Teacher Packet Cover Sheet

Dear Teacher,
This packet is designed to make your job easier. Please read over the **Teacher Packet** carefully before beginning this lab. Included in this packet are the following:

1. **Inventory Sheet**. Please check to see that all the materials listed on the Inventory Sheet have safely arrived to you and store each item appropriately.

2. **Laboratory Preparation Sheet**. Read the Laboratory Preparation carefully for suggestions on setting up this PCR activity in your classroom.

3. **Informed Consent Release (optional)**. All students participating in PCR amplification of their own DNA may need to sign an Informed Consent Release form provided in this packet. Please check the requirements at your school to determine whether these forms are required or not. If they are required, please keep these in your records. Fill in the appropriate spaces (date, class, school, phone number, signature) before photocopying this form and passing it out to students.

4. **Thermal Cycler Grid**. Schematics showing the layout of the spaces in the GeneAmp 2400 and GeneAmp 9700 thermal cyclers are provided for students to record where they put their samples.

5. **Debriefing Form**. After completing PCR, please fill out the enclosed Debriefing Form and send it back to Shary Rosenbaum. This form helps us monitor the success of PCR-based curricula.

6. **Teacher's Manual**. The teacher's version of the lab manual includes both the student version and important notes at points in the protocol where it is easy to make mistakes. Please make sure to read the lab manual carefully and closely monitor student technique during these steps.

If you have questions please contact me by email at sharyrosenbaum@hotmail.com or by phone at (925) 376-4041.

Shary Rosenbaum
EBBEP Coordinator

Inventory Sheet

Listed below are the reagents and consumables provided in the PCR kit you should receive as well as additional reagents and consumables you will need to acquire/provide yourself. Make sure to also read the list of equipment needed for this PCR activity. If any reagents are missing please contact Shary Rosenbaum ASAP. If any of the kit materials are missing, please contact your cluster leader immediately (or the teacher that you got the kit from)

1 reagent kit contains reagents for 40 reactions. A class of 38 students will have just enough reagents for each student and a positive and negative control.

PCR Reagents Provided in BABEC/Applied Biosystems/EBBEP Kit

ã	ITEM	STORAGE	VOLUME PER KIT (tube)	VOLUME STUDENT
	5 % Chelex 5% weight/volume Chelex 100 Resin Bio-Rad catalog # 143-2832	Refrigerator	10 mL	200 µL/student
	Master Mix** 2.5X PCR buffer II, 3.75 m*M* $MgCl_2$, 2 m*M* dNTP blend, 0.075 Units/µL AmpliTaq Gold ® DNA polymerase	Freezer until ready to use, then Refrigerator	1000 µL (1 mL)	(20 µL/reaction)
	Primer Mix** 0.5 µM each primer 5'- GGATCTCAGGGTGGGTGGCAATGCT –3' 5'- GAAAGGCAAGCTACCAGAAGCCCCAA –3'	Freezer until ready to use, then Refrigerator	1000 µL (1 mL)	(20 µL/reaction)
	+ Control DNA 1.0 ng/µL female genomic DNA 2.0 Novagen catalog # 70605	Refrigerator	50 µL	10 µL/reaction (at least 1 reaction/class)
	MW Marker 50 µg.mL100 bp ladder New England Biolabs #N3231L	Refrigerator	50 µL	5 µL/gel

****Master and primer mixes should come to you frozen. If they are defrosted, keep in refrigerator until ready for use. DO NOT REFREEZE!!!**

Other PCR materials provided in EBBEP PCR kit

ITEM	COMMENTS
PCR tubes (2 per student)	One tube for 200µl Chelex during DNA extraction, one tube for PCR reaction
0.9 % saline	10 mL per student for the mouthwash and an additional 30 µL per student during the DNA preparation.
1.0x or 0.5 X TBE buffer	Roughly 300 mL/gel for agarose electrophoresis and 45 mL per agarose gel. Store at room temperature.
Agarose	2 % agarose gel, approx. 1g agarose per gel. Store at room temperature.
Loading Dye	5 µL per student. Store at room temperature.
Ethidium Bromide	Use as provided in kit (+/- 0.5 µg/mL solution). Store at room temperature but in the plastic bag in the kit. Pour used ethidium bromide back into original bottle. DO NOT ADD ANYTHING TO ETHIDIUM BROMIDE
1.5 mL microfuge tubes 1/student	These should be clean, but don't need to be autoclaved
Sterile micropipet tips	p20s, p200s for student use and p1000s for teacher aliquotting.
Gloves	Use these when handling ethidium bromide.

