



Biotechnology in the Classroom

PARTNERSHIP FOR BIOTECHNOLOGY & GENOMICS EDUCATION
UNIVERSITY OF CALIFORNIA DAVIS

PCR Kit

POLYMERASE CHAIN REACTION: Amplification of Alu PV92

Adapted from:

Ah, Lou! There really are differences between us!

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POLYMERASE CHAIN REACTION

Amplification of Alu PV92 Insert

Introduction

Several recent studies suggest that a small piece of DNA, called an Alu element, found its way into the genome less than one million years ago. In a given group of individuals, some carry this 300 base pair insertion while others do not.

During this laboratory, students will extract their own DNA from a cell sample taken from the lining of their mouth. They will then use a powerful molecular biology technique, called the Polymerase Chain Reaction, to amplify this Alu region so that it can be visualized using gel electrophoresis.

Attached readings and activities will provide more information about the Alu region and help illustrate the process of PCR and its importance in modern day biology.

Objectives

1. Isolate DNA from cheek cells and prepare a reaction for PCR amplification.
2. List and explain the importance of each component of PCR.
3. Electrophorese the Alu repeat to determine your genotype for the Alu insertion.
4. Understand the difference between heterozygous and homozygous.
5. Calculate allele and genotype frequencies.

Materials

For Each Student

- gloves
- safety goggles*
- disposable transfer pipet
- two 1.5 ml microtubes
- 200 μ l of 5% Chelex in a 1.5 ml tube
- 0.2 ml PCR tube with lid
- microtube cap lock
- paper cup with 10 ml of 0.9% NaCl *

For Each Lab Group

- 20 μ l micropipet
- 100 μ l micropipet
- box of 20 – 200 μ l tips
- microtube rack
- PCR tube rack
- waterproof pen
- 80 μ l master mix
- 80 μ l primer mix
- 30 μ l 0.9% NaCl *
- cup of crushed ice*

Common Materials

- two dual gel boxes
- power supply
- carboy with 1X TBE solution
- 2.0% agarose gel
- microcentrifuge
- heat block & thermometer
- thermal cycler & 96 well PCR tray
- UV transilluminator
- MiniVisionary system
- PCR grid
- gel electrophoresis grid



Instructor Use

- 1000 µl micropipet
- 100 µl micropipet
- 20 µl micropipet
- box of 1000 µl tips
- box of 20 – 200 µl tips
- 100 bp ladder
- control DNA
- loading dye with SYBR® Green
- sterile water
- lab tape
- PCR and microcentrifuge tube rack
- 4 gel trays
- 4 10-well combs
- 4 12 well combs

* Materials provided by instructor

Advance Preparation

DAY 1

1. Mix 4.5 g of non-iodized table salt in a 500 ml bottle of drinking water to create a 0.9% NaCl solution.
 - **Since students will be using the NaCl solution as a mouthwash, be sure to use containers and measuring equipment that is designated for use with “edible only” experiments.**
2. For each student, aliquot 200 µl 5% Chelex in a 1.5 ml tube.
 - **Use a 1000 µl micropipet so the beads will not clog the tip and swirl the tube frequently to ensure each student gets a 5% solution.**
3. For each lab group, aliquot 100 µl of 0.9% NaCl solution. You may use some of the leftover 0.9% NaCl solution from Step 1.
4. Set heat block to 99°C (5.5 on the high dial)
5. For each student, aliquot 10 ml of 0.9% NaCl solution in a paper cup

DAY 2:

1. Buy/prepare crushed ice for each lab station.
 - **It is very important to keep the Master and Primer mix cold while students are preparing their PCR reactions.**
2. For each lab group, aliquot:
 - a) 80 µl Master Mix (keep on ice)
 - b) 80 µl Primer Mix (keep on ice)
3. Prepare a positive and negative control solution.
 - a) **Positive Control:**
20 µl Master Mix
20 µl Primer Mix
10 µl control DNA
 - b) **Negative Control:**
20 µl Master Mix
20 µl Primer Mix
10 µl sterile water
4. Place the paper PCR grid copied from lab manual next to the thermal cycler.
 - **Make sure the 96 well tray is in the thermal cycler.**
5. Follow the startup instructions on the thermal cycler lid.

