Lab 14 – PCR investigation I

A. Objectives
Become familiar with
1. PCR technique;
2. Isolating cheek cells

B. Before coming to lab
Read
- The following lab exercise "Ah, Lou! There really are differences between us!" No protocol is necessary.
- Pp. 403-405 in Campbell et al. *Biology.*

C. During lab
- Working by yourself, perform the exercise as outlined in the following protocol

D. After lab
- Enjoy life!

E. Ah, Lou! There really are differences between us!

(written by Maria C. Abilock (BABEC) and Frank H. Stephenson, Ph.D. (PE Biosystems), used with permission)

We gratefully acknowledge David Micklos of the DNA Learning Center at Cold Spring Harbor Laboratory for his generous help. Some materials for this exercise were adapted, by permission, from the *Genomic Biology: Advanced Instructional Technology for High School and College Biology Faculty* laboratory manual, Cold Spring Harbor Laboratory, copyright 1999.

Introduction

As you know, the human **genome** (the complete DNA component of our diploid cells) is made up of approximately 6 billion base pairs distributed on 46 chromosomes. All cells in your body, except red blood cells, sperm, and eggs, contain 46 chromosomes. Only 1.5 percent of this enormous amount of DNA, however, is actually used to directly code for the proteins required for supporting cellular metabolism, growth, and reproduction. The protein-encoding regions are scattered throughout the genome. These regions may be separated by many thousands of base pairs. Furthermore, most protein-coding DNA regions in the human organism are themselves broken into smaller protein-encoding segments, called **exons**, with, in many cases, hundreds or thousands of base pairs intervening

between them. Since introns have an ill-defined or possibly even non-essential role, they have been referred to by many as "junk DNA". Whatever their function, examination of these intervening DNA regions 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. They got their name from the fact that most carry within them the base sequence AGCT, the recognition site for the *Alu* I **restriction endonuclease**, a type of enzyme that cuts DNA at a specific site. There are over 500,000 *Alu* repeats 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 own genome at the time you were conceived. 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.

The Polymerase Chain Reaction

Objectives:

You should be able to list and explain the importance of each component of PCR.

You should be able to associate the temperature changes with the cycling steps of PCR.

The polymerase chain reaction (PCR) is a method used by scientists to rapidly copy, in a test tube, specific segments of DNA. By mimicking some of the DNA replication strategies employed by living cells, PCR has the capacity for churning out millions of copies of a particular DNA region. It has found use in forensic science, in the diagnosis of genetic disease, and in the cloning of rare genes. One of the reasons PCR has become such a popular technique is that it doesn't require much starting material. It can be used to amplify DNA recovered from a plucked hair, from a small spot of blood, or from the back of a licked postage stamp.

There are some essential reaction components and conditions needed to amplify DNA by PCR. First and foremost, it is necessary to have a sample of DNA containing the segment you wish to amplify. This DNA is called the **template** because it provides the pattern of base sequence to be duplicated during the PCR process. Along with template DNA, PCR requires two short single-stranded pieces of DNA called **primers**. These are usually about 20 bases in length and are complementary to opposite strands of the template at the ends of the target DNA segment being amplified. Primers attach (**anneal**) to their complementary sites on the template and are used as initiation sites for synthesis of new DNA

strands. **Deoxynucleotides** containing the bases A, C, G, and T are also added to the reaction. The enzyme **DNA polymerase** binds to one end of each annealed primer and strings the deoxynucleotides together to form new DNA chains complementary to the template. The DNA polymerase enzyme absolutely requires the metal ion magnesium (**Mg^{++}**) for its activity. It is supplied to the reaction in the form of $MgCl_2$ salt. A **buffer** is used to maintain an optimal pH level.

PCR is accomplished by cycling a reaction through several temperature steps. In the first step, the two strands of the template DNA molecule are separated, or **denatured**, by exposure to a high temperature (usually 94° to 96°C). Once in a single-stranded form, the bases of the template DNA are exposed and are free to interact with the primers. In the second step of PCR, called **annealing**, the reaction is brought down to a temperature usually between 37˚C to 55 ˚C. At this lower temperature, stable hydrogen bonds can form between the complementary bases of the primers and template. Although human genomic DNA is billions of base pairs in length, the primers require only seconds to locate and anneal to their complementary sites. In the third step of PCR, called **extension**, the reaction temperature is raised to an intermediate level (65˚C to 72˚C). During this step, the DNA polymerase starts adding nucleotides to the ends of the annealed primers. These three phases are repeated over and over again, doubling the number of DNA molecules with each cycle. After 25 to 40 cycles, millions of copies of DNA are produced. The PCR process taken through four cycles is illustrated on the following page (Figure 1).