EQUIPMENT YOU NEED TO GET:

1. **POLAROID 667 FILM FOR PHOTOGRAPHING GELS (THIS CAN BE ORDERED FROM REED'S CAMERA IN WALNUT CREEK, 1524 Locust St., 925-938-4200)**
2. **Ice**
3. **Paper cups for DNA extraction and waste tips**
4. **Permanent markers**
5. **Disposable Staining trays** (weigh boats work fine for this)
6. **Goggles** (use when handling ethidium bromide)

ALL OTHER NEEDED EQUIPMENT SHOULD BE IN THE KIT. CHECK THE KIT INVENTORY SHEET

Laboratory Preparation Sheet

What to do prior to DNA Extraction (Lab Day 1)

1. Dispense 200 µL of 5% Chelex into 0.2 mL PCR tubes if you plan to use the thermal cycler as your heat block, one per student. Make sure to keep swirling the Chelex as you pipet so that every aliquot is actually 5% Chelex (and not more or less). Make sure to use a p1000 micropipet so the Chelex beads do not get clogged in the smaller p200 tips.

2. Make 0.9% saline. The easiest way to make this is to buy a 1 liter bottle of drinking water and add 9 grams of NON-IODIZED salt. Swirl to dissolve the salt. Use a sterile graduated culture tube or sterile pipet to transfer 10 mL into Dixie cups for students just prior to class.

3. Aliquot 150 µL of 0.9% saline for each group of 4 students for the cell pellet rehydration.

4. Only the p200 micropipets are needed on this day.

What to do prior to PCR (Lab Day 2)

1. Buy/prepare ice for teacher lab station. It is very important to keep the master mix and primer mix cold while students are preparing their PCR reactions. The DNA polymerase and/or dNTPs may degrade, resulting in no amplification if the reagents warm up too much.

2. Photocopy and place grid(s) next to thermal cycler for students to record their ID# in the correct spot. Two grids are provided, one for the GeneAmp 2400 (24 spaces) and one for the GeneAmp 9700 (96 spaces).

4. Ensure you have the tray that sits in the thermal cycler (it is usually either red, teal, or black and has a notched upper right-hand corner). Without tray, tubes may melt.

5. Only the p20 micropipets are needed on this day.

What to do prior to Electrophoresis (Lab Day 3)

1. Make 0.5X or 1 X TBE buffer for electrophoresis and agarose gels.

2. Make 2% agarose gels (2g agarose for every 100 mL of electrophoresis buffer) for students or allow time to do this with your students. Be sure to use the same buffer as (1) above.

3. Use the p20 micropipets for gel loading.

4. **Be sure to have the Polaroid 667 film on hand to photograph the gels**.

Informed Consent Release

Date: ____________

Dear Parent(s) or Guardian,

The ___________________class at _____________________school has the opportunity to participate in a class exercise in which an important technique in biotechnology will be used to analyze the students' DNA. The technique the students will be using is called the Polymerase Chain Reaction (PCR). It is a method by which a particular piece of DNA can be amplified many million-fold. PCR has a number of applications in the scientific community, including uses in forensics, diagnostics, parentage testing, and evolutionary studies. It is used by forensic laboratories for the identification of possible suspects involved with a crime. It is used for the diagnosis of different genetic diseases. It is routinely used in most molecular biology laboratories for the cloning and characterization of specific genes.

In this laboratory protocol, students will be isolating DNA from their own cheek cells. They will then apply the PCR technique to amplify a particular segment of their DNA. This segment is not known to be associated with any genetic disease and variation between individuals in this region is in no way an indicator of health or genetic fitness. The results of this particular lab exercise are for teaching purposes only and will NOT be used for any diagnostic or identification purposes. Your student's privacy will be protected. The student's name will not be linked to his/her DNA and the results of the lab exercise will remain anonymous.

Participation is voluntary. By signing this permission form, you are allowing your student to participate in this exciting learning experience. If you have any concerns or questions, please contact me at ___________________.

