

## BIO 3A Laboratory

### PCR Lab Part 2 - Analyzing Your DNA Using Gel Electrophoresis

#### Objectives

- To learn how to micropipet
- Understanding the principle of electrophoresis
- Differentiate between introns and exons

#### Introduction

##### What Can Genes and DNA Tell Us?

It is estimated that the 23 pairs, or 46 chromosomes, of the human genome (23 from the mother and the other 23 from the father) contain approximately 30,000 to 50,000 genes. Each chromosome contains a series of specific genes. The larger chromosomes contain more DNA, and therefore more genes, compared to the smaller chromosomes. Each of the homologous chromosomes (pairs) contain similar genes.

Each gene holds the code for a particular protein. Interestingly, the 30,000 to 50,000 genes only comprise 5% of the total chromosomal DNA. The other 95% is non-coding DNA. This non-coding DNA is interspersed in blocks between functional segments of genes and within genes, splitting them into segments. The exact function of the non-coding (intergenic) DNA is not yet known, although it is thought that non-coding DNA allows for the accumulation of mutations and variations within organisms.

When DNA is first transcribed into RNA, exons are separated from each other by introns. While the RNA is still in the nucleus, intergenic sequences, or introns, are removed from the molecule. Meanwhile the exons are spliced together to form the complete messenger RNA coding sequence for each protein. This process is called RNA splicing and is carried out by specialized enzymes called spliceosomes.

Surprisingly, introns often vary in their size and sequence among individuals while exons do not. This variation is thought to be the result of the differential accumulation of mutations in DNA throughout evolution. These mutations are silently passed on to our descendants. We do not notice mutations in our non-coding DNA because they do not affect our phenotypes. However these differences in our DNA represent the molecular basis of DNA fingerprinting used in human identification and studies in population genetics.

##### The Target Sequence-Can You Say "Alu"?

The genetic code contains small repetitive DNA elements that have become randomly inserted into the human genome over millions of years. One such repetitive element is called the "Alu sequence" (Figure 1). This is a DNA sequence about 300 base pairs long that is repeated, one copy at a time, almost 500,000 times throughout the human genome. The origin and function of these randomly repeated sequences is not yet known.

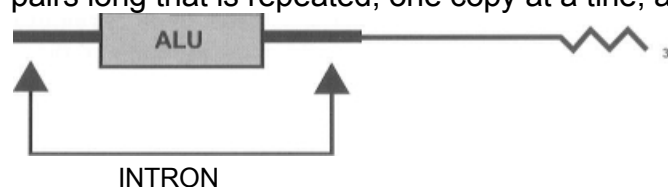


Figure One. Location of an Alu repetitive element within an intron.

Some of these Alu sequences have characteristics that make them very useful to geneticists. When present within introns of certain genes, they can either be associated with a disease or merely used to estimate relatedness among individuals. In this exercise, analysis of a single Alu repeat is used to estimate its frequency in the population and as a simple measure of molecular genetic variation with no reference to disease or relatedness among individuals.

In this lab you will be hunting for an Alu element in the PV92 region of chromosome 16. This particular Alu element is dimorphic, meaning that the element is present in some individuals and not others. Some people have the insert in one copy of their 16<sup>th</sup> chromosomes (one allele), others may have the insert in both copies of their 16<sup>th</sup> chromosome, while others may not have the insert on either copy of the 16<sup>th</sup> chromosome. The presence or absence of this insert can be detected using the polymerase chain reaction followed by agarose gel electrophoresis

Since you are amplifying a region of DNA contained within an **intron**, the region of DNA is never really used in your body. So if you don't have it, don't worry.

The primers in this kit are designed to bracket the region within the PV92 region that is 641 base pairs in length if the intron does not contain the Alu insertion or 941 base pairs in length if Alu is present. This increase in size is due to the 300 base pair sequence contributed by the Alu insert. When your PCR products are electrophoresed on an agarose gel, there are three distinct outcomes that can be visualized.

If both chromosomes contain Alu inserts, then each amplified PCR product will be 941 base pairs long. On a gel these will migrate at the same speed so there will be one band that corresponds to 941 base pairs. If neither chromosome contains the insert then each amplified PCR product will be 641 base pairs and they will migrate as one band that corresponds to 641 base pairs. If you have an Alu insert on one chromosome but not the other, then there will be one PCR product of 641 base pairs and one of 941 base pairs. The resulting gel will reveal two bands.