DAY 3:

1. Make a 1X TBE solution from 10X stock and place in carboy.

Figure on about 1L per class:

$$M_1V_1=M_2V_2$$
$$(10X)(x) = (1X)(1,000 \text{ ml})$$
$$x = 100 \text{ ml}$$

*Use 100 ml of (10X) buffer and add **distilled** water to bring the final concentration to 1,000 ml*
2. Pour four 2% agarose gels. Cover bench with lab paper prior to pouring gels.



3. Set up a gel run area with two dual gel boxes and one power supply. Tape the paper grid included in this packet near each box. Put gel trays in box.
4. Add 5 ul of loading dye to each of the student sample tubes. Be sure to change tips for every tube.

➤ **Remember the SYBR Green DNA stain is in the loading dye.**

DAY FOUR:

1. Retrieve gels from refrigerator.
2. Set up MiniVisionary photo system according to instructions included with system in the kit box.

Teaching Tips

CHELEX

Chelex is a resin containing organic chemicals that remove metal ions that might degrade the DNA during boiling.

USE OF MICROPIPETTES:

These extremely precise and expensive instruments need to be handled gently.

- **Do not wind the micropipet dial past the maximum amount indicated on the top of the plunger.**
- **Always hold the micropipet in a vertical position.**
- **Always change tips between aliquots to reduce risk of contamination.**

USE OF THERMAL CYCLER

- **Make sure you have a tray installed in the heat block of the thermal cycler. Students' tubes should be in this tray. Without a tray, the tubes may melt!**

After the run is complete, tubes will be held at 4°C until you quit the procedure. Be sure to end the procedure prior to turning the power off.

Instructions for operating the thermal cycler are taped to the machine. All cycling protocols that you will need are pre-programmed into the machine.

USE OF SYBR® GREEN:

SYBR® Green is a fluorescent dye used to stain DNA and RNA. Tests have found it to be less mutagenic than ethidium bromide. This dye has been pre-added to the loading dye students add to the DNA sample and will fluoresce when exposed to UV light.

This chemical should never be handled without gloves. Used gels, tips, tubes, and TBE buffer should be returned to the Biotechnology in the Classroom program for proper disposal.

In this experiment, the agar is never touched without gloves. Please see enclosed safety binder for more information about SYBR® Green.

USE OF POWER SUPPLIES:

The power supply produces a voltage that is high enough to cause severe electrical shock if handled improperly.

- **Do NOT plug power supply into wall receptacle until the safety cover is positioned on the cell and all other electrical connections are properly made.**

USE OF THE UV TRANSILLUMINATOR:

When students are ready to look at their gels, view them only through the UV blocking cover. Do not attempt to operate the transilluminator with the UV blocking cover raised. Any attempt to override the safety interlock and view the gel directly could result in serious eye and skin damage.

- **Do not use the transilluminator if the UV blocking cover becomes cracked or broken.**



BUFFER SOLUTION:

Tris/Borate/EDTA (TBE) buffer is commonly used in electrophoresis systems. This salt solution both conducts the electric current and controls the pH of the solution during separation of DNA fragments. Once the gels have run, the instructor should pour the used TBE solution down the drain.

DISTILLED WATER:

Minerals in regular tap water will quickly stain equipment. Please rinse and air-dry both the gel trays and gel boxes in distilled water. Be careful not to dislodge the wiring at the base of the gel box.

USE OF THE MINIVISIONARY SYSTEM

Please review the enclosed instruction manual for setup of the MiniVisionary cables. Note that the camera hood should remain on the camera and that the SYBR[®] green filter is in place.

➤ **Do not remove the hood from the camera or the filter from the hood.**

Follow these instructions when taking a picture of your gel electrophoresis results:

1. Plug in the power cords to both the controller and the printer. Turn both pieces of equipment on.
2. Set the integration time to 100 using the buttons on top of the controller.
3. With the transilluminator OFF, place the gel tray directly on the platform. There is no need to remove the gel from the tray.

➤ **The camera hood is strictly for photographic purposes. Do not attempt to use the camera hood to view subjects on the transilluminator. Serious skin or eye injuries may result from improper use.**

4. Turn on the transilluminator.
5. Print the image by using the large print button on the front panel of the controller. Do not use the small button on the front panel of the thermal printer.
6. Turn off the transilluminator. Remove and dispose of the gels in the regular trash.