In the following laboratory exercise, 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 for this experiment. DNA is easily obtained from the human body. A simple saltwater mouthwash will release cheek cells, from which you will extract the DNA. After you amplify the *Alu* repeat region, 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 electrophoresing your PCR sample on an agarose gel. Finally, using a program developed by the DNA Learning Center at Cold Spring Harbor Laboratory, you will determine how rare this *Alu* sequence is in the human population and make some assessment as to when and where it arose.

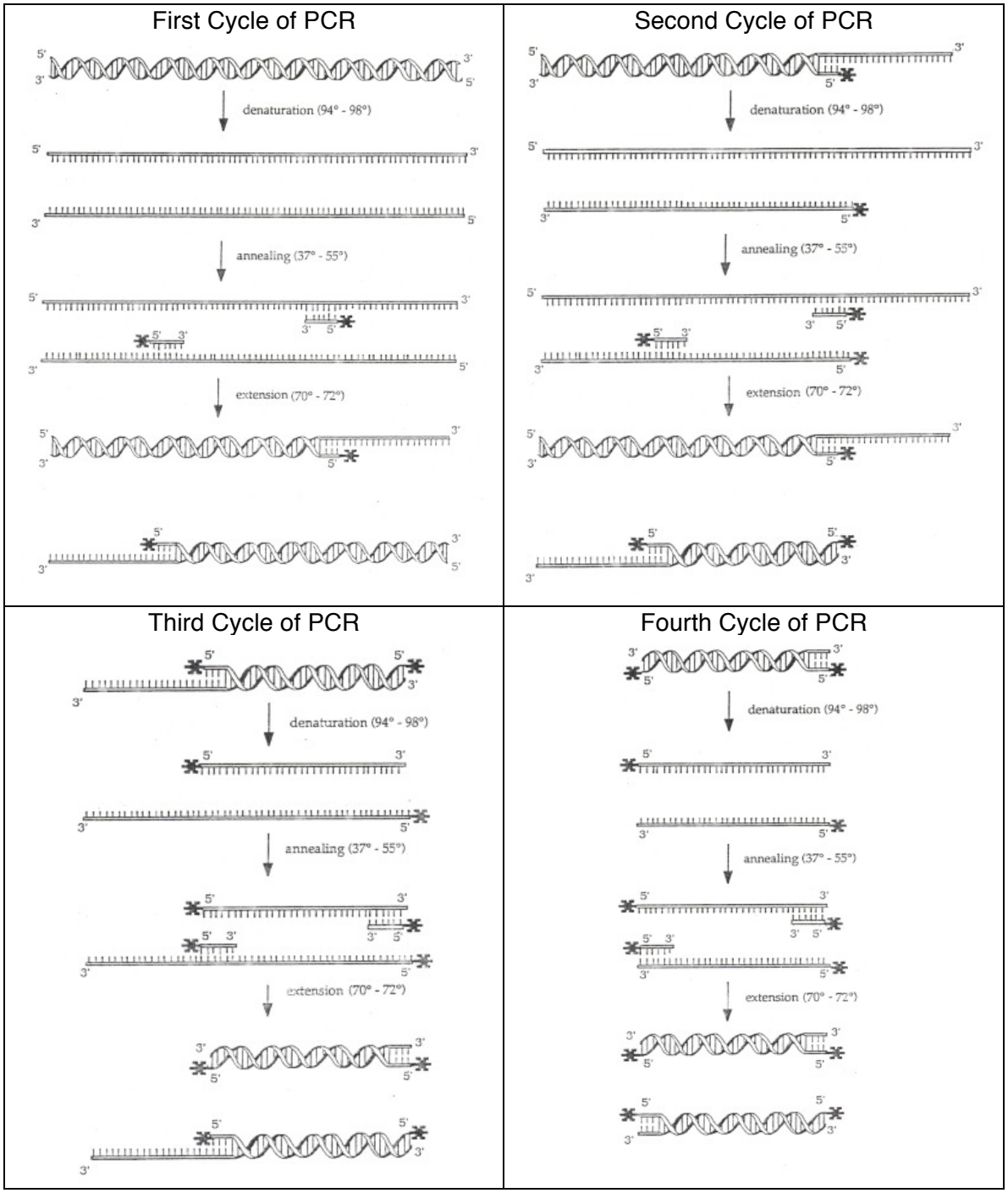


Figure 1. The first four cycles of the polymerase chain reaction.

