.com

Table of Contents

Experiment Components	3
Experiment Requirements	4
Background Information	5
Experiment Procedures	
Experiment Overview and General Instructions	9
Module I-A: Isolation of DNA from Human Cheek Cells	10
Module I-B: Isolation of DNA from Human Hair	11
Module II: Amplification of the PV92 Locus	12
Module III: Separation of PCR Reaction Products by Agarose Gel Electrophoresis	13
Module IV: Staining Agarose Gels	15
Study Questions	18
Instructor's Guidelines	19
Pre-Lab Preparations	20
Experiment Results and Analysis	23
Study Questions and Answers	24
Appendices	25
A Troubleshooting Guides	26
B Preparation and Handling of PCR Samples With Wax	28
C Bulk Preparation of Agarose Gels	29

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



333.140702

EDVOTEK, The Biotechnology Education Company, and InstaStain are registered trademarks of EDVOTEK, Inc. UltraSpec-Agarose, PCR EdvoBeads, and FlashBlue are trademarks of EDVOTEK, Inc.

Experiment Components

Experiment # 333 contains material for up to 25 human DNA typing reactions.

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

Component	Storage	Check (✓)
A Tubes with PCR EdvoBeads™ Each PCR EdvoBead™ contains <ul style="list-style-type: none"> • dNTP Mixture • Taq DNA Polymerase Buffer • Taq DNA Polymerase • MgCl₂ • Reaction Buffer 	Room Temperature	<input type="checkbox"/>
B PV92 Primer mix concentrate	-20°C Freezer	<input type="checkbox"/>
C 200 base pair ladder	-20°C Freezer	<input type="checkbox"/>
D Control DNA concentrate	-20°C Freezer	<input type="checkbox"/>
E TE buffer	-20°C Freezer	<input type="checkbox"/>
F Proteinase K	Room temperature	<input type="checkbox"/>

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

Reagents & Supplies

Store all components below at room temperature.

Component	Check (✓)
• UltraSpec-Agarose™	<input type="checkbox"/>
• Electrophoresis Buffer (50x)	<input type="checkbox"/>
• 10x Gel Loading Solution	<input type="checkbox"/>
• InstaStain® Ethidium Bromide	<input type="checkbox"/>
• FlashBlue™ Liquid Stain	<input type="checkbox"/>
• Conical tube (15 ml)	<input type="checkbox"/>
• Microcentrifuge Tubes	<input type="checkbox"/>
• PCR tubes (0.2 ml - for thermal cyclers with 0.2 ml template)	<input type="checkbox"/>
• Disposable plastic cups	<input type="checkbox"/>
• Salt packets	<input type="checkbox"/>
• Wax beads (for waterbath option or thermal cyclers without heated lid)	<input type="checkbox"/>

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

Experiment Requirements (NOT included in this experiment)

*If you do not have a thermal cycler, PCR experiments can be conducted, with proper care, using three waterbaths. However, a thermal cycler assures a significantly higher rate of success.

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

EDVO-TECH Service

1.800.EDVOTEK

(1.800.338.6835)

Mon-Fri
8am-5:30pm ET

Please Have the Following Info:

- Experiment number and title
- Kit lot number on box or tube
- Literature version (in lower right corner)
 - Approx. purchase date

FAX 202.370.1501 • info@edvotek.com • www.edvotek.com

Visit Us Online!

www.edvotek.com

Download
Edvotek kits
with
Protocols!

Browse
our online
catalog!

Order
Products
online!

Receive
Technical
Support!



333.140702

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.