Answers to Student Activity

1. *Answers will vary with class data.*
2. *Answers will vary with class data*
3. *Answers will vary with class data*
4. *Yes, the results should add up to one.*
5. *Answers will vary with class data.*
6. *Answers will vary with class data*
7. *Answers will vary with class data*
8. *Answers will vary with class data*
9. *68*
10. *0.7*
11. *0.8*
12. *0.42*



POLYMERASE CHAIN REACTION

Amplification of Alu PV92 Insert



Background Reading

ALU REGION

Only three to ten percent of the nearly six billion base pairs of DNA in your body is actually used to directly code for proteins. The protein-encoding regions are scattered throughout the genome and the genes may be separated by many thousands of base pairs. Furthermore, most genes are themselves broken into smaller protein-encoding segments called exons and intervening regions with an ill-defined or non-essential role called introns. Whatever their function, examination of these introns has revealed the presence of unique genetic elements that can be found in a number of different locations within the genome. One of the first such repeating elements identified is Alu.

Alu repeats are approximately 300 base pairs in length. So named because most carry within them the base sequence AGCT, the recognition site for the Alu I restriction endonuclease, over 500,000 of these repeats are scattered throughout the human genome. On average, one can be found every 4,000 base pairs along a human DNA molecule. How they arose is still a matter of speculation but evidence suggests that the first one may have appeared in the genome of higher primates about 60 million years ago. Approximately every 100 years since then, a new Alu repeat has inserted itself in an additional location in the human genome. Alu repeats are inherited in a stable manner; they come intact in the DNA your mother and father contributed to your genome.

Some Alu repeats are fixed in a population, meaning all humans have that particular Alu repeat. Others are said to be dimorphic; different individuals may or may not carry a particular Alu sequence at a particular chromosomal location. In this lab we will be analyze a dimorphic Alu region.

PV92 is a human-specific Alu insertion on chromosome 16. The PV92 genetic system has only two alleles indicating the presence (+) or absence (-) of the Alu transposable element on each of the paired chromosomes. This results in three PV92 genotypes (++ , +- , or --). The + and - alleles can be separated by size using gel electrophoresis.

POLYMERASE CHAIN REACTION

In order to analyze a particular gene out of the tens or hundreds of thousands of genes in a genomic DNA sample, sensitive and sequence specific techniques are needed. One such methodology, Polymerase Chain Reaction (PCR), has been developed to amplify specific DNA sequences in a DNA sample, even with minute amounts of starting material. The PCR technique is commonly used in the molecular analysis of genes, but it has also been used to amplify, and subsequently analyze, the DNA from many sources once thought impossible. For example, using PCR scientists have analyzed DNA from a single sperm, museum specimens of extinct animals and millions of year-old plant and animal fossils. In addition, PCR can be used to analyze the DNA of a blood or skin sample that might be evidence in a criminal investigation. Following is a step-by-step description of the PCR process.

The hypothetical DNA segment below will be amplified through PCR. The ???????? may be a very long or short set of deoxyribonucleotides--it doesn't matter.

```
AATTGCCCCGGGAAATT  ??????????????????  AAATTTGGGCCCAA
TTAACGGGGCCCTTAA  ??????????????????  TTAAACCCGGGT
```



STEP 1 – SYNTHESIZE PRIMERS

Synthesize **primers** of about 20 deoxyribonucleotides long. A primer is a small segment of DNA that matches a known series of bases on the target strand. This step is accomplished by using a machine called a DNA synthesizer.

In this example, we will make one primer exactly like the lower right-hand sequence, and one primer exactly like the upper left-hand sequence.

Target DNA strand:

AATTGCCCGGGAAATT ??????????????????
TTAACGGGGCCCTTAAA ??????????????????

AAATTGGGCCCAA
TTTAAACCCGGGT

Primer 1:
AATTGCCCGGGAAATT

Primer 2
TTTAAACCCGGGT

STEP 2 – DENATURE DNA

Target DNA is heated, which causes the paired strands to separate. The single strands are now accessible to primers.