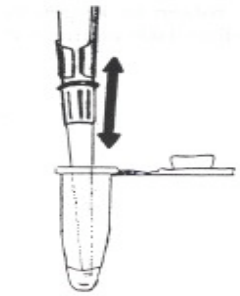
An excellent animated tutorial showing the steps of PCR is available at the Cold Spring Harbor web site. **http://vector.cshl.org/shockwave/pcranwhole.html**

Laboratory Exercise

Objectives:

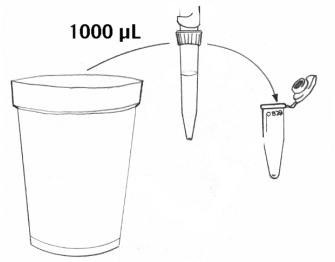
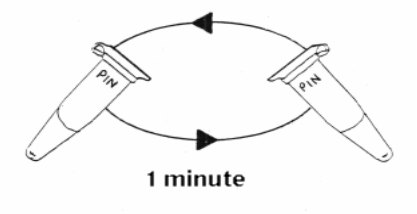
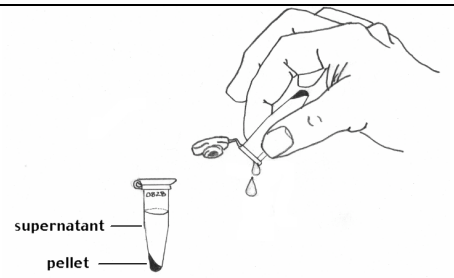
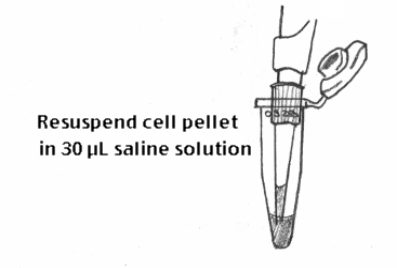
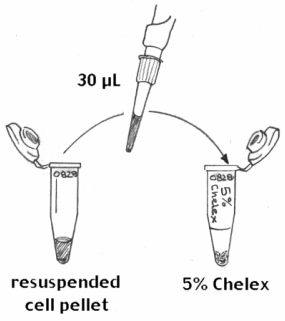
You should be able to successfully isolate DNA from cheek cells.

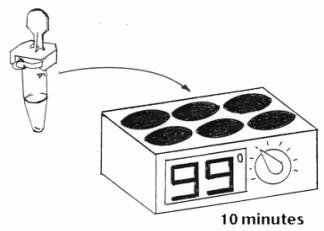
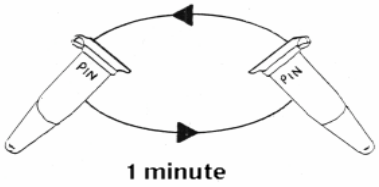
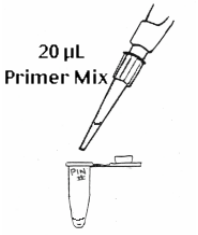
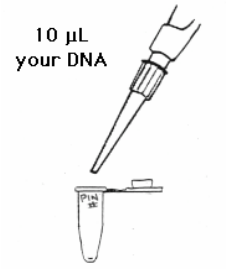
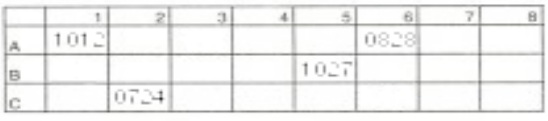
You should be able to prepare a reaction for PCR amplification of an *Alu* insert.

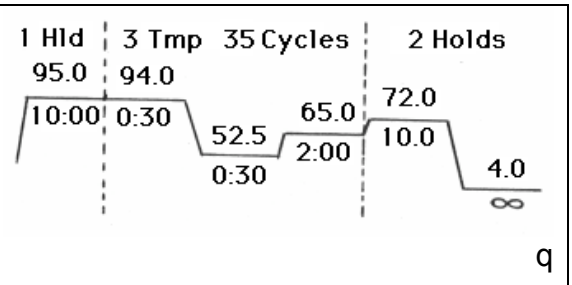
IMPORTANT LABORATORY PRACTICES	
Add reagents to the bottom of the reaction tube, not to its side. You should add each additional reagent directly into previously-added reagent and pipet the combined liquid up and down several times to ensure proper mixing. Pipet slowly to prevent contaminating the pipette barrel. 	Change pipette tips between each delivery. You should change the tip even if it is the same reagent being delivered between tubes. 

Place a check mark in the box of each step as it is completed.

DNA Preparation Using a Saline Mouthwash	
1. Swirl 10 mL of 0.9% saline in your mouth for 30 seconds.	
2. Expel saline into a cup and swirl to mix the cells.	