Electrophoresis separates DNA fragments according to their relative size (molecular weight). DNA fragments are loaded into an agarose gel slab, which is placed into it chamber filled with a conductive liquid buffer solution. A direct current is passed between wire electrodes at each end of the chamber. DNA fragments are negatively charged, and when placed in an electric field will be drawn toward the positive pole and repelled by the negative pole. The matrix of the agarose gel acts as a molecular sieve through which smaller DNA fragments can move more easily than larger ones. Over a period of time smaller fragments will travel farther than larger ones. Fragments of the same size stay together and migrate in what appear as single "bands" of DNA in the gel. In the sample gel below (Figure 2), PCR amplified bands of 941 bp and 641 bp are separated based upon their size.

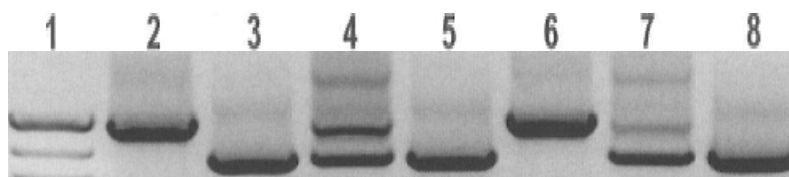


Figure Two. **Separation of DNA bands based on size.** This gel depicts the electrophoretic separation of the EZ Load DNA molecular mass ruler, which contains 1,000 bp, 700 bp, 500 bp, 200 bp and 100 bp.

by fragments (lane 1), two homozygous (+/+) 941 bp fragments (lanes 2 and 6), three homozygous (-/-) 641 bp fragments (lanes 3, 5, and 8), and two heterozygous (+/-) 941/641 bp fragments (lanes 4 and 7).

## **Analyzing Your DNA Using Gel Electrophoresis**

Materials and supplies that should be present at your workstation

<u>Student workstations</u>	<u>Number/Station</u>
Agarose gel	1
PCR samples	1/student
MMR-DNA standard	1
LD loading dye	1
P-20 micropipet	1
Pipet tips (filter type), 2 20 pl	11 tips
Lab marker	1
Styrofoam microtube rack	1
Gel box and power supply	1
Gel staining tray	1
Waste container	1
Copy of Quick Guide or protocol	1

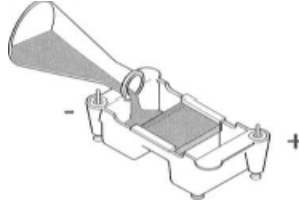
### **Common workstation**

1x TAE electrophoresis buffer	275 ml/gel box
Bio-Safe DNA stain -I x solution	500 ml
MMR-DNA standard	1
Positive control samples (two each)	6
PV92 homozygous (+ /+ )	
PV92 homozygous (-/-)	
PV92 heterozygous (+ /-)	
Centrifuge	
Shaking platform	

### **Lab Protocol for Agarose Electrophoresis**

1. Your PCR samples will be stored in the microtube rack. Place your PCR tubes them in the adaptor and pulse spin the tubes in the centrifuge( about 3 seconds at 2,000 x g) to bring the condensation that formed on the lids to the bottom of the tubes. Add 10 µL of 5x loading dye to each of your PCR tubes.
2. Obtain melted bottle of agarose and pour 30 – 35 mL into your casting gel with the rubber dams and blue comb affixed. Make sure the blue comb is placed towards the end of the casting gel, not the middle.
3. Pour the agarose gel and allow it to solidify undisturbed for 10 – 20 minutes. Once solidified, it should look opaque in color. Carefully remove the rubber dams and comb as directed by your instructor.

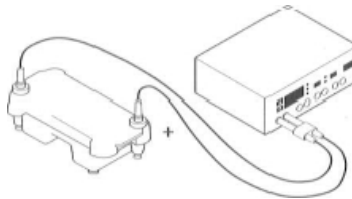
- Place the casting tray with the solidified gel in it, into the platform in the gel box. The wells should be at the cathode (negative) end of the box, where the black lead is connected. Very carefully remove the comb from the gel by pulling it straight up.
- Once all the gels are in the electrophoresis chamber, your instructor will pour the electrophoresis buffer into the electrophoresis chamber until it just covers the wells.



- Using a separate tip for each sample, load the samples into the six wells of the gel. There will be only one control gel, the remaining gels will have the student samples. The control gel will be loaded as follows:

Lane	Sample	Volume
1	MMR-DNA Standard	10 $\mu$ L
2	homozygous (+/+)	20 $\mu$ L
3	homozygous (-/-)	20 $\mu$ L
4	heterozygous (+/-)	20 $\mu$ L
5	Instructor	20 $\mu$ L
6	Student 1	20 $\mu$ L

- Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect electrical leads to the power supply.
- Turn on the power supply. Set it to 100 V and electrophorese the samples for 30 minutes.



- When the electrophoresis is complete, turn off the power and remove the lid From the gel box. Carefully remove the gel tray and the gel from the gel box. Be careful, the gel is very slippery. Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray. We will combine two gel into one staining container.