AATTGCCCGGGAAATT ?????????????????? **AAATTGGGCCCAA**

TTAACGGGGCCCTTAAA ?????????????????? **TTTAAACCCGGGT**

STEP 3 – ANNEALING

Add the primers you created earlier. The amount should be large relative to the amount of DNA being amplified. Cool the reaction mixture to allow double-strands to form again. Because of the large excess of primers, the two strands will always bind to the primers, instead of with each other.

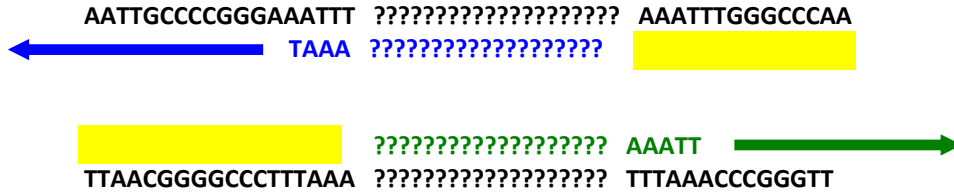
AATTGCCCGGGAAATT ?????????????????? **AAATTGGGCCCAA**
Primer 2

Primer 1
TTAACGGGGCCCTTAAA ?????????????????? **TTTAAACCCGGGT**



STEP 4 - EXTENSION

Add the four deoxyribonucleotides and an enzyme that can read the opposing strand's sentence and extend the primer's sentence by hooking letters together in the order in which they pair across from one another. This particular enzyme is called a **DNA polymerase**. One such enzyme used in PCR is called **Taq polymerase** (originally isolated from a bacterium that can live in hot springs). This enzyme can withstand the high temperature necessary for DNA-strand separation and can be left in the reaction.



If you look at this arrangement, you can see that primer 2 paired to the upper strand is extended to the left in the direction of the arrow and primer 1 paired to the lower strand and is being extended to the right in the direction of the arrow, Remember that the ??? also represent deoxyribonucleotides.

There are now four strands, where originally there were only two. If one leaves everything in the test tube and repeats the procedure, there will be eight strands. Do it again and there will be 16, etc. Therefore, about 20 cycles will theoretically produce approximately one-million copies of the original sequences.

With this amplification potential, there is enough DNA in one-tenth of one-millionth of a liter (0.1 microliter) of human saliva to use the PCR system to identify a genetic sequence as having come from a human being.

In the following laboratory, you will use PCR to amplify a dimorphic *Alu* repeat (designated PV92) found on your number 16 chromosome. You will use your own DNA as template. After you amplify the *Alu* repeat region using PCR, you will determine whether or not you carry this particular *Alu* sequence on one or both of your number 16 chromosomes. This will be accomplished by running your PCR product on an agarose gel.

Attached readings explain the PCR process and the significance of *Alu* regions in more detail. In addition, an excellent animated tutorial showing the steps of PCR is available at the Cold Spring Harbor web site: <http://vector.cshl.org/shockwave/pcranwhole.htm>



Objectives

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2. List and explain the importance of each component of PCR.
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5. Calculate allele and genotype frequencies.

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- safety goggles*
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- 0.2 ml PCR tube with lid
- microtube cap lock
- paper cup with 10 ml of 0.9% NaCl *

For Each Lab Group

- 20 μ l micropipet
- 100 μ l micropipet
- box of 20 – 200 μ l tips
- microtube rack
- PCR tube rack
- waterproof pen
- 80 μ l master mix
- 80 μ l primer mix
- 30 μ l 0.9% NaCl *
- cup of crushed ice*

Common Materials

- two dual gel boxes
- power supply
- carboy with 1X TBE solution
- 2.0% agarose gel
- microcentrifuge
- heat block & thermometer
- thermal cycler & 96 well PCR tray
- UV transilluminator
- MiniVisionary system
- PCR grid
- gel electrophoresis grid

Instructor Use

- 1000 μ l micropipet
- 100 μ l micropipet
- 20 μ l micropipet
- box of 1000 μ l tips
- box of 20 – 200 μ l tips
- 100 bp ladder
- control DNA
- loading dye with SYBR[®] Green
- sterile water
- lab tape
- PCR and microcentrifuge tube rack
- 4 gel trays
- 4 10-well combs
- 4 12 well combs