3. Transfer 1000 µL of the liquid into a 1.5 mL microfuge tube, labeled with your PIN.	
4. In a balanced centrifuge, spin sample for 1 minute.	
5. Observe your cell pellet at the bottom of the tube. Pour off the supernatant, being careful not to lose your cell pellet. **Note**: It is okay if some supernatant is left in the tube.	
6. Resuspend your cell pellet in 30 µL of saline. Make sure the entire cell pellet is thoroughly mixed by vortexing, pipeting 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.	
7. Withdraw 30 µL of the cell suspension and add it to a 1.5 mL tube containing 200 µL of 5% Chelex. **Note**: Do not pipet up and down at this step or else you will clog the tip with Chelex beads.	

8. Secure your tube with a cap lock and place it in the 99˚C heat block or boiling water bath for 10 minutes.	
9. Shake your tube well or briefly vortex it and then place it in a balanced centrifuge. Spin for 1 minute.	
• Label a 200 µL PCR tube with your initials. Note that this will wash off in the thermal cycler	 q
• Change your tip and dispense **10 µL of Master Mix** into your PCR tube.	 q
• Change your pipet tip and add **10 µL of Primer Mix** into your PCR tube.	 q
• With a new pipet tip, add **7 µL of your purified DNA** into your PCR tube. Make sure not to pick up Chelex beads! **Note**: Slowly pipet up and down several times to mix all the reagents in your reaction tube.	 q
• Place your reaction into the thermal cycler and record the location of your tube on the grid provided by your teacher.	

• The cycling protocol for amplification of this Alu region is: 95˚C, 10 minutes; 94˚C, 30 seconds; 52.5˚C, 30 seconds; X 35 cycles 65˚C, 2 minutes; 72˚C, 10 minutes; 4˚C, hold	

Controls
+ Control: 10 µL Master Mix, 10 µL Primer Mix, 7 µL +Control DNA
- Control: 7 µL sterile water, 10 µL Master Mix, 10 µL Primer Mix

F. Review

What was the purpose of _____ in our PCR experiment.

1. using saline solution
2. keeping cells cold
3. centrifuging cells in saline
4. using chelex
5. heating cells and chelex
6. centrifuging cells and chelex
7. using primer mix
8. using our own cells
9. using Taq polymerase
10. using deoxynucleotides
11. using ATP
12. using MgCl
13. using buffer
14. heating the DNA up to 94.0 °C and then to 65 °C

15. cooling the DNA down to 52.5 °C and then to 4.0 °C?
16. What is an alu element?
17. How many alu elements do humans have?
18. How many alu elements are we testing for?
19. What is the function of alu elements?
20. How does this exercise relate to DNA fingerprinting?
21. What does mean that our alu insert is “dimorphic”?

Lab 15 – PCR II

A. Objectives

- Learn to interpret agarose gels depicting DNA samples
- Learn how to calculate allele and genotype frequencies

B. Before coming to lab

- Read the following lab exercise. No protocol is necessary.

C. During lab

- Perform the laboratory exercise as outlined in Part E below. Report your results in your laboratory notebook.

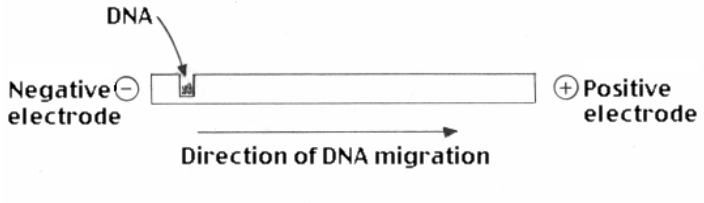
D. After lab

- Answer the review questions for this lab.

E. Agarose Gel Electrophoresis

To determine whether or not you carry the *Alu* repeat, you will need to visualize the products of your amplification. This will be done using a process called **electrophoresis** in which electric current forces the migration of DNA fragments through a special gel material. Since DNA is negatively charged, it will migrate in an electric field towards the positive electrode (Figure 2). When electrophoresed through a gel, shorter fragments of DNA move at a faster rate than longer ones. The *Alu* repeat adds 300 base pairs of length to a DNA fragment and thus will slow its migration during electrophoresis.

Figure 2. Side view of an agarose gel showing DNA loaded into a well and the direction of DNA fragment migration during electrophoresis.