Sincerely,

____
Print Student's Name

___________________________________ ________________
Student Signature Date

___________________________________ ________________
Parent's Signature Date

GeneAmp 2400 Thermal Cycler

	1	2	3	4	5	6	7	8
A								
B								
C								

GeneAmp 9700 Thermal Cycler

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Debriefing Form

Name:	**School:**
Date:	
Subject/Level:	**Number of Students:**

1. How were the reagents stored before use (and for how long)? Please check the appropriate boxes below and make comments.

Reagent	**Refrigerator Freezer**		**Room Temperature**
Chelex			
Master Mix			
Primer Mix			
+ control DNA			
MW Marker			

2. What previous micropipetting experience did your students have?

3. Please fill out the chart below describing your Alu PV92 PCR results.

Sample	**Number run**	**Number of homozygotes without insert (415 bp)**	**Number of homozygotes with insert (715 bp)**	**Number of heterozygotes (415 bp, 715 bp)**	**Number of ambiguous or no results**
Student					
Positive control					
Negative control					
MW Marker					
Did bands look distinct and readily readable?					
Did you see bands other than at 415 bp or 715 bp? If so, what size(s)?					

4. If your results did not turn out as expected, what problem(s) do you think may have occurred? Please attach a copy of best and worst gel photos.

5. How was the support you received?

6. What additional support would have been helpful?

7. What was your students' overall reaction to the lab/unit?

OTHER COMMENTS: (on back, please)

Please return this form to Shary Rosenbaum, EBBEP Coordinator, 37 Lynwood Place, Moraga, CA 94556

Ah, Lou! There really are differences between us!
Teacher's Guide

Maria C. Abilock Frank H. Stephenson, Ph.D.
BABEC Applied Biosystems
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

We are humans. We are bipedal and stand upright. We have hands, feet, fingers, and toes. You can look at the student next to you and easily recognize that person to be human too. What makes us look similar to each other while different from frogs, fish, or fuchsias is the molecule **deoxyribonucleic acid** (**DNA**).

The basic building block of DNA is the **nucleotide** comprising a deoxyribose sugar, a phosphate, and one of the four bases A (adenine), C (cytosine), G (guanine), or T (thymine). In the DNA molecule, nucleotides are linked together in a chain. DNA is a **double helix**; two chains of nucleotides are wound around each other to form a spiral structure. Interactions (hydrogen bonds) between the bases on the opposing strands hold the double helix together. The A's on one strand hydrogen bond with the T's on the other strand. The G's on one strand interact with the C's on the other. Therefore, A's and T's are said to be **complementary** as are G's and C's. Complementary bases, when hydrogen bonding in the double helix, are called **base pairs** (**bp**). It is the order of the bases along the strands of the DNA molecule that makes each species unique.

Our bodies are caldrons for thousands of chemical reactions carried out to support the process of life. We ingest food for energy and for the raw materials needed to build the structures of the cell. We breathe oxygen; it assists in the moving of electrons from one molecule to another. We manufacture protein molecules called **enzymes** needed for the building or breakdown of still other molecules. We all look like humans because we all share the same cellular makeup.

The information for the construction of all the enzymes in the cell and all the proteins giving the cell its shape and function is stored within DNA's sequence of bases. One particular base sequence may carry the information for the assembly of hemoglobin, a protein that carries oxygen to your cells. Another sequence of bases may direct the manufacture of an actin molecule, a protein found in muscle. The region of bases on DNA that holds the information directly needed for the construction of a particular protein is called a **gene**. The average gene is approximately 10,000 base pairs long. There are approximately 140,000 genes in human DNA.

The human **genome** (the total sum of our genetic makeup) 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 these 46 chromosomes (sperm and egg cells contain only 23

chromosomes). Only 3 to 10 percent of this enormous amount of DNA, however, are 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. Genes may be separated by many thousands of base pairs. Furthermore, most genes 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. These intervening regions are called **introns** and they make up between 90 to 97 percent of the entire genome. 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.