* Materials provided by instructor



Precautions

- **The SYBR[®] Green stain found in the loading dye is a hazardous chemical. Although it is present in an extremely low concentration, it still should not be touched. After electrophoresis, the gels should only be handled with gloves. Always leave gel in its tray.**
- **When using the microcentrifuge, always make sure the tubes are loaded in the rotor symmetrically; each tube should be balanced by another tube directly opposite it. Never run the centrifuge with the lid open or the rotor missing. Never put hands in the rotor area unless the rotor is completely stopped.**
- **Ultraviolet (UV) radiation can cause severe eye and skin damage! The camera hood is strictly for photographic purposes. Do not attempt to use the camera hood to view samples on the transilluminator. Make sure that the hood is seated correctly and there are no raised edges that would allow UV light to leak out.**
- **If you choose to view the gel prior to making a photograph, be sure the UV blocking cover is down before turning the power on.**
- **Follow your teacher's instructions regarding micropipet handling.**
- **The power supply produces a voltage that is high enough to cause severe electrical shock if handled improperly. Assure that you are thoroughly drilled in the procedures regarding the use of this unit, making electrical connections, and that the teacher directly supervises you.**



Procedure

DNA PREPARATION USING A SALINE MOUTHWASH

1. Gather all required lab supplies and think of a 4 digit identification number (PIN) to write down on your lab sheet.
2. Get a paper cup with 10 ml of 0.9% NaCl solution from your instructor. Swirl the solution in your mouth for 30 seconds.
3. Expel the NaCl solution back into the cup and swirl to mix the cells.
4. Label the cap of a 1.5 ml microtube with your PIN. Using a disposable pipet, transfer 1000 μ l of the saliva & NaCl solution into the tube. Close the cap tightly. Discard cup.
5. In a balanced microcentrifuge, spin sample for 1 minute to pellet the cells.
6. Observe your cell pellet at the bottom of the tube. Pour off and discard the supernatant, being careful not to lose your cell pellet.
➤ **Note: It is okay if some supernatant is left in the tube.**
7. Resuspend your cell pellet in 30 μ l of 0.9% NaCl. Make sure the entire cell pellet is thoroughly mixed by pipetting up and down several times or “racking” your tube.
➤ **Note: To “rack” your sample, be sure the top of the tube is closed, hold tube firmly at the top, and pull it across a microfuge rack 2-3 times.**
8. Label a 1.5 ml tube of 5% Chelex with your PIN number.
9. Withdraw 30 μ l of the cheek cell suspension and add it to the Chelex tube.
➤ **Note: Do not pipet up and down at this step or else you will clog the tip with Chelex.**
10. Secure your tube with a cap lock and place it in the 99°C heat block for 10 minutes.
11. Remove tube from heat block and take off your cap lock. Shake your tube well and then place it in a balanced centrifuge. Spin for 1 minute.
12. Withdraw 60 μ l of supernatant (no Chelex beads) to a clean tube, labeled with your PIN. Discard all other tubes.
➤ **Note: This stored sample is your “DNA” tube.**
13. Clean up your lab station and wash your hands.

⊕ **STOP POINT:** Place your DNA tube in the class microtube rack so that your teacher can refrigerate your isolated DNA until you are ready to prepare your PCR amplification.



POLYMERASE CHAIN REACTION

1. Gather all required lab supplies and retrieve your DNA tube from the class microtube rack.
2. Label a 200 μ l PCR tube with your 4 digit PIN.
3. Put a new tip on your 20 μ l micropipet and dispense 20 μ l of Master Mix into your PCR tube.
4. Change your pipet tip and add 20 μ l of Primer Mix into your PCR tube.
5. Change your pipet tip and add 10 μ l of your purified DNA into your PCR tube.