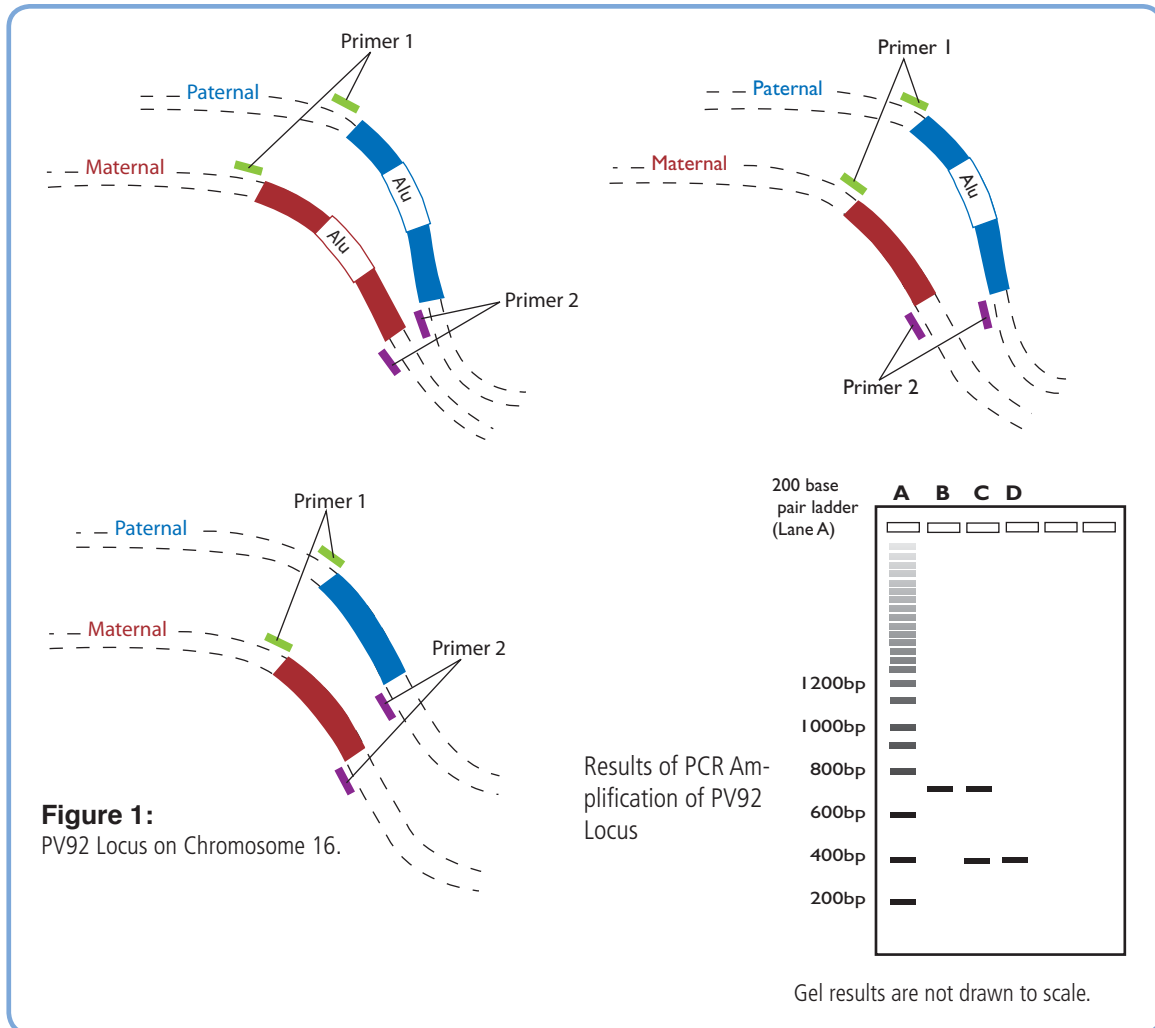
Humans (and other primates) possess hundreds of thousands of Alu elements throughout their genome. Most of them are fixed, meaning that both chromosomes have the same insertion. However, other Alus are dimorphic, meaning that the element may or may not be present at a specific chromosomal location. These 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 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 groundbreaking technology, Mullis was awarded the Nobel Prize in Chemistry in 1993.



Alu-Human DNA Typing



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



Alu-Human DNA Typing

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 Mg²⁺, 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 “un- zip” (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.