First Cycle of PCR	Second Cycle of PCR
Third Cycle of PCR	Fourth Cycle of PCR

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

An excellent animated tutorial showing the steps of PCR is available at the DNA Learning Center web

site. **http://www.dnalc.org/resources/BiologyAnimationLibrary.htm**

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. ***Note to teachers****: Make sure you use sterile containers for the saline.*	
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. ***Note to teachers****: If you don't have p1000 micropipets, students can use 1 mL transfer pipets or the p200 set at 200 µL (five times).*	

4. In a balanced centrifuge, spin sample for 1 minute. ***Note to teachers****: With the small, black microcentrifuges, you may need to spin for 3-5 minutes.*	
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. ***Note to teachers****: If there is still ~100µL of saline in the cell pellet tuve, do not add more saline. Simply resuspend the pellet in the existing volume.*	
7. Withdraw 30 µL of the cell suspension and add it to a 0.2 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. Place your 0.2 mL tube with 200 µL of Chelex and 30 µL of cell suspension in the 99°C thermal cycler for 10 minutes. **Note:** Remember to record the location of your tube in the thermal cycler.	

9. Shake your tube well or briefly vortex it and then place it in a balanced centrifuge. Spin for 1 minute.	

10. Withdraw 60 µL of supernatant (no Chelex beads) to a clean tube, labeled with your PIN. **Note**: This stored sample is your "DNA" tube. ***Note to teachers****: This step allows you to ensure that no Chelex beads have been transferred into the DNA tube. If you see beads, have students put the DNA back into their chelex tube, re-spin, and transfer 60 µL into a NEW tube.*	
11. Place your DNA tube in the class microfuge rack so that your teacher can refrigerate your isolated DNA until you are ready to prepare your PCR amplification. ***Note to teachers****: If you have time, you may skip this step, collect the p200 and p1000 micropipets, pass out the p20s, and begin setting up PCR.*	

Polymerase Chain Reaction	
1. Label a 200 µL PCR tube with your 4 digit PIN.	
2. Change your tip and dispense **20 µL of Master Mix** into your PCR tube.	
3. Change your pipet tip and add **20 µL of Primer Mix** into your PCR tube.	

4. With a new pipet tip, add **10 µL 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.	
5. Place your reaction into the thermal cycler and record the location of your tube on the grid provided by your teacher.	
6. The cycling protocol for amplification of this Alu region is: 95°C, 10 minutes; 94°C, 30 seconds; 60.0°C, 30 seconds; X 30 cycles 72°C, 2 minutes; 72°C, 10 minutes; 4°C, hold	

Controls
+Control: 20 µL Master Mix, 20 µL Primer Mix, 10 µL +Control DNA
-Control: 10 µL sterile water, 20 µL Master Mix, 20 µL Primer Mix

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, 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 (100%) (M_i) = (2%) (50 g)
i = initial 100 (M_i) = 100 g
f = final M_i = 1 g agarose powder
50 g – 1 g = 49 g = 49 mL buffer
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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**. ***Note to teachers****: If you are using TAE buffer, keep a close eye on the gels because they may heat up and begin to melt if they are run too high.*	
6. Gels may be stored in 70% ethanol for one day in the refrigerator before staining and photographing.	

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 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 415 bp fragment if the repeat is not present. If the repeat is present, an 715 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.

We use the term **allele** to describe different forms of a gene or genetic site. For those who have the *Alu* repeat (they have at least one 715 bp band), we can say that they are positive for the insertion and denote that allele configuration with a "+" sign. If the *Alu* repeat is absent (a 415 bp band is generated in the PCR), 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 student is said to be **heterozygous** at this site and the designation "+/-" is assigned. A person's particular combination of alleles is called their **genotype**.

Figure 6 on the following page shows a representation of a possible experimental outcome in which all possible allele 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 re 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 415 bp band and an 715 bp band.