The gel material to be used for this experiment is called **agarose.** When agarose granules are placed in a buffer solution and heated to boiling, they dissolve and the solution becomes clear. A casting tray is set up with a comb to provide a mold for the gel. The agarose is allowed to cool slightly and is then poured into the casting tray. Within about 15 minutes, the agarose solidifies into an opaque gel having the look and feel of coconut Jell-O. The gel, in its casting tray, is placed in a buffer chamber connected to a power supply and buffer is poured into the chamber until the gel is completely submerged. The comb can then be pulled out to form the wells into which your PCR sample will be loaded.

Loading dye is a colored, viscous liquid containing dyes (making it easy to see) and sucrose, ficoll (high molecular weight sucrose-polymers), or glycerol (making it dense). You will add loading dye to your amplification reaction and then pipet an aliquot of the mixture into one of the wells of your agarose gel. When all wells have been loaded with sample, your instructor will switch on the power supply. The samples should be allowed to electrophorese until the blue loading dye is 1 to 2 cm from the bottom. The gel can then be stained with ethidium bromide and photographed.

You will need a 2% agarose gel for electrophoresis of your PCR products. If your agarose gel casting tray holds 50 mL, then you can calculate the amount of agarose you will need as follows:

$$(C_i)\,(M_i) = (C_f)\,(M_f)$$

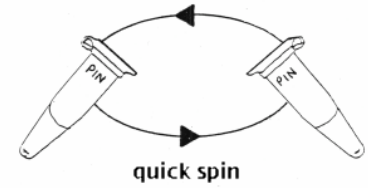
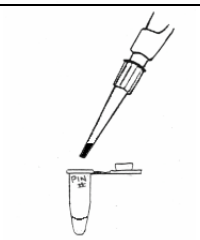
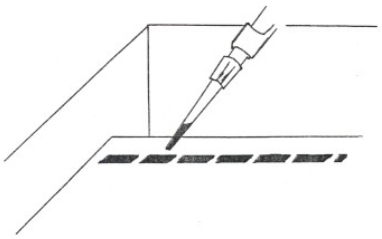
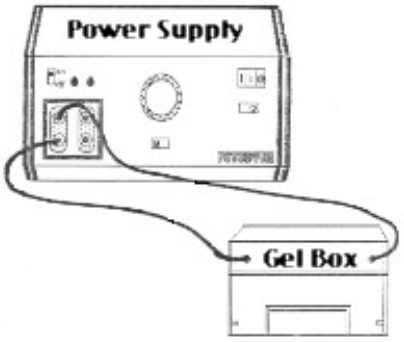
C = concentration
M = Mass
i = initial
f = final

$(100\%)\,(M_i) = (2\%)\,(50\text{ g})$
$100\,(M_i) = 100\text{ g}$
$M_i = 1$ g agarose powder
$50\text{ g} - 1\text{ g} = 49\text{ g}$

The buffer used to make the agarose solution is a liquid and not a solid, so we need the amount to be in units of volume, not grams. Since the density of the buffer is approximately 1g/ml:

49 g = 49 mL buffer

Therefore, you should dissolve 1 g of agarose powder in 49 ml buffer.

Electrophoresis of Amplified DNA	
1. Retrieve your PCR tube and spin it briefly to bring the liquid to the bottom of the reaction tube. **Make sure the centrifuge is balanced before you begin spinning your sample!**	
2. Add **5 µL** of loading dye to your PCR tube. Slowly pipet the mixture up and down until the contents in the tube are uniformly colored.	
3. Carefully load **15 – 20 µl** of your reaction into a well in your gel. Avoid poking the pipette tip through the bottom of the gel or spilling sample over the sides of the well. Use a new tip for each sample.	
4. One student (or the instructor) should load **5 µl** of the 100 bp ladder (molecular weight marker) into one of the wells of each gel.	
5. When all samples are loaded, attach the electrodes from the gel box to the power supply. Have your teacher check your connections and then electrophorese your samples at **125 Volts** for **45-50 minutes**.	

Staining and Photographing Agarose Gels

Your teacher will stain your agarose gel and take a photograph for you so that you may analyze your Alu results. Gel staining is done as follows.

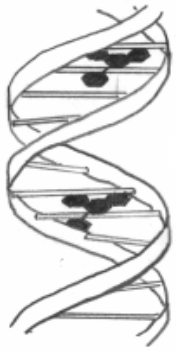
1. Place the agarose gel in a staining tray.
2. Pour enough ethidium bromide (0.5µg/ml) to cover the gel. Wait 15 minutes.