Alu-Human DNA Typing

The Experiment

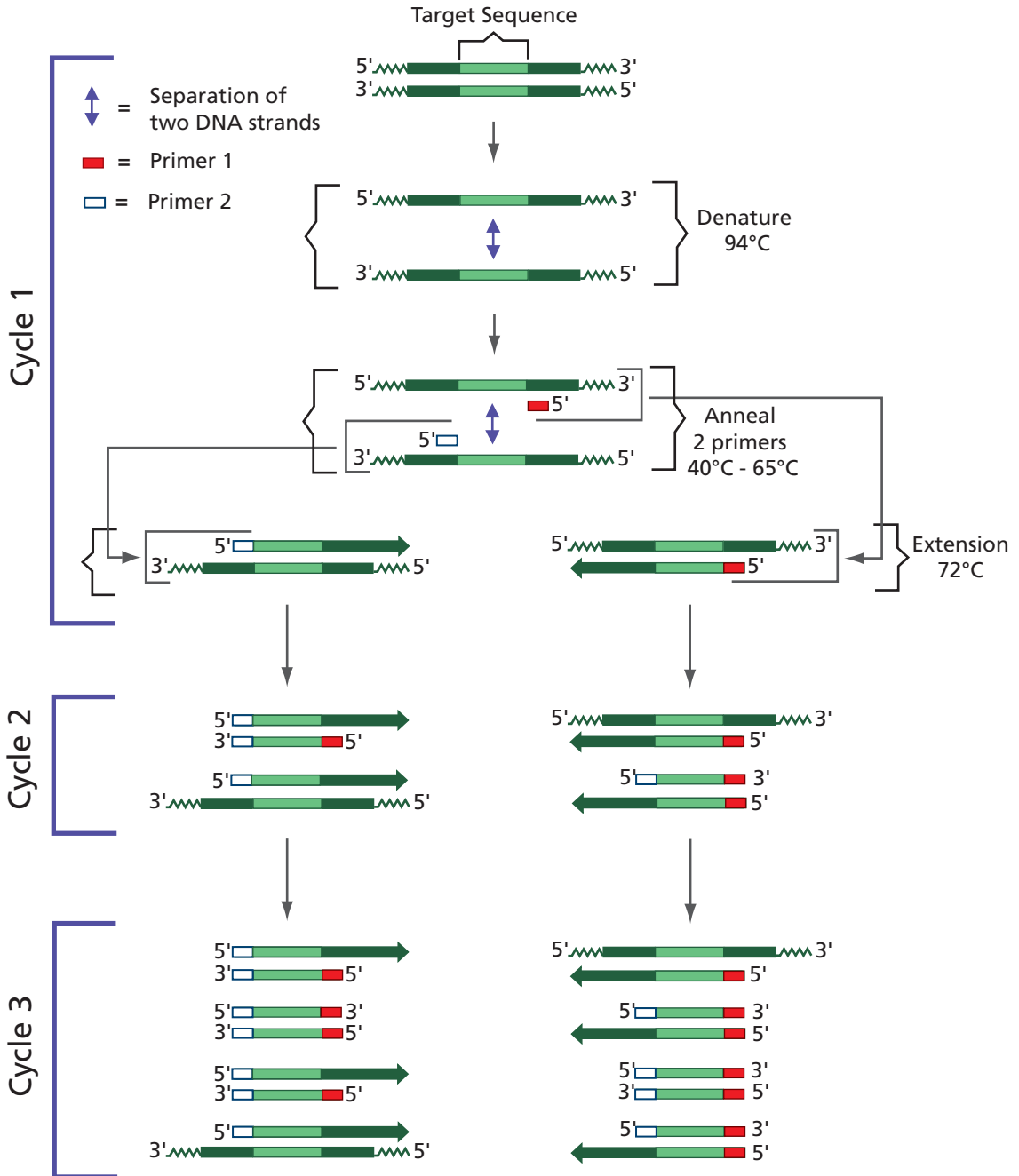


Figure 2: Polymerase Chain Reaction



Duplication of any part of this document is permitted for non-profit educational purposes only. Copyright © 2000-2014 EDVOTEK, Inc., all rights reserved. 333.140702

Experiment Overview and General Instructions

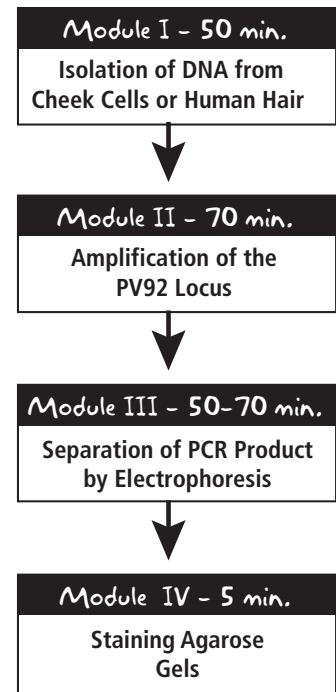
EXPERIMENT OBJECTIVE:

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).