***Note to teachers**: The following "*Alu* Gel Analysis" pages are not in the Student Manual.*

Alu Gel Analysis

Desired *Alu* results. The bands of the 100 bp ladder are sharp and well-resolved. Student samples should contain either one band (for students homozygous -/- or +/+) or two bands (for students heterozygous +/- for the *Alu* insertion). For heterozygous genotypes, it is typical that the larger 715 bp band will be less intense than the smaller 415 bp band. This is called **preferential amplification** and results from the fact that shorter fragments are amplified by PCR more efficiently than larger fragments.	
Artifact bands. This gel shows a number of the artifact bands that might be generated during the *Alu* PCR amplification. In the +/- student lane, we see the desired 415 bp and 715 bp products but also a number of other bands, **primer dimer** (at the bottom of the gel) forms by an interaction between the primers. We also see a number of large fragments that we believe are **heteroduplex** molecules generated by annealing between a 550 base single strand and an 850 base single strand. Such a heteroduplex will migrate down the gel at a slower rate than might be expected for its actual length. Shown in the -/- lane is a band that migrates at about 300 bp. This may appear as a single band, or, if the gel is run longer, two bands in this area might appear. These probably result from **nonspecific amplification**; primers annealing elsewhere on the template and generating a PCR fragment. In the +/+ lane, a band at 415 bp appears. Since this band is less intense than the 715 bp band (the opposite of what we should expect from preferential amplification), it is an artifact. It can result from spill-over from the lane next to it or from contamination.	

100 bp ladder shows bands but no sample bands are present. Reactions failed to produce amplification. This might result from: 1. Inadequate template amounts added to the reaction. 2. Some component of the reaction was not added. 3. The reagents were inactive due to improper storage. Reagents should be stored frozen or in the refrigerator until use. 4. Master Mix was vortexed violently resulting in loss of AmpliTaq DNA Polymerase activity.	
No bands. If no bands are present on the gel… not even for the 100 bp marker lane, then the possibilities are: 1. Samples were not properly loaded into the wells. 2. The staining solution does not have adequate ethidium bromide stain. Staining solution should contain ethidium bromide at a concentration of 0.5 µg/mL and staining should be allowed to proceed for at least 15 minutes at room temperature. 3. Gel was stored too long in buffer prior to staining and the bands diffused out of the gel.	

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? 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: 2 allelesstudent×100 students= 200 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:
2 "+" alleleshomozygous "+/+" student×20 homozygous "+" students=40 "+" 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
\+ <u>50 "+" alleles from the heterozygotes</u>
90 "+" alleles
The frequency of the "+" allele in this class, therefore, is: 90 "+" alleles200 total alleles=0.45

The frequency for the PV92 "-" allele is calculated in a similar manner. There are 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
\+ <u>50 "-" alleles from the heterozygotes</u>
110 "-" alleles
The frequency of the "-" allele in this class, therefore, is 110 "-" alleles200 total alleles=0.55
Notice that the sum of the frequencies for the "+" and "-" alleles is 1.0.
0.45 "+" allele frequency
\+ <u>0.55 "-" allele frequency</u>
1.0
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:

Note to Teachers: *In a class of 30 students, there would be 60 alleles.* **2 allelesstudent×______students=_______alleles**

Number of "+" and "-" alleles:

Genotype	**Number of Students**	**Number of "+" Alleles**	**Number of "-" Alleles**
+/+			**0**

+/-			
-/-		0	
Total			

Allele frequencies: "+" **allele frequency=______total "+"alleles_______total alleles=________"-" allele frequency=______total "-"alleles_______total alleles=________**

Do these allele frequencies add up to 1.0? ________

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 (page 14) 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 (hint: use data on page 15).
Actual “+/+” genotype = ______________________________
Actual “+/-“ genotype = ______________________________
Actual “-/-“ genotype = ______________________________

Name______________________________

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?

Using the Allele Server at the CSHL DNA Learning Center

Objectives:

You should be able to view your class data in the Cold Spring Harbor Laboratory *Alu* PV92 database.
You should be able to plot the frequency of the *Alu* PV92 insert for different world populations on a map.
You should be able to formulate a hypothesis describing the origin and spread of the *Alu* PV92 insert across the globe.
You should be able to use Chi Square analysis to determine differences between your class data and data sets from other human populations.
You should be able to brainstorm reasons for genetic similarities and differences between populations.

The DNA Learning Center at Cold Spring Harbor Laboratory has developed a number of **bioinformatics** tools for student use. Bioinformatics tools are computer programs used to help scientists make sense of biological data and solve biological problems. You will be using the Allele Server for four different activities to help you learn more about *Alu* PV92 in human populations. In the first exercise, you will check your calculations for allele and genotype frequencies. Next, you will access data from a database to plot the “+” allele frequency from different world populations. In the third activity you will check to see if your class could be in Hardy-Weinberg equilibrium. The last activity includes computer analysis to find human populations similar to and different from your class.