CAUTION**: Ethidium bromide is a carcinogen. Always wear gloves and safety glasses when handling.**

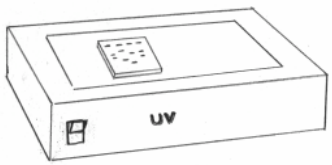
3. Pour the ethidium bromide solution back into its storage bottle. Pour enough water into the staining tray to cover the gel. Wait 5 minutes.
4. Pour the water out of the staining tray into a hazardous waste container and place the stained gel on a UV light box.

CAUTION**: Ultraviolet light can damage your eyes and skin. Always wear protective clothing and UV safety glasses when using a UV light box.**

5. Place the camera over the gel and take a photograph.

Figure 3. Ethidium bromide molecules stacked between DNA base pairs.	

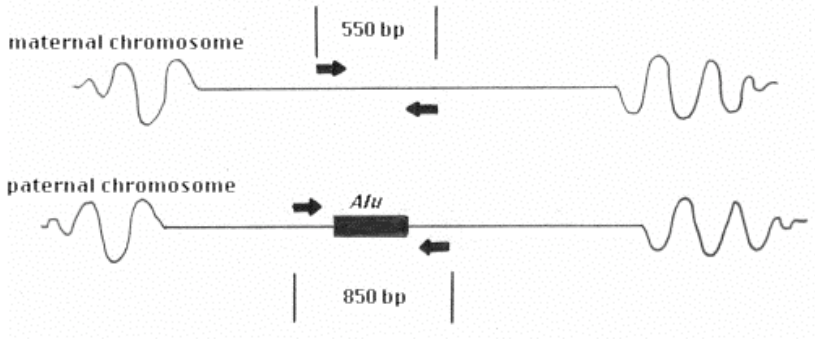
The PCR products that run on your agarose gel are invisible to the naked eye. If you look at your gel in normal room light, you will not be able to see the amplified products of your reaction. In order to "see" them, we must stain the gel with a fluorescent dye called **ethidium bromide**. Molecules of ethidium bromide are flat and can nestle between adjacent base pairs of double stranded DNA (Figure 3). When this interaction occurs, they take on a more ordered and regular configuration causing them to fluoresce under ultraviolet light (UV). Exposing the gel to UV light after staining, allows you to see bright, pinkish-orange bands where there is DNA (Figure 4).

Figure 4. After staining an agarose gel with ethidium bromide, DNA bands are visible upon exposure to UV light.	

Results

By examining the photograph of your agarose gel, you will determine whether or not you carry the *Alu* repeat on one, both, or neither of your number 16 chromosomes. PCR amplification of this *Alu* site will generate a 550 bp fragment if the repeat is not present. If the repeat is present, an 850 bp fragment will be made. Figure 5 shows the structure of an individual's two number 16 chromosomes in a case where one carries the *Alu* repeat and the other does not.

Figure 5. The chromosomes you inherit from your parents may or may not carry the *Alu* repeat on Chromosome 16.



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.

For those who have the *Alu* repeat (they have at least one 850 bp band), we can say that they are positive for the insertion and denote that configuration with a "+" sign. If the *Alu* repeat is absent (a 550 bp band is generated in the PCR), and we assign a "-" designation. If a student has a single band, whether it is a single 550 bp band or a single 850 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 550 bp band and an 850 bp band during PCR, the student is said to be **heterozygous** at this site and the designation "+/-" is assigned.

Figure 6 on the following page shows a representation of a possible experimental outcome in which all possible genetic sequence combinations have been generated.