➤ **Note: Slowly pipet up and down several times to mix all the reagents in your reaction tube.**

6. Your instructor has prepared two PCR control tubes:

Positive Control:

20 μ l Master Mix
20 μ l Primer Mix
10 μ l Control DNA

Negative Control

20 μ l Master Mix
20 μ l Primer Mix
10 μ l sterile water

7. Place your reaction tube into the thermal cycler and record the location of your tube on the grid provided by your teacher.
8. Your instructor will begin the thermal cycler. The cycling protocol for amplification of this Alu region is:

95°C	10 minutes	} Repeat for 30 cycles
94°C	30 seconds	
60°C	30 seconds	
72°C	2 minutes	
72°C	10 minutes	
4°C	hold	

➤ **Note: This process will take longer than your class period so your instructor will remove the tubes from the machine upon completion.**

⊕ **STOP POINT:** Instructor – The thermal cycler may be left on overnight. Upon removal from the machine, place the amplified DNA tubes in the class PCR tube rack. Refrigerate the samples until you are ready to run your agarose gels



ELECTROPHORESIS OF AMPLIFIED DNA

1. Gather necessary lab supplies and put on gloves and safety goggles.
2. Retrieve your PCR tube and tap it lightly on the counter to bring the liquid to the bottom of the reaction tube.
3. Confirm that your instructor has already loaded 5 μ l of loading dye to your PCR tube.
4. Carefully load 15 - 20 μ l of your reaction into a well on one of the gels your teacher has prepared. On the grid next to the gel box, write down your PIN in the appropriate lane number.
➤ **Note: Avoid poking the pipette tip throughout the bottom of the gel or spilling sample over the sides of the well. Use a new tip for each sample.**
5. Your instructor will load 5 μ l of the 100 bp ladder into one of the wells of each gel.
6. Your instructor will load one lane in each gel with 10 μ l of a positive control and one lane with 10 μ l of a negative control.
7. When all samples are loaded, your instructor will attach the electrodes from the gel box to the power supply and electrophorese your samples at 125 volts for 45 – 50 minutes.

⊕ **STOP POINT: Instructor** – Gels may be stored in a Tupperware container filled with 70% ethanol or kept in the TBE buffer for one day before photographing



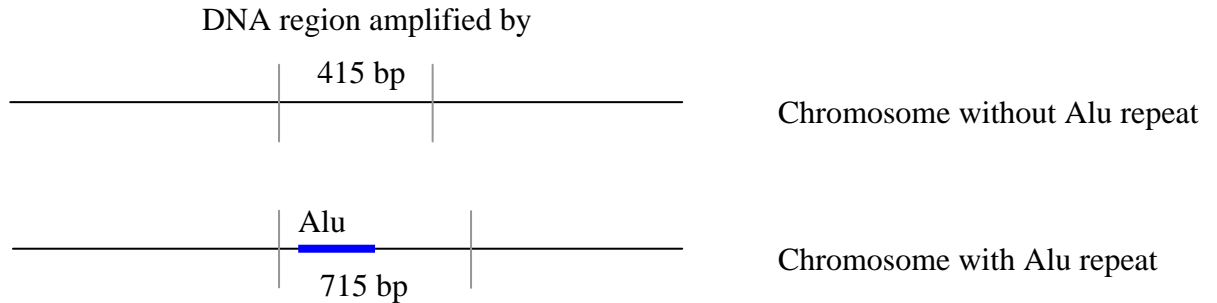
PHOTOGRAPHING AGAROSE GELS

1. Put on gloves and safety glasses. Retrieve your gel tray from your instructor
2. Make sure the power to the UV transilluminator is OFF. Lift the UV blocking cover and place gel tray on the black platform. Close the lid completely before turning machine on. UV radiation can cause severe eye and skin damage.
3. Briefly observe the number, placement and relative brightness of the DNA bands. Turn the transilluminator OFF.
4. With the transilluminator OFF, your instructor will place the hooded CCD camera over the black platform. Make sure that the hood is seated correctly and there are no raised edges that would allow UV light to leak out.
5. Turn ON the transilluminator. Your instructor will print an image for each member of your lab group.
6. Turn OFF the transilluminator.
7. Remove the CCD camera/hood from the transilluminator.
8. Follow your teacher's instructions for clean up. Discard all gels into the plastic disposal bags. Do not touch the gel! Gel boxes and plates must be rinsed in distilled or deionized water and air-dried. Please take great care with the equipment so many more students may enjoy these experiments.
9. Wash hands and clean lab station.
10. Analyze your results and complete the attached student activity sheet.



