Exercise 5: Detection of a Human *Alu* Element by PCR

(adapted from Dolan DNA Learning Center, Cold Spring Harbor Laboratory, NY and Science Outreach, Washington University, St. Louis, MO)

Background Information

Although the DNA from different individuals is more alike than different, there are many regions of the human chromosomes that exhibit a great deal of diversity. Such variable sequences are termed **polymorphic**, meaning many forms. These polymorphic sequences provide the basis for genetic disease diagnosis, forensic identification, and paternity testing. In the group research projects, the polymerase chain reaction (PCR**)** will be used to amplify nucleotide sequences from two different human genes to look for polymorphisms that are associated with the ability to taste bitter compounds or remain lactase persistent into adulthood, respectively. However, you will first amplify a nucleotide sequence from your own 8th chromosome, to look for an insertion of a short DNA sequence, called ***Alu***, within the tissue plasminogen activator (TPA) gene.

Alu elements are classified as SINEs, or Short INterspersed Elements. All *Alu* elements are approximately 300-bp in length and derive their name from a single recognition site for the endonuclease *Alu I*, located near the middle of the *Alu* sequence. Human chromosomes contain about 1,000,000 *Alu* copies, which equal 10% of the total genome. An estimated 500-2000 different *Alu* elements are found scattered across the human genome. A few have appeared relatively recently, within the last one million years, and are not present in all individuals. One such *Alu* element, called TPA-25, is found within an intron of the tissue plasminogen activator (TPA) gene. This insertion is **dimorphic**, meaning that it is present in some individuals and not in others. PCR can be used to screen individuals for the presence, or absence, of the TPA-25 insertion.

In this experiment, oligonucleotide primers flanking the insertion site will be used to amplify a segment of the TPA gene. The expected product of the PCR reaction will be a 400-bp fragment when TPA-25 is present and a 100-bp fragment when it is absent. Each of the three possible genotypes – **homozygous** for presence of TPA-25 (400-bp fragment only), homozygous for the absence of TPA-25 (100-bp fragment only), and **heterozygous** (400-bp and 100-bp fragments) – will be able to be determined following electrophoresis of our PCR reaction products in an agarose gel.

Description of Lab Exercise

Our source of template DNA will be a sample of several thousand cells obtained from inside your own mouths! The cells will be suspended in a solution containing **Chelex**, a resin that binds metal ions (and that can also inhibit a PCR reaction). The cells will be **lysed**, or broken open, by boiling and then **centrifuged** to remove cell debris. A sample of **supernatant** containing your genomic DNA will be mixed with ***Taq* DNA polymerase**, **oligonucleotide primers**, the four **deoxynucleotides** (A, T, C, G), and the **cofactor** $MgCl_2$. Temperature cycling will be used to **denature** the template DNA, **anneal** the primers, and **extend** a complementary DNA strand. The size of the amplification product(s) will depend upon the presence or absence of the *Alu* insertion at the TPA-25 locus on each copy of chromosome 8.

We will compare the genotypes of different individuals by loading aliquots of your amplified samples into wells of an **agarose gel**. The lab instructor will load **DNA size markers** into other wells. The bands of the markers will be used to estimate the sizes of your **PCR products**. Following **electrophoresis**, amplification products appear as distinct bands in the gel – the distance a DNA fragment travels from the starting well is inversely proportional to its molecular size. The larger TPA product (containing the 300-bp *Alu* element) will not migrate as far from the well as the smaller TPA product (missing the 300-bp *Alu* element). In a successful experiment, one or two amplified bands will be observed, indicating that you are either homozygous or heterozygous for the TPA-25 locus.

Materials Needed

0.5 ml of 10% Chelex in sterile microcentrifuge tube
15 ml centrifuge tube with 10 ml of 0.9% NaCl
50 ml centrifuge tube
15-ml and 50-ml centrifuge tube racks
clinical centrifuge & high-speed microcentrifuge
sterile plastic transfer pipette
boiling water bath & tube float for water bath
forceps/tongs to remove float
jar with sterile 1.5 ml microcentrifuge tubes
jar with sterile 0.2 ml PCR tubes
microcentrifuge and PCR tube racks
ice/ice container
Sharpie markers
SML latex gloves
P20 and P200 micropipettors
rack of sterile P20/200 yellow tips
minifuge, purple, 6 place
TPALU 1 primer (Stock, 25 pmol/ml)
TPALU 2 primer (Stock, 25 pmol/ml)
PCR master mix: Primers +
- 5X Green GoTaq® PCR buffer
- 10 mM dNTP's (40X)
- GoTaq® DNA polymerase
- sterile, nuclease-free water

thermocycler

Gel Analysis of Alu PCR Products
Owl Horizontal Gel System 12x13.5 cm
agarose (2% gel)
1X TBE
100-bp ladder (0.13 mg/ml stock in OG)
5X Orange G Loading Dye
1 mg/ml ethidium bromide
Tupperware staining dish w/lid
UV transilluminator
UV face shield/goggles
Digital camera, hood and printer
HP Everyday Photo paper