IMPORTANT

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

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

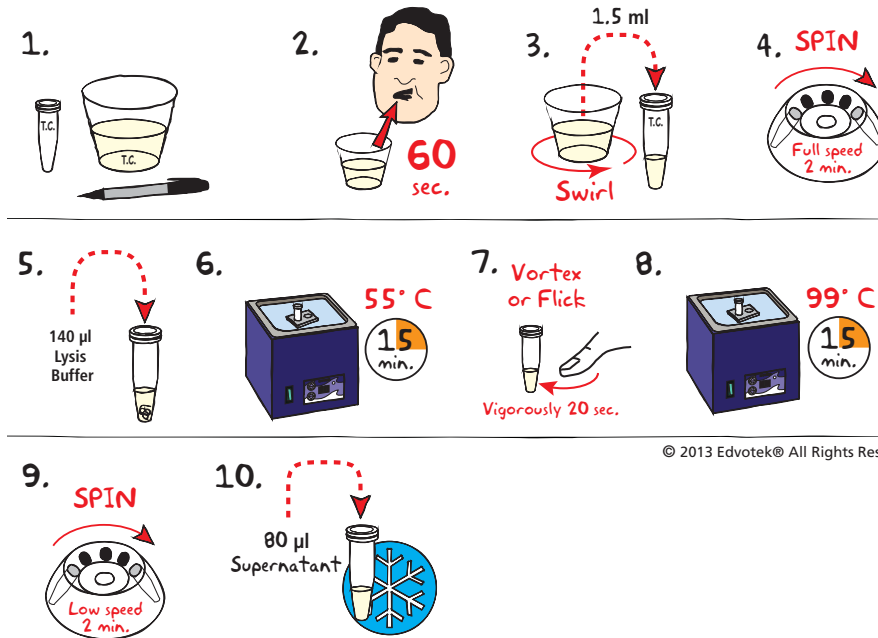


NOTE: Experimental times are approximate.

The Experiment

Preferred Method

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



© 2013 Edvotek® All Rights Reserved.

Warning!

Students should use screw-cap tubes when boiling samples.

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

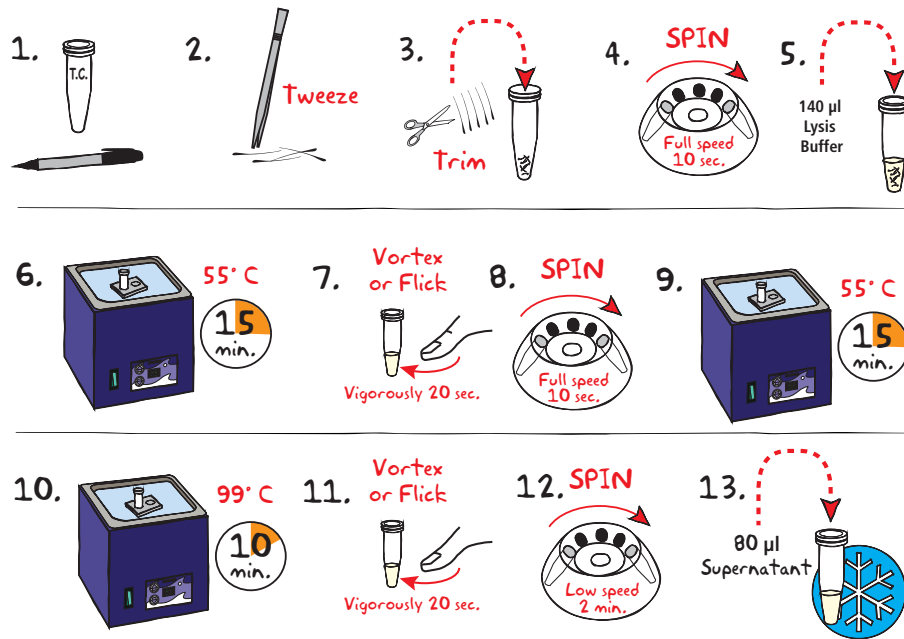
**OPTIONAL STOPPING POINT:**

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



Duplication of any part of this document is permitted for non-profit educational purposes only. Copyright © 2000-2014 EDVOTEK, Inc., all rights reserved. 333.140702

Module I-B: Isolation of DNA from Human Hair



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

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

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

1. **LABEL** a 1.5 ml screw top microcentrifuge tube with your lab group and/or initials.
2. Using tweezers, **GRASP** 2-3 hair shafts at the base and **PULL** quickly. **COLLECT** at least 5 hairs that include the root and the sheath (a sticky barrel-shaped layer of cells that encircles the root end of the hair).
3. Using a clean scalpel or scissors, **TRIM** away any extra hair from the root (leave about 1 cm in length from the root). **TRANSFER** the roots to the labeled tube using forceps.
4. **CAP** the tube and **CENTRIFUGE** the sample for 10 seconds at full speed to collect the roots at the bottom of the tube.
5. **ADD** 140 µL lysis buffer to the tube. For best results, completely **IMMERSE** the follicles in the solution.
6. **CAP** the tube and **PLACE** it in a waterbath float. **INCUBATE** the sample in a 55° C waterbath for 15 min.
7. **MIX** the sample by vortexing or flicking the tube vigorously for 20 seconds.
8. **CENTRIFUGE** the sample for 10 seconds at full speed to collect the roots at the bottom of the tube.
9. **INCUBATE** the sample at 55° C for an additional 15 min.
10. **MOVE** the sample to a 99° C waterbath. **INCUBATE** for 10 min. Be sure to use screw-cap tubes when boiling samples.
11. **MIX** the sample by vortexing or flicking the tube vigorously for 20 seconds.
12. **CENTRIFUGE** the cellular lysate for 2 min. at low speed (6000 rpm).
13. **TRANSFER** 80 µl of the supernatant to a clean, labeled microcentrifuge tube. **PLACE** tube in ice.
14. **PROCEED** to Module II: Amplification of the PV92 Locus.



