

Lab 2: PCR of PV92 Alu

About 95% of the human genome is noncoding DNA. Among these sequences are a class of short, repetitive sequences called Alu insertions, named because of the Alu I restriction enzyme recognition site they contain. We will isolate DNA from class members, use PCR to amplify a region that might contain an Alu sequence. The data from this experiment will be used to calculate and compare allele frequencies.

Objectives

- 1) Use boiling prep to obtain DNA template.
- 2) Understand the components and steps in the polymerase chain reaction.
- 3) Obtain genotype data using electrophoresis, and,
- 4) Use this data to think about allele frequencies, inheritance, and evolution.

Introduction

Alu elements are a type of transposable element that arose in the primate evolutionary lineage. These 300 base-pair sequences account for about 5% of the human genome. Recently (within the last 1 million years) an Alu transposon in an early human moved into the PV92 locus on chromosome 16. The genotype of this locus (+ or -) has not become fixed in the human population, so individuals may be +/+, +/-, or -/-. This locus is commonly used in forensics, paternity, and other genetic testing. In this lab, we are not interested in establishing relatedness between individuals or using your genotype as identification, but we will use class data to think about allele frequencies in populations.

Lab Question

What is each student's genotype with regard to the PV92 Alu insertion? What are the frequencies of the alleles in the class? How do these compare to the predicted allele frequencies for human populations?

The presence or absence of the PV92 Alu element will be detected by assessing the electrophoretic

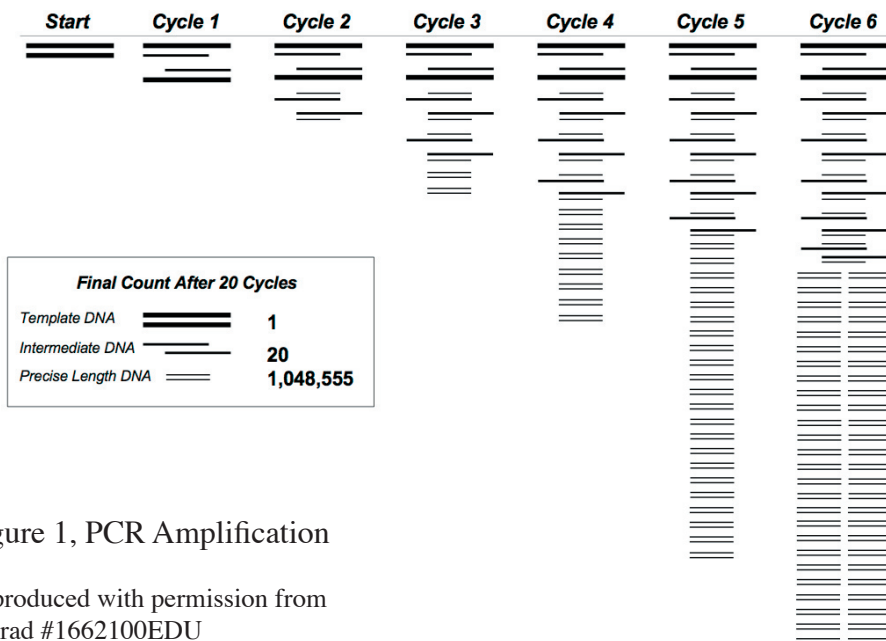


Figure 1, PCR Amplification

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mobility of a PCR product. DNA will be extracted from a small tissue sample, and used as a template in a PCR reaction. This should give you a good idea of how PCR revolutionized the field of molecular genetics. In theory, even the template from a single cell should be enough to amplify a usable amount of product. (See Figure 1.) This DNA will be combined with primers, buffer, substrates, and *Taq* polymerase in a thermal cycler. For a good animated introduction to PCR, see the Cold Spring Harbor Laboratory:

<http://www.dnalc.org/shockwave/pcranwhole.html>

Products will be separated electrophoretically, and detected by staining. The PV92 allele lacking the insertion should yield a 641 base pair product, while the

