Lesson 3 Gel Electrophoresis of Amplified PCR Samples and Staining of Agarose Gels

What Are You Looking At?

Before you analyze your PCR products, let's take a look at the target sequence being explored.

What Can Genes and DNA Tell Us?

It is estimated that the 23 pairs, or 46 **chromosomes**, of the human genome (23 chromosomes come from the mother and the other 23 come from the father) contain approximately 30,000–50,000 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 chromosome pairs contains similar genes.

Each gene holds the code for a particular protein. Interestingly, the 30,000–50,000 genes only comprise 5% of the total chromosomal DNA. The other 95% is noncoding DNA. This noncoding DNA is found not only between, but within genes, splitting them into segments. The exact function of the noncoding DNA is not known, although it is thought that noncoding DNA allows for the accumulation of mutations and variations in genomes.

When RNA is first transcribed from DNA, it contains both coding and noncoding sequences. While the RNA is still in the nucleus, the noncodong **introns** (**in = stay within** the nucleus) are removed from the RNA while the **exons** (**ex = exit the nucleus**) are spliced together to form the complete messenger RNA coding sequence for the protein (Figure 10). This process is called **RNA splicing** and is carried out by specialized enzymes called **spliceosomes**.

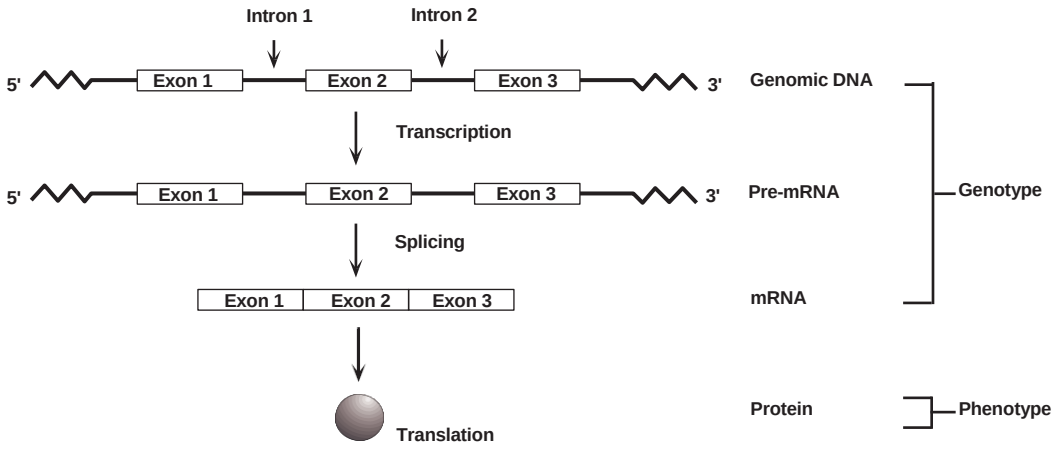


Fig. 10. Splicing of introns from genes.

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 in our noncoding DNA are silently passed on to our descendants; we do not notice them 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

The human genome contains small, repetitive DNA elements or sequences that have become randomly inserted into it over millions of years. One such repetitive element is called the "Alu sequence"[7] (Figure 11). This is a DNA sequence about 300 base pairs long that is repeated almost 500,000 times throughout the human genome.[8] The origin and function of these repeated sequences is not yet known.

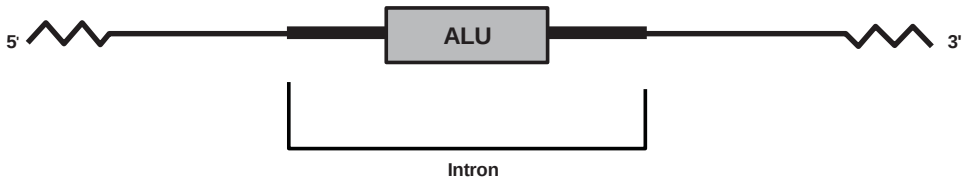


Fig. 11. Location of an Alu repetitive element within an intron.

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 be 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 laboratory activity you will look at 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 chromosome 16 (**one allele**), others may have the insert in both copies of chromosome 16 (**two alleles**), while some may not have the insert on either copy of the chromosome (Figure 12). The presence or absence of this insert can be detected using PCR 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 a sequence within the PV92 region that is 641 base pairs long if the intron does not contain the Alu insertion, or 941 base pairs long 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, three distinct outcomes are possible. If both chromosomes contain Alu inserts, each amplified PCR product will be 941 base pairs long. On a gel they will migrate at the same speed so there will be one band that corresponds to 941 base pairs. If neither chromosome contains the insert, each amplified PCR product will be 641 base pairs and they will migrate as one band that corresponds to 641 base pairs. If there is an Alu insert on one chromosome but not the other, there will be one PCR product of 641 base pairs and one of 941 base pairs. The gel will reveal two bands for such a sample.