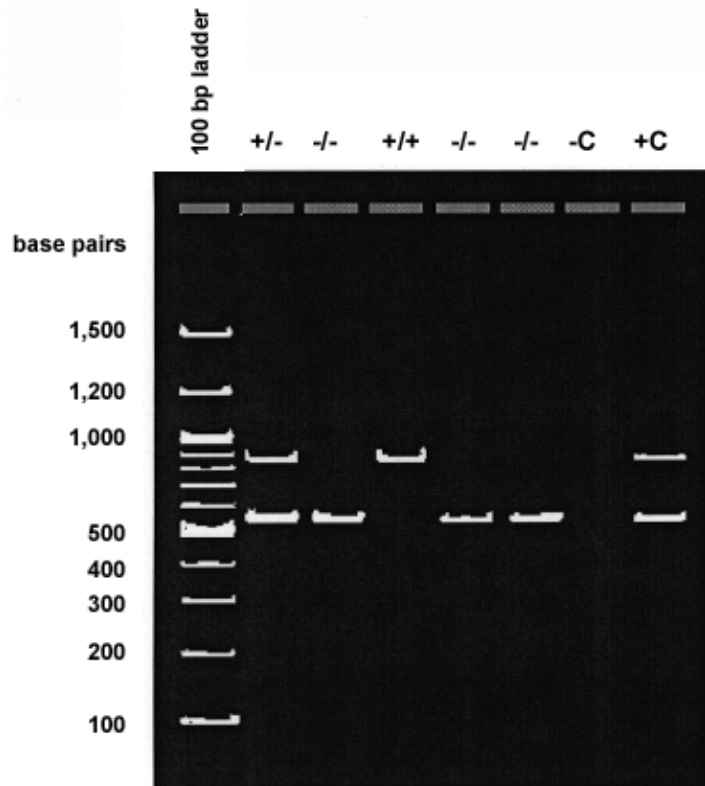
ANALYZING RESULTS

By examining the photograph of your agarose gel, you will determine whether or not you carry the Alu repeat on one, both, or none of your number 16 chromosomes. PCR amplification of this Alu site will generate a 415 bp fragment if the repeat is not present. If the repeat is present, an 715 bp fragment will be made.



When you examine the photograph of your gel, it should be readily apparent that there are differences between people at the level of their DNA. Even though you amplified only one site, a site that every one has in their DNA, you will notice that not all students have the same pattern of bands. Some students will have only one band, while others will have two.

We use the term allele to describe different forms of a gene or genetic site. For those who have the Alu repeat, we can say that they are positive for the insertion and denote that allele configuration with a “+” sign. If the Alu repeat is absent, we assign a “-” allele designation. If a student has a single band, whether it is a single 415 bp band or a single 715 bp band, then both their number 16 chromosomes must be the same in regards to the Alu insertion. They are said to be homozygous and can be designated with the symbols “+/+” or “-/-”, respectively. If a student’s DNA generates a 415 bp band and an 715 bp band during PCR, the students is said to be heterozygous at this site and the designation “+/-” is assigned. A person’s particular combination of alleles is called their genotype.





POLYMERASE CHAIN REACTION

Amplification of Alu PV92 Insert



PIN #: _____ Your ¹⁶Genotype: _____ +/+ _____ +/- _____ -/-

Vocabulary

Allele: One of the variant forms of a gene at a particular locus, or location, on a chromosome. In this laboratory, we are investigating a locus with two possible alleles: (+) or (-).

Genotype: The genetic identity of an individual that does not show as outward characteristics. This characteristic is based on the alleles present at a given locus. In this laboratory, there are three possible genotypes: (+/+), (+/-), or (-/-).

Allele Frequency

Within your class, how unique is your particular combination of *Alu* alleles? An allele frequency is the percentage of a particular allele within a population of alleles. It is expressed as a decimal. You can calculate an allele frequency for the *Alu* PV92 insertion in your class by combining all your data.

Example 1: Imagine that there are 100 students in your class and the genotype distribution within the class is as follows:

Genotype	Students	+ alleles	- alleles
+/+	20	40	0
+/-	50	50	50
-/-	30	0	60
Total	100	90	110

Since each person in your class has two number 16 chromosomes, there must be twice as many total alleles as there are people. Therefore, in this sample of 100 students, there are 200 hundred alleles, 90 (+) alleles and 110 (-) alleles.