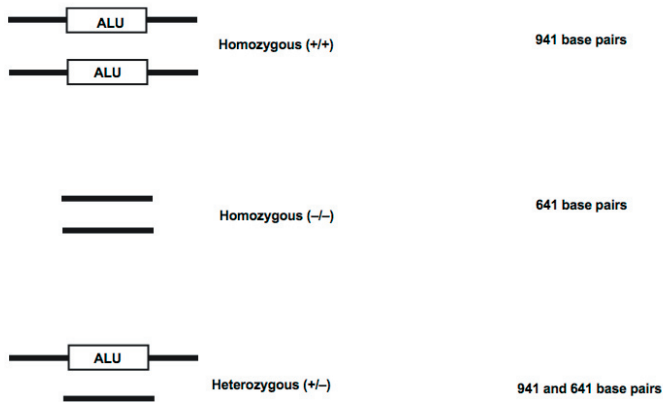


Figure 2, PV92 Products

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allele with the Alu insertion produces a 941 base-pair product. (Figure 2.) From this data, we should be able to calculate the frequency of each allele in this population (class), and compare our data to that of other human populations as well as to an idealized Hardy-Weinberg population, described by $p^2 + 2pq + q^2 = 1$.

You should complete this exercise with a lab partner. The entire exercise takes about three to five hours over two lab periods. There is a stopping point following PCR amplification, so you should plan on being there with the rest of the class at the end of the first lab period. Your lab report is due Monday 23 February in lab. Each lab group should work independently.

Procedure

Section A - DNA Isolation

Follow the steps on page 1 of the PV92 Quick Guide. Briefly, cells are collected and lysed by boiling to release their contents, including genomic DNA. In this prep, the beads are Chelex polyanions, and chelate the divalent cations required as cofactors by DNA modifying enzymes. What would happen if these beads were transferred along with the template to your PCR reaction?

Section B - PCR

To save time and resources, your instructor has prepared a 2x PCR master mix for you from stock solutions. Before you can use this mix, though, you and your lab partner must independently pass the PCR quiz, showing that you understand the steps, components and concentrations of a PCR reaction. The master mix contains 2x buffer diluted from a 10x stock, 3 mM $MgCl_2$, diluted from a 25 mM stock, 400 μM of each dNTP, diluted from a stock that was 25 mM in each dNTP, 1 μM of each primer (forward and reverse), diluted from a stock that was 100 μM , and 0.05 units/ μl *Taq* polymerase, diluted from a 1 unit/ μl stock. What will these concentrations be when you mix 20 μl of this mix with 20 μl of your template prep? You should begin by organizing the above information in a table in your notebook.

A good online description of the components of a PCR reaction can be found at

http://www. Roche-applied-science.com/pcr/application_hints_01_1c.htm

You should plan to complete the quiz with at least an hour remaining in the lab, so you may have to spend some prep time thinking about these components and concentrations, as well as the steps of each PCR cycle. When you have completed the quiz, get your instructor's signature, and follow the directions on page 2 of the Quick Guide.

Section B Approval: _____

Section C - Electrophoresis

Prepare a 1% agarose gel with 0.05 $\mu\text{g/ml}$ ethidium bromide. One or two small gels should be enough for the entire lab. Load and run the gels for about 30 minutes at about 100 V. Visualize and photograph the gels.

Section D - Analysis

Compare the class results with the control lanes on the gel. Assign a genotype to each student. Note that the interpretation of PCR product presence or absence on the gel may require some subtlety. Make sure you take notes on the class discussion so that you can write a quality lab report. Use this data to assign allele frequencies to each of the PV92 alleles in our population (p and q). Because there is no discernible phenotype for this Alu insertion, we might expect that the distribution of genotype would be determined from the allele frequencies by $p^2 + 2pq + q^2 = 1$. (Not all Alu insertions are benign - an insertion in the NF1 gene is the cause of neurofibromatosis I.) Given these allele frequencies, what would be the genotype frequencies predicted by the Hardy Weinberg equation?

How do the class data compare to these predicted allele frequencies, and to the genotype frequencies in the USA population?

Genotype	Frequency
(+ / +)	0.242
(+ / -)	0.553
(- / -)	0.205

What allele frequencies would this data predict?

To continue your analysis, point one web browser to vector.cshl.org, and log onto the allele server. The login and password are both "evvdna". Your group is UEB331S05. The group password is also "evvdna". After entering the data as a group, you can access and analyze it individually. Use the Chi-Square test to see if the class is significantly different from available

comparison populations. The Genetic Drift analysis predicts the time since the last common ancestor in the two populations.