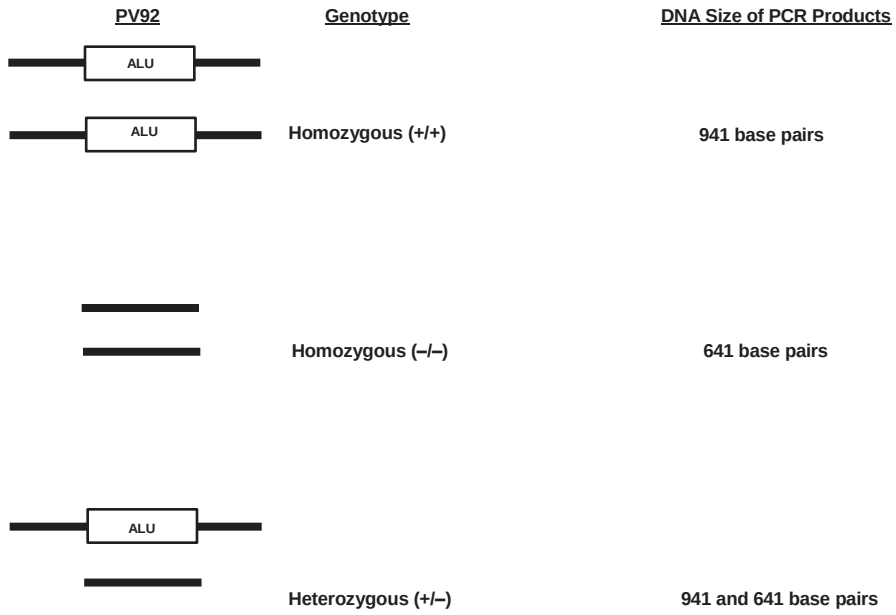


Fig. 12. The presence or absence of the Alu insert within the PV92 region of chromosome 16.

Electrophoresis separates DNA fragments according to their relative sizes. DNA fragments are loaded into an agarose gel slab, which is placed into a chamber filled with a conductive 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 appears as a single “band” of DNA in the gel. In the sample gel below (Figure 13), PCR-amplified bands of 941 bp and 641 bp are separated based on their sizes.

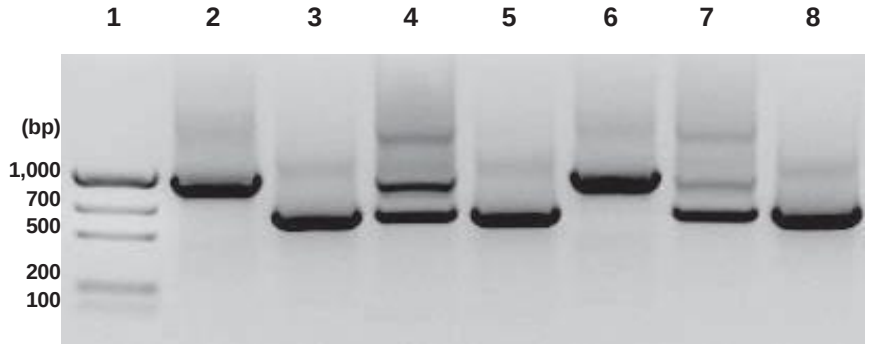


Fig. 13. Electrophoretic separation of DNA bands based on size. EZ Load DNA molecular mass ruler, which contains 1,000 bp, 700 bp, 500 bp, 200 bp, and 100 bp fragments (lane 1); two homozygous (+/+) individuals with 941 bp fragments (lanes 2, 6); three homozygous (–/–) individuals with 641 bp fragments (lanes 3, 5, and 8), and two heterozygous (+/–) individuals with 941 and 641 bp fragments (lanes 4 and 7).

Lesson 3 Gel Electrophoresis of Amplified PCR Samples and Staining of Agarose Gels (Lab Protocol)

Workstation Checklist

Materials and supplies that should be present at the workstations prior to beginning this lab are listed below.