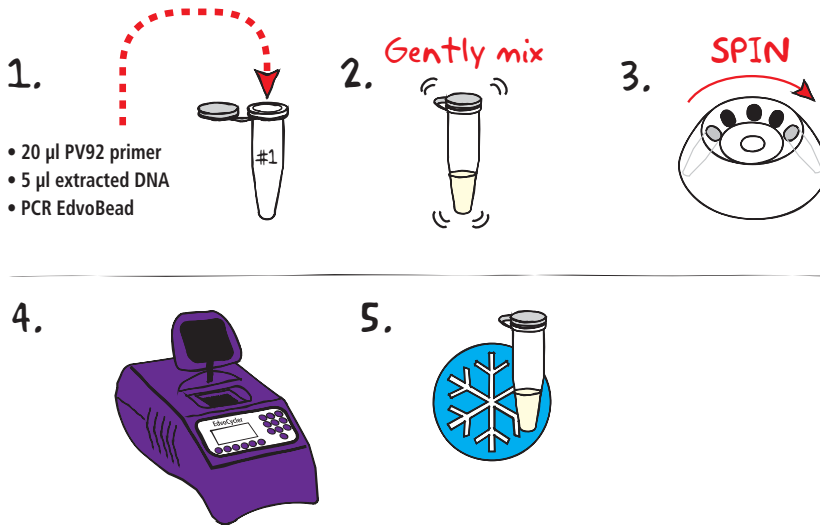
OPTIONAL STOPPING POINT:

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

The Experiment



Module II: Amplification of the PV92 Locus

**NOTES AND REMINDERS:**

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

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

- ADD** 20 µL PV92 primer mix, 5 µl extracted DNA (or control DNA) and one PCR EdvoBead™ to a labeled 0.2 ml or 0.5 ml PCR tube (depending on the Thermal Cycler).
- MIX** the PCR sample. Make sure the PCR EdvoBead™ is completely dissolved.
- CENTRIFUGE** to collect the sample at the bottom of the tube.
- 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

NOTE:

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

**OPTIONAL STOPPING POINT**

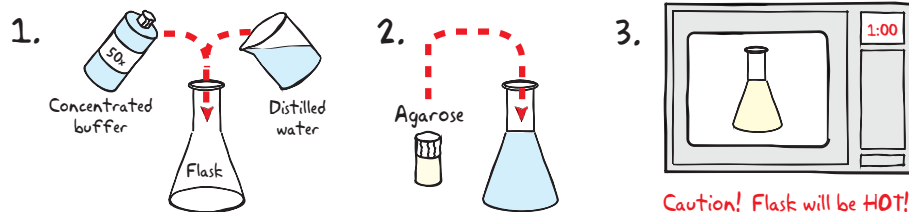
The PCR samples may be stored at -20°C for electrophoresis at a later time.



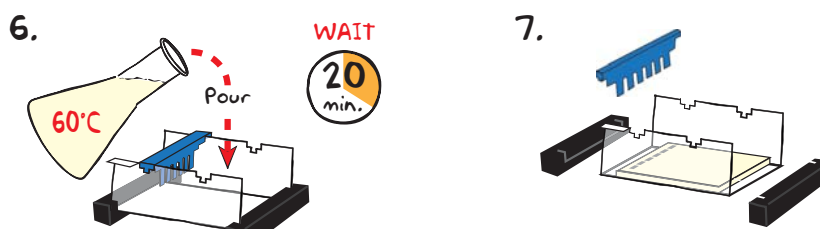
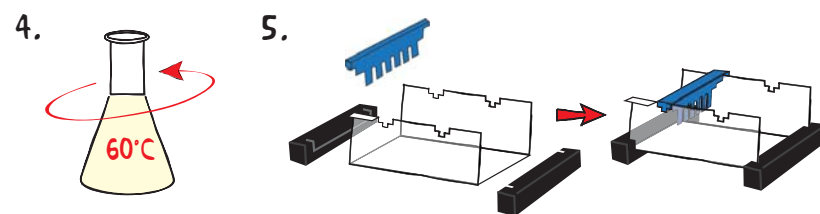
Module III: Separation of PCR Reaction Products by Electrophoresis



Wear gloves and safety goggles



Caution! Flask will be HOT!