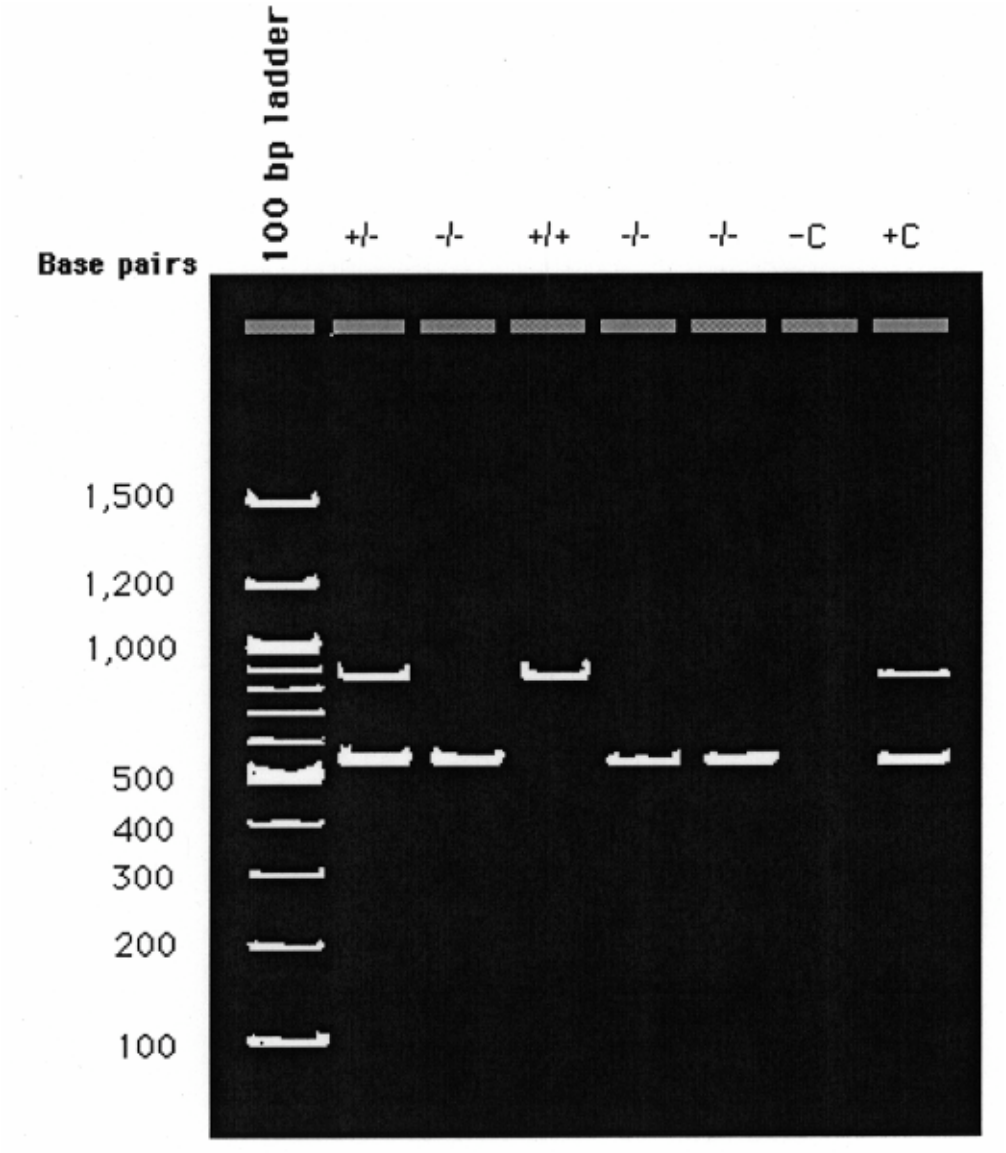


Figure 6. Agarose gel of homozygous and heterozygous individuals for the PV92 *Alu* insertion. A 100 base pair ladder is loaded in the first lane and is used as a size marker. The bands differ by 100 bp in length. The 500 bp band and the 1,000 bp band are more intense when stained with ethidium bromide than the other bands of the ladder. The next 5 lanes contain the results of homozygous and heterozygous individuals. A negative control (-C) does not contain any template DNA and should therefore contain no bands. The positive control (+C) is heterozygous for the *Alu* insertion; it contains a 550 bp band and an 850 bp band.

Calculating Allele and Genotype Frequencies

Objectives:

- You should be able to calculate allele frequencies.
- You should be able to calculate genotype frequencies.

Allele Frequencies

Within your class, how unique is your particular combination of *Alu* alleles (note that we use the term allele here to distinguish between the genetic sequence on chromosome 16 that contains and the one that does not contain the *Alu* insert. Traditionally, the terms “gene” and “allele” have been restricted to describe protein-coding regions.) By calculating an allele frequency**,** you can begin to answer this question. 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. For example, imagine that there are 100 students in your class and the genotype distribution within the class is as follows:

Genotype	Number of Students having that Genotype
+/+	20
+/-	50
-/-	30

Since each person in your class has two number 16 chromosomes (they are diploid for chromosome 16), there must be twice as many total alleles as there are people:

$$\frac{2 \text{ alleles}}{\text{student}} \times 100 \text{ students} = 200 \text{ alleles}$$

To calculate allele frequencies for the class, therefore, 200 will be used as the denominator value. To calculate the “+” allele frequency, we must look at all those students who have a “+” in their genotype. There are 20 students who are “+/+”; they are homozygous for the insertion. Since these 20 students have two copies of the *Alu* insert on their chromosomes, they contribute 40 “+” alleles to the overall frequency:

$$\frac{2 \text{ "+" alleles}}{\text{homozygous "+/+" student}} \times 20 \text{ homozygous "+" students} = 40 \text{ "+" alleles}$$