Note to Teachers: *When you have collected the student data in the "Class Results for the PV92* Alu *Insertion," you will need to enter it into the Cold Spring Harbor Laboratory DNA Learning Center Allele Server.*

Entering Class Data into Allele Server

These instructions for entering class data into the Allele Server are not in the Student Guide.

1. Click on the icon for your Internet Service Provider to open the application. (This might be America Online, Netscape Navigator, Microsoft Internet Explorer, etc.)	
2. In the internet address box, type in the following web address: http://www.bioservers.org/bioserver and press the Enter key on the keyboard.	
3. The Bioservers page should appear on the screen. If you have not registered with Bioservers before, click on the REGISTER button below the Allele Server. If you are already registered, enter your Username and Password in the Allele Server section, click the LOGIN button and go to Step 5.	

4. In the Registration window, fill in the entry boxes and press the "Register" button. A note to confirm your registration will then appear on the screen. Click the OK button.

5. When you complete Step 4, the screen will advance to a split window. The top window, entitled “Using Allele Server” provides a brief overview of the site. You may close this window by clicking the small box in its upper right hand corner. (Note: Be sure that you are not in Sequence Server at this point. If you are, close the window, get back into Allele Server, enter your new Username and Password, and press the LOGIN button.) The larger window tiled below this first one is the Allele Server workspace. Click on the MANAGE GROUPS button to bring up the MANAGE GROUPS window. **NOTE:** There is currently a bug in the Bioservers that automatically puts you into the Sequence Server instead of the Allele Server. Click on the small link “return to Bioservers” at the top left of the Sequence Server page and find your way to the Allele Server.	
6. In the upper right hand corner of the MANAGE GROUPS window, is a popup menu. Scroll to “Your Groups” and click on the ADD GROUP button. This will bring up the “Create Group” window.	

7. Fill in each box of the CREATE GROUP window. In the “Type” box, scroll to choose “Public”. Do not fill in the lower two boxes that would allow your students to enter data. Click the OK button. You will be returned to the MANAGE GROUPS window.	
8. In the MANAGE GROUPS window, press the EDIT GROUP and then the “INDIVIDUALS” tab (at the top of the window). This will bring up the EDIT GROUP window.	

9. Enter the data collected from the class (on the "Class Results for the PV92 Alu Insertion" table). After you have filled in the information for each person, click the "Add" button. When all students have been added, click the "Done" button. This will take you back to the MANAGE GROUPS window. Click the "OK" button. You may then close the application. Your class data will be saved in the MANAGE GROUPS window under the "Teachers" data.	

Class Results for the PV92 *Alu* Insertion

Date: ___________________________

Class: ___________________________

Genotype (Enter +/+, +/-, or -/-)	**Gender** (Male or Female)	**Mother's Origin***	**Father's Origin***

* Enter: Native American (North), Native American (South), African, Asian, Australasian, European, or All (origin unknown).

Note to Teacher: *This genotype grid is not in the Student Manual.*

Using the Allele Server to Check Your Allele and Genotype Frequencies

Your teacher has added your class results into a database at the Cold Spring Harbor Laboratory using the Allele Server program. In this activity you will also use the Allele Server to access your class data so that you can check your allele and genotype calculations.

Looking at Allele and Genotype Frequencies	
1. Click on the icon for your Internet Service Provider to gain access to the internet. (This might be America Online, Netscape Navigator, Microsoft Internet Explorer, etc.)	
2. In the internet address box, type in the following URL: http://www.bioservers.org/bioservers and press the Enter (or Return) key on the keyboard. The Allele Server main page will be brought up.	

3. In the Bioservers page, type in your username and password in the Allele Server field and then click LOGIN. If you do not have a username and password, you must first click the REGISTER button and complete the registration form.	
4. Two windows will appear. The top window, "Using Allele Server", provides additional instructions for this site that you may want to reference later. You may click on the second, larger window to bring it to the foreground. This window is the Allele Server workspace. Click on the MANAGE GROUPS button. This will bring up the MANAGE GROUPS window.	
5. In the upper right hand corner of the MANAGE GROUPS window, is a popup menu. Select "Teachers" from this menu. In the "Teachers" window, scroll to find your class and then click the VIEW button next to it. The GROUP INFORMATION window will be brought forward.	