NOTES:

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

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

The Experiment

- DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
- MIX** agarose powder with 1X buffer in a 250 ml flask (see Table B, page 14).
- 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).
- COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
- 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.
- 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.
- REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A 1x Electrophoresis Buffer (Chamber Buffer)

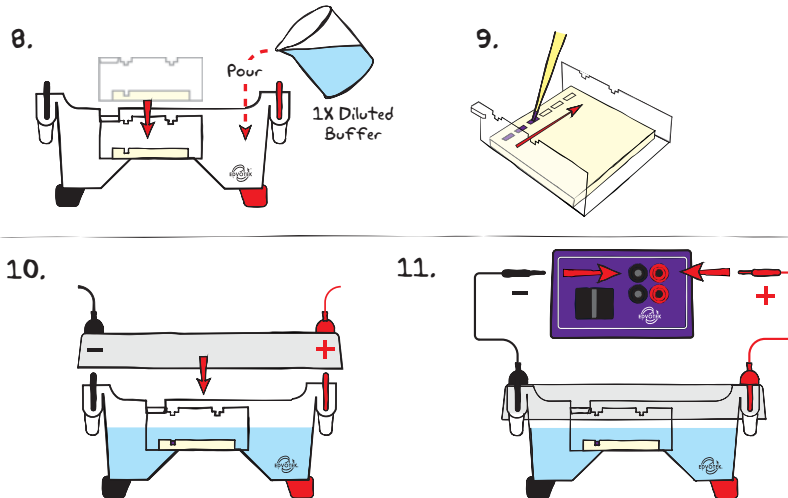
EDVOTEK Model #	Total Volume Required	Dilution 50x Conc. Buffer + Distilled Water	
M6+	300 ml	6 ml	294 ml
M12	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml



Module III: Separation of PCR Reaction Products by Electrophoresis

Reminder:

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



The Experiment

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

Table 1

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

* Optional, or additional student sample.

Table B

Individual 1.5% UltraSpec-Agarose™ Gel

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

Table C

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

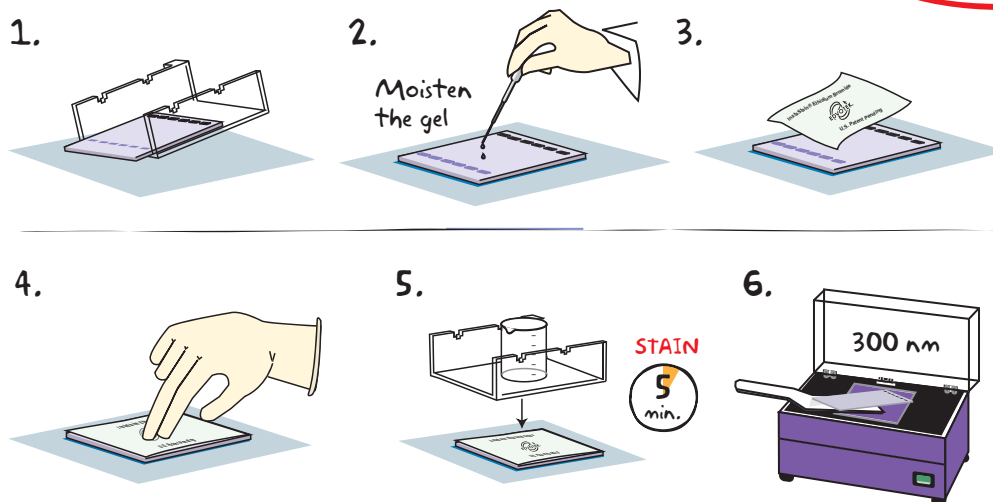
Volts	Recommended Time	
	Minimum	Maximum
125	55 min.	1 hour 15 min.
70	2 hours 15 min.	3 hours
50	3 hours 25 min.	5 hours



Duplication of any part of this document is permitted for non-profit educational purposes only. Copyright © 2000-2014 EDVOTEK, Inc., all rights reserved. 333.140702

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

Preferred Method

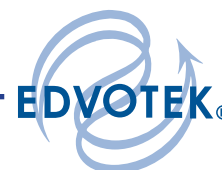


WEAR GLOVES AND GOGGLES WHEN USING THIS PRODUCT.