The frequency of the (+) allele in this class is:

$$\frac{90 \text{ (+) alleles}}{200 \text{ total alleles}} = 0.45$$

The frequency of the (-) allele in this class is:

$$\frac{110 \text{ (-) alleles}}{200 \text{ total alleles}} = 0.55$$

If the allele frequencies do not add up to 1.0 then you have made an error in the math.



1. Fill in the chart below.

Genotype	Students	+ alleles	- alleles
+/+			
+/-			
-/-			
Total			

2. Calculate the (+) allele frequency = _____ *you will need this number later*
3. Calculate the (-) allele frequency = _____ *you will need this number later*
4. Do these allele frequencies add up to 1.00? _____

Genotype Frequencies

A genotype frequency is the percentage of individuals within a population having a particular genotype. You can calculate the frequency of each genotype in your class by counting how many students have a particular genotype and dividing that number by the total number of students.

Example 2: In a class of 100 students, 20 students have the **(+/+)** genotype. The genotype frequency for the **(+/+)** genotype in this class is $20/100 = 0.2$.

Use the genotype information in the chart above to calculate the genotype frequencies for your class.

3. (+/+) frequency = _____
4. (+/-) frequency = _____
5. (-/-) frequency = _____



Expected Genotype Frequencies (Hardy-Weinberg Law)

If within an infinitely large population no mutations are acquired, no genotypes are lost or gained, mating is random, and all genotypes are equally viable, then that population is said to be in Hardy-Weinberg equilibrium. In such populations, the allele frequencies will remain constant generation after generation. Genotype frequencies within this population can then be calculated from allele frequencies by using the equation:

$$p^2 + 2pq = q^2 = 1.0 - p \text{ \& } q \text{ are allele frequencies for the two forms of a genetic site}$$

The genotype frequency of the homozygous condition is either p^2 or q^2 depending on which allele you assign to p and which to q . The heterozygous genotype frequency is $2pq$.

Example 3: Use our fictitious class to calculate expected genotype frequencies. In example 1 we determined the following allele frequencies. *We will assign p to the (+) allele and q to the (-) allele.*

$$p=0.45$$

$$q=0.55$$

The genotype frequency for (+/+) is equal to p^2 :

$$p^2 = (0.45)^2 = 0.2025$$

The frequency for the "+/-" genotype is $2pq$:

$$2pq = 2(0.45)(0.55) = 0.495$$

The frequency for the "-/-" genotype is q^2 :

$$q^2 = (0.55)^2 = 0.3025$$

To convert these decimal numbers into numbers of students, we multiply each by the total number of students. Since there are 100 students in this fictitious class, the number of students in the class expected to have the (+/+) genotype is:

$$100 \times 0.2025 = 20.25$$

The number of students who should be (+/-) is

$$100 \times 0.495 = 49.5$$

The number of students who should be (-/-) is

$$100 \times 0.3025 = 30.25$$

In our example, the expected numbers of students with a specific genotype matches the observed very closely. This is not always the case.



6. Use the allele frequencies you calculated earlier in questions 2 & 3 to determine the expected class genotype frequencies. Following that, record the actual genotype frequencies:

p (+ allele) = _____ q (- allele) = _____

Genotype	Actual Genotype Frequency (questions 5-7)	Expected Genotype Frequency	Total # of Students	Expected # of Students with Specific Genotype	Actual # of Students with specific genotype
+/+		$p^2=$			
+/-		$2pq=$			
-/-		$q^2=$			

Review Questions

7. A class is looking at a dimorphic *Alu* insert on chromosome #3. How many total alleles are there in a class of 34 students for this *Alu* site?
8. The “-” allele frequency for the class is 0.3, What is the “+” allele frequency?
9. A class in Hardy-Weinberg equilibrium has a “+/+” genotype frequency of 0.64. What is the “+” allele frequency?
10. The “+/+” genotype frequency for a class is 0.49 and the “-” genotype frequency is 0.09. What is the “+/-” genotype frequency if the class is in Hardy-Weinberg equilibrium?