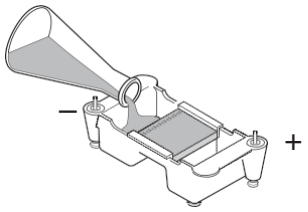
Student workstation	**Quantity per Station**	**(✔)**
Agarose gel	1	❒
Student PCR samples	1 per student	❒
MMR (DNA standard)	1 tube	❒
PV92 XC DNA loading dye	1 tube	❒
P-20 micropipet	1	❒
Pipet tips (filter type), 2–20 µl	12	❒
Permanent marker	1	❒
PCR tube holder	1	❒
Gel box and power supply	1	❒
Fast Blast™ DNA stain, 1x or 100x solution	120 ml per 2 stations	❒
Gel support film (optional)	1	❒
Clear acetate sheets for tracing gels (optional)	1	❒
Warm tap water for destaining gels (if performing quick staining protocol)	1.5–2 L per 2 stations	❒
Large containers for destaining (if performing quick staining protocol)	1–3 per 2 stations	❒
Copy of Quick Guide or protocol	1	❒
Waste container	1	❒

Instructor's workstation	**Quantity per Class**	
1x TAE electrophoresis buffer	275 ml per gel box	❒
Amplified positive control samples (4 each)	12	❒
PV92 homozygous (+/+)		
PV92 homozygous (–/–)		
PV92 heterozygous (+/–)		
Shaking platform (optional)*	1	❒
Microcentrifuge	1	❒
or mini centrifuge	4	❒

*** Strongly recommended.**

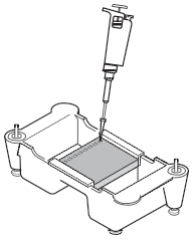
Lesson 3 Gel Electrophoresis of Amplified PCR Samples (Lab Protocol)

1. Collect your PCR samples from front. Pulse-spin your PCR tube in the centrifuge (~3 seconds at 2,000 x g) to bring the condensation that formed on the lids to the bottom of the tubes.
2. Add 10 µl of PV92 XC loading dye to each PCR tube and mix gently.
3. Obtain an agarose gel (either the one you poured or one pre-poured by your teacher). Place the casting tray with the solidified gel in it, onto the platform in the gel box. The wells should be at the cathode (–) end of the box, where the black lead is connected. Very carefully remove the comb from the gel by pulling it straight up, slowly.
4. Pour ~275 ml of electrophoresis buffer into the electrophoresis chamber, until it just covers the wells.

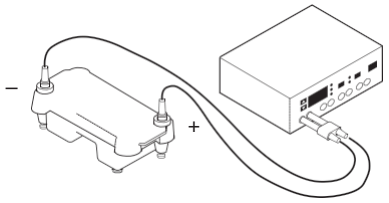


5. Using a clean tip for each sample, load the samples into the wells of the gel. Load your DNA ladder into the well that matches your lab station number. (This will help us identify your lab group's gel.)

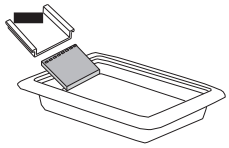
Lane	Sample	Load Volume
1	MMR (DNA standard)	10 µl
2	Homozygous (+/+) control	10 µl
3	Homozygous (–/–) control	10 µl
4	Heterozygous (+/–) control	10 µl
5	Student 1	20 µl
6	Student 2	20 µl
7	Student 3	20 µl
8	Student 4	20 µl



6. 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 the electrical leads to the power supply.
7. Turn on the power supply. Set it to 200 V and electrophorese the samples for 20 minutes.



8. When 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.



Staining of Agarose Gels

The moment of truth has arrived. What is your genotype? Are you a homozygote or a heterozygote? To find out, you will have to stain your agarose gel. Since DNA is naturally colorless, it is not immediately visible in the gel. Unaided visual examination of gel after electrophoresis indicates only the positions of the loading dyes and not the positions of the DNA fragments. DNA fragments are visualized by staining the gel with a blue dye called Fast Blast DNA stain. The blue dye molecules are positively charged and have a high affinity for the DNA. These blue dye molecules strongly bind to the DNA fragments and allow DNA to become visible. These visible bands of DNA may then be traced, photographed, sketched, or retained as a permanently dried gel for analysis.

Directions for Using Fast Blast DNA Stain

Using Fast Blast DNA stain DNA bands can be visualized in 12–15 minutes.

WARNING
Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes.

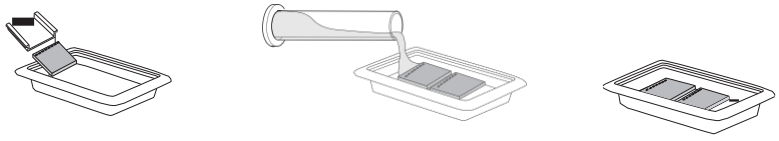
Protocol 1: Quick Staining of Agarose Gels in 100x Fast Blast DNA Stain

This protocol allows quick visualization of DNA bands in agarose gels within 15 minutes. For quick staining, Fast Blast DNA stain (500x) should be diluted to a 100x concentration. We recommend using 120 ml of 100x Fast Blast to stain two 7 x 7 cm or 7 x 10 cm agarose gels in individual staining trays provided in Bio-Rad's education kits. If alternative staining trays are used, add a sufficient volume of staining solution to completely submerge the gels.