6. The GROUP INFORMATION window will show the data for your class. **a. In the spaces provided, fill in the allele and gentopye frequencies for your class as displayed in the GROUP INFORMATION window.** **"+" Allele Frequency:_________** **"-" Allele Frequency:__________** **"+/+" Genotype Frequency:_______** **"+/-" Genotype Frequency:_______ "-/-" Genotype Frequency:________** **b. Do these allele and genotype frequencies the actual allele and genotype frequencies that you calculated (see pages 15 and 17 of the student manual)? _________ If you answered "No", check your calculations again.** Close the window by clicking the small close box at its top. **Do not** click the "Done" button. This will return you to the MANAGE GROUPS window.	

Using the Allele Server to Look at Different World Populations

Humans, monkeys, mice, canines, and corn have them running rampant through their genomes. They move undetectably from chromosome to chromosome. They are the so-called "jumping genes" and the *Alu* element is one of them. Although *Alu* is found only in primates, there are other related "jumping genes" that have found their way into the DNA of most eukaryotic organisms on Earth.

Alu is classified as a **retroposon** - a genetic element that uses the enzyme reverse transcriptase to copy itself from one chromosomal location to the next. For this reason, calling *Alu* a "jumping gene" can be misleading. *Alu* doesn't "jump" in the sense that it leaves one location to occupy another. When *Alu* moves, it leaves a copy of itself behind.

The *Alu* element first appeared tens of millions of years ago and since that time, it has been increasing within our genome at the rate of about one copy every 100 years. It is difficult to tell how *Alu* arose. It shows a striking similarity to a gene (called 7SL RNA) that performs a vital function in our metabolism. But *Alu* , it seems, has no function. It is self-serving and, like a parasite, takes advantage of us for its own replication without providing us any advantage to our own survival.

Most *Alu* elements are "fixed"; they are found at the same chromosomal site in every person on the planet. Fixed *Alu* elements must have arose very early in our evolution, well before Homo sapiens appeared. When modern humans did arise some 200,000 years ago, the vast majority of our *Alu* insertions came to us already intact in our DNA. The *Alu* PV92 insertion, however, is not fixed. This insertion may or may not be present on

one or both of a person's number 16 chromosomes. Since not everyone has the *Alu* PV92 element, it must have arisen after the initial human population began growing. It is a widely held belief that modern humans originated in Africa and then disseminated across the planet. Did the *Alu* PV92 insert arise in Africa or on some other continent during our spread across the globe? In the following exercise, you will

plot the "+" allele frequencies for various populations on a world map and make some determination as to where this *Alu* arose and how it might have spread across continents.

Plotting the Alu PV92 Insert on the World Map	
1. Return to the MANAGE GROUPS window and select "Reference" from the popup menu on the upper right corner of the page. Click on the boxes to the left of at least 10 population groups from representative places around the world. When done, click the OK button. This will place these groups in the Allele Server workspace.	
2. Click on the OPEN button to the right of the population group at the top of the list. This will bring up the window displaying details about the population.	
3. Record the "+" allele frequency for that population on the world map provided (page 23). Close the window by clicking the close box at the top of the window and open the next population group. Record that "+" allele frequency on your map. Repeat this process for all the population groups listed on your workspace. When you are finished, clear the workspace by checking each box to the left of each population group and then pressing the CLEAR button.	

Name_______________________________

Name_______________________________

Review Question: Plotting *Alu* PV92

Look at the "+" allele frequencies for the various world populations that you entered on your world map. Formulate an explanation for where you believe the *Alu* PV92 insert originated and how it spread throughout different world populations. You may use the following map.

Using Allele Server to Test if Your Class is in Hardy-Weinberg Equilibrium

On page 17, you calculated the expected genotype frequencies for your class using the Hardy-Weinberg

equation. Are the expected genotype frequencies you calculated similar to the actual class frequencies? If they are different, then it may mean that the population in your class is not in Hardy-Weinberg equilibrium. If we do observe differences, how can we account for them? How do we even know when there is actually a significant difference between the observed genotype frequencies and the expected genotype frequencies? You will use the Allele Server program to address these questions.