There are 50 students heterozygous (“+/-”) for the *Alu* insertion. Each heterozygous
individual, therefore, contributes one “+” allele to the overall frequency, or 50 “+” alleles. Adding all “+” alleles together gives us:

40 “+” alleles from the homozygotes
\+ 50 “+” alleles from the heterozygotes
90 “+” alleles

The frequency of the “+” allele in this class, therefore, is:
The frequency for the PV92 “-” allele is calculated in a similar manner. There are

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

30 students homozygous for the “-” allele. This group, then, contributes 60 “-” alleles to the frequency. There are 50 students heterozygous for the *Alu* insertion. They contribute 50 “-” alleles to the frequency. Adding all “-” alleles together gives us:

60 “-” alleles from the homozygotes
\+ 50 “-” alleles from the heterozygotes
110 “-” alleles

The frequency of the “-” allele in this class, therefore, is

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

Notice that the sum of the frequencies for the “+” and “-” alleles is 1.0.

0.45 “+” allele frequency
\+ 0.55 “-” allele frequency
1.00

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

Use the spaces below to calculate the “+” and “-” allele frequencies for your class.
Number of total alleles:

$$\frac{\textbf{2 alleles}}{\textbf{student}} \times \text{\_\_\_\_\_}\textbf{students} = \text{\_\_\_\_\_}\textbf{alleles}$$

Number of “+” and “-” alleles:

Genotype	Number of Students	Number of “+” Alleles	Number of “-” Alleles
+/+			**0**
+/-			
-/-		**0**	
Total			

Allele frequencies:

$$\text{"+" allele frequency} = \frac{\text{\_\_\_\_\_total "+" alleles}}{\text{\_\_\_\_\_total alleles}} = \text{\_\_\_\_\_}$$

$$\text{"-" allele frequency} = \frac{\text{\_\_\_\_\_total "-" alleles}}{\text{\_\_\_\_\_total alleles}} = \text{\_\_\_\_\_}$$

Do these allele frequencies add up to 1.00? _____

Genotype Frequencies

How does the distribution of *Alu* genotypes in your class compare with the distribution in other populations? For this analysis, you need to calculate a **genotype frequency**, the percentage of individuals within a population having a particular genotype. Remember that the term *allele* refers to one of several different forms of a particular genetic site whereas the term *genotype* refers to the specific alleles that an organism carries. 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. For example, in a class of 100 students, let's say that there are 20 students who have the "+/+" genotype. The genotype frequency for "+/+", then, is 20/100 = 0.2. Given the ethnic makeup of your class, might you expect something different? How can you estimate what the expected frequency should be?

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:

$$p^2 + 2pq + q^2 = 1.0$$

where p and q are the allele frequencies for two alternate 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.

Let's use our fictitious class again to calculate expected genotype frequencies. We determined the following allele frequencies (we will assign p to the "+" allele and q to the "-" allele):

$$p = 0.45$$
$$q = 0.55$$

We expect, therefore, that the genotype frequency for "+/+" is equal to p^2 which is

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

The frequency for the "+/-" genotype is

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

The frequency for the "-/-" homozygous genotype is expected to be

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

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 x 0.2025 = 20.25 students who should be "+/+"

The number of students who should be "+/-" is

100 x 0.495 = 49.5

The number of students who should be "-/-" is

100 x 0.3025 = 30.25

On page 15, you calculated the allele frequencies found in your class. Use these frequencies to determine the expected class genotype frequencies. (Let p represent the "+" allele and q the "-" allele.)

Expected "+/+" genotype frequency:

p^2 = _____ = _____

Expected "+/-" genotype frequency:

2pq = _____ = _____

Expected "-/-" genotype frequency:

q^2 = _____ = _____

Use the table below to calculate how many students in your class should have each genotype.

Genotype	Expected Genotype Frequency	Total Number of Students in Class	Expected Number of Students with Specific Genotype
+/+			
+/-			
-/-			

Now, calculate the **actual** genotype frequencies for this class.
Actual "+/+" genotype = _____

Actual "+/-" genotype = _____

Actual "-/-" genotype = _____

F. Review Questions: Allele and Genotype Frequencies

1. A class is looking at a dimorphic *Alu* insert on chromosome number 3. How many total alleles are there in a class of 34 students for this *Alu* site?

2. The "-" allele frequency for the class is 0.3. What is the "+" allele frequency?

3. A class in Hardy-Weinberg equilibrium has a "+/+" genotype frequency of 0.64. What is the "+" allele frequency?

4. 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?

5. What errors could lead to a student not having any bands in his/her lane?

6. How can you explain bands representing 20 bp fragments at the bottom of the gel?