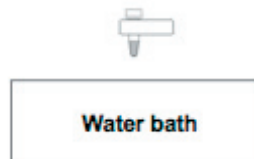
Assignment - Lab Report

Write a complete lab report with introduction, methods, results, and discussion. Your report should use the format you learned in Biology 107 and Biology 320. External sources will be required for your introduction. You might want to include the Batzer et al. paper from the website as a start. The results section must include all of your data, presented in proper graph/table/photograph format, and must include an answer to the lab question that is supported by your data. Do not work with other lab groups. You may work with your lab partner, but each student must write her or his own report, including graphs, tables, and photograph labels. Remember to submit your entire lab report to turnitin.com before the due date.

Quick Guide

Lesson 1 DNA Template Preparation

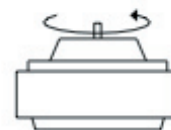
1. Label two screwcap tubes that contain 200 μ l of InstaGene™ matrix with your initials. Label the tubes as “tube 1” and “tube 2”.
2. Using a sterile 20–200 μ l filter pipet tip, gently scrape the inside of both cheeks 10 times each with the tip. You should see a small volume of white cells in the pipet tip. Visually inspect the pipet tip to ensure that ~0.5–1.0 mm of a cell plug is present.
3. Place the tip that contains your cheek cells into the screwcap tube labeled “tube 1”.
4. Using a second sterile 20–200 μ l filter pipet tip, gently scrape the insides of both cheeks 10 times each with the tip. Place the tip that contains your cheek cells into your screwcap tube labeled “tube 2”.
5. When a P-200 micropipet becomes available, set it to 100 μ l and place a tip containing cheek cells on the end. Pipet up and down 5 times into the InstaGene matrix to transfer your cheek cells into the matrix. Repeat with the second tip.
6. Screw the caps tightly on the tubes. Shake or vortex to mix the contents.
7. Place the tubes in the foam micro test tube holder, and incubate at 56°C for 10 minutes in a water bath. At the halfway point (5 minutes), remix the contents of the tubes by shaking or vortexing gently, then place back in the 56°C water bath for the remaining 5 minutes.
8. Remove the tubes, remix the contents by gently shaking or vortexing, and place the tubes in a boiling water bath (100°C). Incubate at 100°C for 6 minutes.
9. Remove the tubes from the boiling water bath and shake or vortex the contents to resuspend. Pellet the matrix by spinning at 6,000 x g for 5 minutes (or 2,000 x g for 10 minutes) in a centrifuge.
10. Remove 170 μ l of the supernatant from tube 2 and transfer it to tube 1. Discard tube 2.
11. Store your screwcap tube in the refrigerator until the next laboratory period (or proceed to step 2 of Lesson 2).



56°C, 10 min



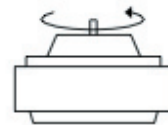
100°C, 6 min



Centrifuge

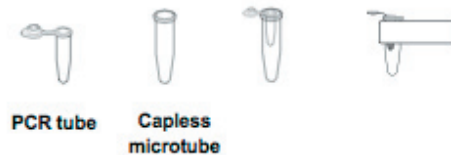
Lesson 2 PCR Amplification

1. Obtain the screwcap tube with your cheek cell DNA template from the refrigerator and place in the rack. Spin the screwcap tube for 2 minutes at 6,000 x g (5 minutes at 2,000 x g) in a centrifuge.

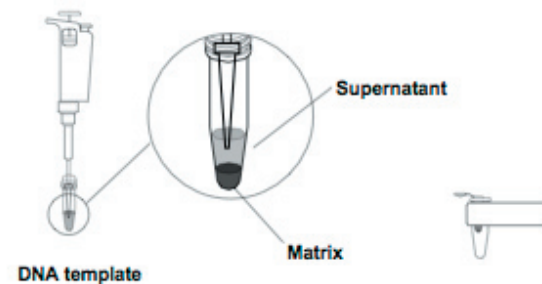


Centrifuge

2. Label a PCR tube and a capless microtube with your initials, place the PCR tube in the capless tube as shown, and place both in the foam holder.



3. Transfer 20 μ l of the DNA template (the supernatant) from the screwcap tube into the bottom of the PCR tube. Be very careful **not** to transfer any of the matrix beads into the PCR tube.



4. Locate the tube of yellow master mix on ice and transfer 20 μ l of the master mix into the PCR tube. Mix by pipetting up and down 2–3 times. Cap the PCR tube tightly. The mixture should be yellow.



5. Place the PCR tube into the Gene Cyclor or MyCycler. The reaction will undergo 40 cycles of PCR amplification.