Chi Square Analysis of Your Class Data	
1. In the MANAGE GROUPS "Teachers" window, locate you class and place a check mark in the box to its left. Click the OK button at the bottom of the window. This will bring you back to the ALLELE SERVER workspace window. Your class data will have been placed in the workspace.	
2. Click on the box to the left of your class name in the workspace. In the scroll box to the left of the ANALYZE button, make sure it is on "Chi-Square". Below the ANALYZE button is a circular radio button (a small hand on the right side of the screen points to it). Click on it then click on the ANALYZE button.	
3. The CHI SQUARE window that appears displays your observed genotype frequencies for your class and the genotype frequencies that would be expected if your class is in Hardy-Weinberg equilibrium. Do these look similar? Close the CHI SQUARE window when you are finished.	

By following the above steps, you have directed Allele Server to use a test called **Chi-square**, a statistical test used for comparing observed frequencies with expected frequencies. The Allele Server analysis gives you a Chi-square value and a p-value. The larger the chi-square value, the greater is the difference between the observed and the expected values. When using the Chi-square analysis, we test the null hypothesis that there is no difference between samples (observed and expected) and we assume that if there is any difference, then it arose simply by chance and is not real. For this study, our null hypothesis is that your class is in Hardy-Weinberg equilibrium. Whether or not we can accept the null hypothesis is given by the p-value. If the calculated p-value is less than 0.05, the null hypothesis is disproved; the population is not in Hardy-Weinberg equilibrium. If the p-value is greater than 0.05, the population may be in Hardy-Weinberg equilibrium; we can not prove that it is not in Hardy-Weinberg equilibrium.

As an example, let's say that Chi-square analysis of your data gives a p-value of 0.17. This means that there is a 17% probability that the difference between the observed and the expected values is due to chance. It also means that there is an 83% (100% - 17% = 83%) probability that the difference is not due to chance; the difference is real.

What is the Chi-square value for your class? ____________
What is the p-value for your class data? ___________
Alu Teacher's Guide (rev. 6/18/02) page 31

Name_____________________________

Review Questions: Hardy-Wienberg Equilibrium Analysis
1. What is the probability that the difference between the observed and the expected genotype frequencies calculated for your class is due to chance?
6. Based on the Chi-square and p-values, do you believe your class is in Hardy-Weinberg equilibrium? Why or why not?
Alu Teacher's Guide (rev. 6/18/02) page 32

Using Chi-Square to Compare Two Population Groups
In this part of the exercise, you will use chi-square analysis to determine whether or not there is any difference in the genotype distribution between your class and another population group. If the p-value that is calculated for this comparison is less than 0.05, then the difference between your class and the other population group is probably real. If the p-value is greater than 0.05, then there is probably no difference between your class and the other population group.

Chi Square Analysis of Two Populations	
1. In the Allele Server workspace, click the MANAGE GROUPS button. This will take you to the MANAGE GROUPS window. Select "Teachers" from the popup menu, select your class and then click the OK button.	
2. You should now have your class data on your Allele Server workspace.	
3. Return to the MANAGE GROUPS window and select "Reference" from the popup menu. This will bring up a list of different populations in the world for which the *Alu* PV92 insertion has been determined. Click the box to the left of the population group that you believe should most resemble your class and click the OK button at the bottom of the screen. This will import the data for that group onto the Allele Server workspace.	

4. Click the boxes to the left of both population groups in your workspace. Make sure the popup menu immediately to the right of the COMPARE button displays "Chi-Square". Click the COMPARE button. This will bring up the CHI SQUARE window.	

5. The CHI SQUARE window will display the Chi-square and p-values for these two population groups. **The population group you are comparing your class to is ______________________________.What is the p-value for the Chi-square test? ______ Based on the p-value, are the genotype frequencies of your class and the other population most probably identical or significantly different? ________________________**	
6. When you are finished, close the CHI SQUARE window. Follow the above steps again to identify a human population that your class data most resembles. Choose at least 5 populations to compare with your class. Record your p-values below. **Population p-value** **The population group your class most resembles is _______________. Chi-square analysis gives a p-value of __________ when these two populations are compared.** You may want to compare your class with another class in the database. Other classes can be found in the "Teachers" section of the MANAGE GROUPS window.	

Name______________________________

Review Question: Comparing Populations

Two populations on the small island of Sardinia in the Mediterranean Sea have significantly different genotype frequencies for the *Alu* PV92 insert. Provide a possible explanation for this observation.

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