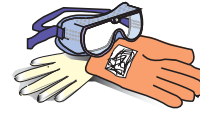
- Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface.
DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.
- MOISTEN** the gel with a few drops of electrophoresis buffer.
- Wearing 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.
- 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.
- PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. **STAIN** the gel for 3-5 min. for an 0.8% gel or 8-10 min. for a gel 1.0% or greater.
- REMOVE** the InstaStain® Ethidium Bromide card(s). **VISUALIZE** the gel using a long wavelength ultra-violet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!

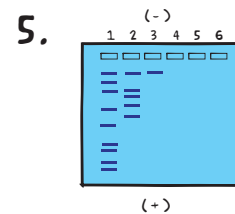
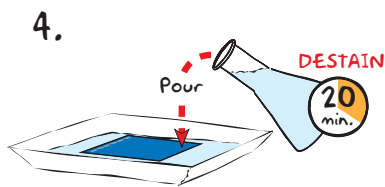
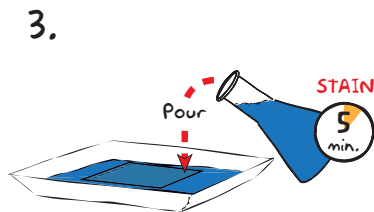
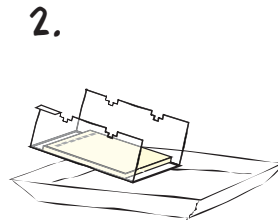
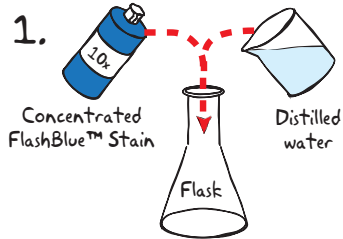
The Experiment



Module IV-B: Staining Agarose Gels using FlashBlue™



Wear gloves
and safety goggles



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

Alternate Protocol:

1. **DILUTE** one mL of concentrated FlashBlue™ stain with 149 mL dH₂O.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.



Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

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



This experiment has been revised and updated. PCR Cycling Conditions have changed. Please review your PCR program before performing the experiment.

The results will differ slightly from previous versions of this kit. Please refer to page 23 for expected results.



333.140702

Pre-Lab Preparations: Module I

FOR MODULE I-A**Each Student should receive:**

- One cup containing 10 ml of saline solution
- One screw-cap tube
- One microcentrifuge tube

Reagents to be Shared by Two Students:

- 300 μ l 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.

FOR MODULE I-B**Each Student should receive:**

- One screw-cap tube
- One microcentrifuge tube

Reagents to be Shared by Two Students:

- 300 μ l Lysis buffer

MODULE I-A: ISOLATION OF DNA FROM HUMAN CHEEK CELLS**Preparation of Saline Solution**

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

Preparation of Lysis Buffer

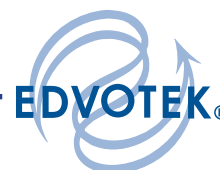
(Prepared no more than one hour before starting the experiment.)

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

MODULE I-B: ISOLATION OF DNA FROM HUMAN HAIR**Preparation of Lysis Buffer**

(Prepared no more than one hour before starting the experiment)

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



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

FOR MODULE II

Each Student should receive:

- One PCR tube and PCR EdvoBead™
- 20 µl Gel Loading Solution

Reagents to be Shared by Two Students:

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

Additional Materials

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

Programming the Thermal Cycler

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

NOTE:

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



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



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

NOTE:

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

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

**FOR MODULE III
Each Student Group
should receive:**

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™ Powder
- Tube of 200 bp ladder (30 µl)
- Control PCR reaction (optional)

PREPARATION OF AGAROSE GELS

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

Individual Gel Preparation:

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

Batch Gel Preparation:

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

Preparing Gels in Advance:

Gels may be prepared ahead and stored for later use. Solidified gels can be 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 200 base pair ladder and samples from 4 or 5 students. The control PCR reaction can also be loaded in one of the wells.

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

Wear gloves
and safety goggles



Pre-Lab Preparations - Module IV: Staining

FOR MODULE IV
Each Student Group
should receive:

- 2 InstaStain® cards per
7 x 14 cm gel

STAINING WITH INSTASTAIN® ETHIDIUM BROMIDE

Preferred Method

InstaStain® Ethidium Bromide provides the sensitivity of ethidium bromide while minimizing the volume of liquid waste generated by staining and destaining a gel. An agarose gel stained with InstaStain® Ethidium Bromide is ready for visualization in as little as 3 minutes! Each InstaStain® card will stain 49 cm² of gel (7 x 7 cm). You will need 2 cards to stain a 7 x 14 cm gel.