10. Pour 60 ml of BioSafe DNA stain (or enough to cover the gels) into your plastic staining tray, cover with plastic wrap, and let the gel sit in the stain overnight. Using the shaking table, gently shake the gels in the stain overnight.



### **The Final Analysis and Interpretation of Results**

The moment of truth has arrived, What is your genotype? Are you a homozygote or a heterozygote to find out, you will have to destain your agarose gel. After 5 - 10 minutes, the excess blue stain will begin to diffuse out of the agarose leaving the remaining dye bound to the DNA in the gel. Destaining increases the contrast and will allow you to visualize the DNA fragments you generated using PCR To increase the contrast between the blue background of the gel and DNA bands, gels can be destained for an additional 6 hours

To make detailed observations of your gel, lay the gel on a white background or you may wish to view your gel with a light box (a light bulb under a piece of white acrylic). The light box will provide a brighter background to view your stained gel. You may also trace the DNA bands in your gel by placing a clear sheet of acetate over the gel. Trace the outline of the gel, the sample wells and the DNA bands with a permanent marker.

### **Analysis and Interpretation of Results:**

Materials and supplies that should be present at your workstation

Water for destaining gels	60 mL
Gel support membrane (optional)	1
Copy of Quick Guide or protocol	1
Acetate for tracing gels	1

### **Protocol**

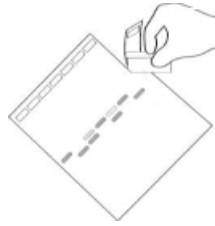
1. Pour off the Bio-Safe DNA stain into a bottle or another appropriate container and destain the gel with 60 ml of water for approximately 5 minutes.



2. Pour the water out of the staining tray. Be sure to place the stain into the appropriate container for disposal.



- Trim away any empty lanes of the gel with a knife or razor blade. Let the gel dry on gel support film or in your staining tray (plastic weigh boats) on your lab bench for 3 - 5 days. When the gel is dry, tape it into your lab notebook for a permanent record.



- With the help of your instructor determine whether you are homozygous or heterozygous for the Alu insertion. First look at the control gel and note the migration pattern of the homozygous (+/+), the homozygous (-/-), and the heterozygous (+/-) samples. You may notice that in the heterozygous sample the smaller 641 base pair fragment is more intense than the larger 941 bp fragment. This difference is due to the fact that the smaller piece is amplified more efficiently than the larger fragment. Copies of the shorter piece can be made at a faster rate than the bigger piece, so more fragments of the shorter piece are created per cycle.

## Analysis

Compare your sample lanes with the control gel lanes using the DNA size marker on both gels as a reference. Mark the location and size of your fragment or fragments by comparing your DNA migration pattern to the controls, determine whether you are homozygous (+/+), homozygous (-/-), or heterozygous (+/-). If your sample lane is blank, discuss with your classmates and teacher the possible reasons for lack of amplification.

Remember that the interpretation of the gel itself allows you to determine your genetic makeup only at the site, or gene locus (location), being studied. There are three possible genotypes for the Alu insert at the location you have amplified. Use the information gathered from your gel to determine the number of sub-populations in your class: homozygous (+ / +), homozygous (-/-), or heterozygous (+/-). Tally the class results in the table on page XX.

A major factor affecting the reliability of DNA fingerprinting evidence in forensics is population genetics and genetic statistics. In humans there are hundreds of loci or DNA segments, like Alu, that can be selected and used for fingerprinting analysis. Depending on demographic factors such as ethnicity or geographic isolation, some populations show much less variation in particular DNA segments than others. The degree of variation will affect the statistical odds of more than one individual having the same sequence. If 33% of a given population has the same frequency in its DNA fingerprinting pattern for a certain DNA segment, then little information will be obtained from using that segment alone to identify an individual.

When performing a DNA fingerprint in identifying a suspect in a criminal case or paternity suit what you want is not a one out of three chance of a match in a population. You want a one in a 10 million chance of a match. The frequency of a particular DNA pattern turning up in a population becomes extremely low when a series of DNA

segments is selected and amplified, rather than just one segment alone. Amplifying multiple DNA segments from a single sample of genomic DNA can serve as a powerful tool to discriminate between individuals in a population. For DNA fingerprinting to be admissible its evidence in a court of law it is necessary for 30 to 40 different DNA segments on multiple chromosomes to be selectively amplified and analyzed. Therefore in analyzing how incriminating the DNA evidence is, one needs to ask the question:

Statistically, how many people in a population may have the same DNA pattern as taken from a crime scene: One in 1,000,000? One in 10,000? Or one in 10?

In actuality, the Alu insert such as the one you have "fingerprinted" in this lab has been used to study the geographic migration patterns of different human populations over the course of human evolution. The data from these studies have been published and your samples can be compared to the data collected from much larger populations.