Following electrophoresis, agarose gels must be removed from their gel trays before being placed in the staining solution. This is easily accomplished by holding the base of the gel tray in one hand and gently pushing out the gel with the thumb of the other hand. Because the gel is fragile, special attention must be given when handling it. We highly recommend using a large spatula or other supportive surface to transfer the gel from one container to another. Destaining requires the use of at least one large-volume container, capable of holding at least 500 ml, at each student workstation. Each student team may utilize separate washing containers for each wash step, or simply use a single container that is emptied after each wash and refilled for the next wash.

1. Stain gels (2–3 minutes)

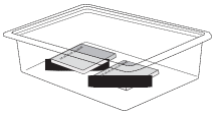
Remove the gel from the gel tray and carefully slide it into the staining tray. Pour approximately 120 ml of 100x stain into the staining tray. If necessary, add more 100x stain to completely submerge the gel. Stain the gels for 2–3 minutes, but not for more than 3 minutes. Using a funnel, pour the 100x stain into a storage bottle and save it for future use. **The stain can be reused at least 7 times.**



2–3 minutes

3. Rinse gels

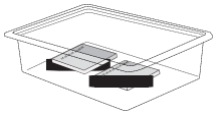
Transfer the gel into a large container containing 500–700 ml of clean, warm (40–55°C) tap water. Gently shake the gel in the water for ~10 seconds to rinse.



10 seconds

4. Wash gels

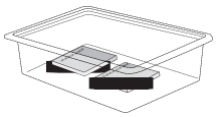
Transfer the gel into a large container with 500–700 ml of clean, warm tap water. Gently rock or shake the gel on a rocking platform for 5 minutes. If no rocking platform is available, move the gel gently in the water once every minute.



5 minutes

5. Wash gels

Perform a second wash as in step 4.



5 minutes

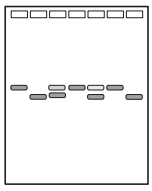
6. Record and analyze results

Examine the stained gels for expected DNA bands. The bands may appear fuzzy immediately after the second wash, but will begin to develop into sharper bands within 5–15 minutes after the second wash. This is due to Fast Blast dye molecules migrating into the gel and binding more tightly to the DNA molecules.

To obtain maximum contrast, additional washes in warm water may be necessary. Destain to the desired level, but do not wash the gels in water overnight. If you cannot complete the destaining in the allocated time, you may transfer the gel to 1x Fast Blast stain for overnight staining. **See Protocol 2.**

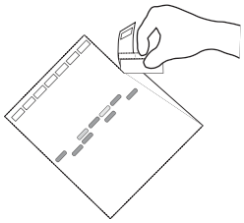
a. Place your gel on a light background and record your results by making a diagram as follows. Place a clear sheet of plastic sheet or acetate over the gel. With a permanent marker, trace the wells and band patterns onto the plastic sheet to make a replica picture of your gel. Remove the plastic sheet for later analysis. Alternatively, gels can be photocopied on a yellow piece of transparent film for optimal contrast.

b. With the help of your instructor, determine whether you are homozygous or heterozygous for the Alu insertion. First look at the control samples and note the migration patterns of the homozygous +/+, the homozygous –/–, and the heterozygous +/– samples (also refer to the example on page 59). You may notice that in the heterozygous sample the smaller 641 base pair band is more intense than the larger 941 bp band. This difference is due to the fact that the smaller fragment is amplified more efficiently than the larger fragment. Copies of the shorter fragment can be made at a faster rate than the bigger fragment, so more copies of the shorter fragment are created per cycle. Refer to pages 69–72 for more information on how to analyze your data.



c. Dry the agarose gel as a permanent record of the experiment.

Trim away any unloaded lanes with a knife or plastic knife. Cut your gel from top to bottom to remove the lanes that you did not load samples into.



Lab 3: Gel Electrophoresis of Amplified PCR Samples Name_____Per___

1. Explain the difference between an intron and an exon.

2. Why do the two possible PCR products differ in size by 300 base pairs?

3. Explain how agarose electrophoresis separates DNA fragments. Why does a smaller DNA fragment move faster than a larger one?

4. What kind of controls are run in this experiment? Why are they important?

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