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

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

STAINING WITH FLASHBLUE™

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

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

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

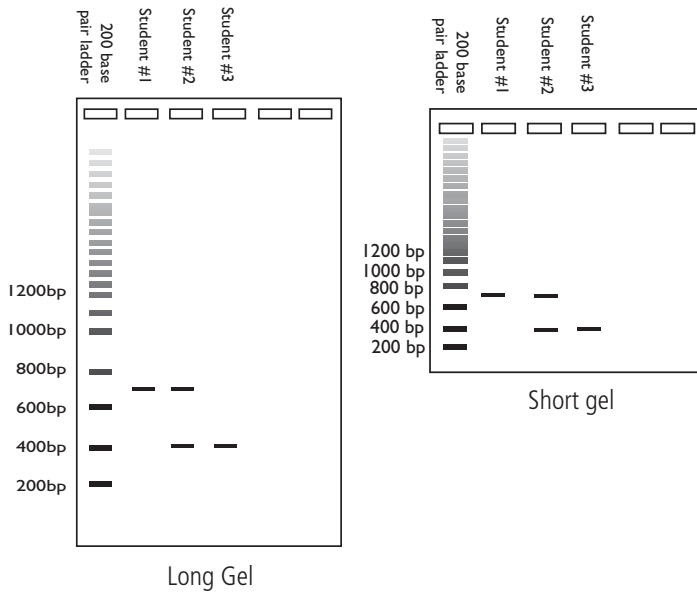
PHOTODOCUMENTATION OF DNA (OPTIONAL)

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



Experiment Results and Analysis

Idealized Schematics



**STOP
READ
ME**

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

Instructor's Guide

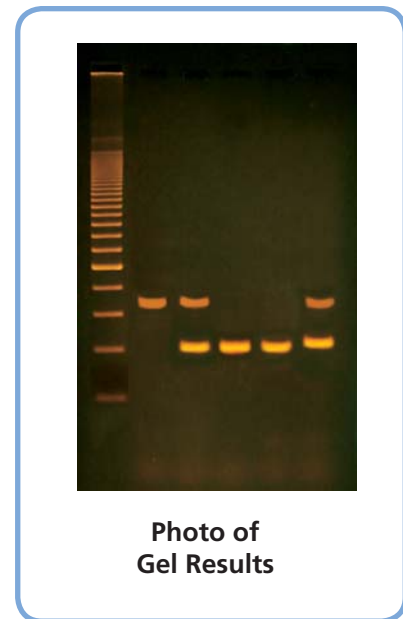
The idealized schematics show the possible PCR products from different genotypes.

Three possible results for PV92 amplification (shown above):

Lane 1	Student #1	Homozygous for Alu insertion (+/+)
Lane 2	Student #2	Heterozygous for Alu insertion (+/-)
Lane 3	Student #3	No genotype 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 non-specific primer binding and the subsequent amplification of these sequences.



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

Appendices

- A EDVOTEK® Troubleshooting Guide
- B Preparation and Handling of PCR Samples With Wax
- C Bulk Preparation of Agarose Gels

Material Safety Data Sheets:

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

EDVO-TECH Service

1.800.EDVOTEK

(1.800.338.6835)



Mon-Fri
8am-5:30pm ET

Please Have the Following Info:

- Experiment number and title
- Kit lot number on box or tube
- Literature version (in lower right corner)
 - Approx. purchase date

FAX 202.370.1501 • info@edvotek.com • www.edvotek.com

Visit Us Online!

www.edvotek.com

<p style="font-size: 10pt; margin: 0;">Download Edvotek kits with Protocols!</p>	<p style="font-size: 10pt; margin: 0;">Browse our online catalog!</p>	<p style="font-size: 10pt; margin: 0;">Order Products online!</p>	<p style="font-size: 10pt; margin: 0;">Receive Technical Support!</p>
---	--	--	--



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.



PCR AND ELECTROPHORESIS

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

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 μ L of 10x Gel Loading Buffer to the sample. Proceed to Module III to perform electrophoresis.



Bulk Preparation of Agarose Gels

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

BULK ELECTROPHORESIS BUFFER

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

Table
D

Bulk Preparation of Electrophoresis Buffer

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

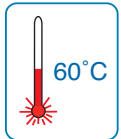
BATCH AGAROSE GELS (1.5%)

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

- Use a 500 ml flask to prepare the diluted gel buffer
- Pour the appropriate amount of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
- With a marking pen, indicate the level of solution volume on the outside of the flask.
- 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.
- 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.
- Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed.
- Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Proceed with electrophoresis (Module II) or store the gels at 4° C under buffer.

Note:

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

Table
EBatch 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