Final PCR Solution/Reaction:
1X PCR Buffer (w/ 1.5 mM $MgCl_2$)
250 μM dNTP's
0.5 μM each primer
1 unit GoTaq® DNA polymerase

Experimental Protocol

NOTE: PCR is extremely sensitive to contamination. A few foreign cells in your preparation can ruin your results. For this reason, it is extremely important to maintain sterility at all times while conducting the following procedures.

Day 1:

A. Cell Extraction and DNA Isolation (adapted from Princeton University)
http://www.hhmi.princeton.edu/Manual/04%20HHMI%20Lab_04%20D1S80.pdf

1. Put on gloves and obtain one 1.5 ml microcentrifuge tube containing 10% Chelex and use a permanent marker to label the top with an **assigned DNA sample number**. (Note that Chelex is not actually a solution, but a slurry of resin coated beads in water. You will see the beads settled at the bottom of the tube.)
2. Also obtain one 15-ml centrifuge tube containing 10 ml of sterile saline (0.9% NaCl) and label the side of the centrifuge tube with the same assigned DNA sample number.
3. Pour the contents (saline) of the conical centrifuge into your mouth and agitate vigorously for 2 min, using your teeth to gently scrape the inner surface of your mouth to dislodge as many cells as possible. The more cells in the sample, the higher the yield of DNA will be. [Note: this works best if you have not eaten/chewed gum for an hour. If you just ate something sticky like Doritos, you should try to rinse you mouth a bit first with water, because otherwise you end up with lots of food particles in the cell suspension.]
4. Expel (i.e., spit) the solution carefully into a clean 50 ml centrifuge tube, then carefully pour the saline/cheek cell suspension back into the original 15 ml centrifuge tube.
5. Place the 15-ml tube in the clinical centrifuge, putting your tube across the rotor from another for balance. Spin the tubes in the centrifuge for 5 min at 3,000 rpm. While the cells are spinning, eat some cookies in the lobby to get the salt taste out of your mouth.
6. When the spin ends, gently pour off the supernatant into the sink, leaving behind the pellet of cheek cells (and whatever else). You should see a white pellet of cells in the tube.
7. Use a sterile, graduated transfer pipette to draw the water away from the settled Chelex beads in the microcentrifuge tube. Use this water and pipette to gently resuspend the cells in the 15-ml tube. Then, transfer the cell suspension back to the microcentrifuge tube with the Chelex. Mix the cell/Chelex suspension well.
8. Firmly shut the microcentrifuge tube and place it in a floating rack with in the boiling water bath for 10 min. DO NOT DELAY BETWEEN STEP 7 AND 8.
9. When the 10 min boiling step has ended, use forceps to remove the tube from the water bath, and place your boiled sample on ice for 1-2 minutes.
10. Spin tube with others (making sure the rotor is balanced) for 5 min in the high-speed microcentrifuge. This will pellet the Chelex beads and cell debris, leaving the DNA suspended in the supernatant above the beads. IF YOU HAVE TO WAIT FOR OTHERS TO SPIN, KEEP YOUR TUBE ON ICE WHILE WAITING.
11. Wearing gloves, use a P200 micropipettor to transfer ONLY 150 µl of the clear supernatant from the top of the solution to a new, sterile microcentrifuge tube. Be careful not to transfer any of the material in the pellet because this will inhibit the PCR reaction.
12. Label the new tube with its respective DNA sample number. You have now isolated a small quantity of human genomic DNA. Keep the tube on ice until you are ready to set up your PCR reaction. The sample can also be frozen for later use.

B. PCR Amplification

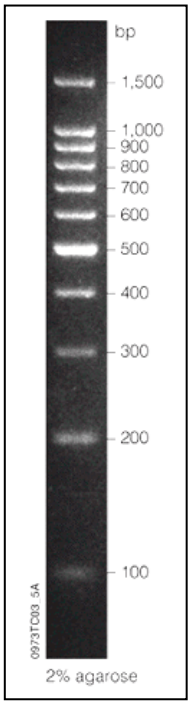
1. Put on gloves. Use a permanent marker to label the top of a sterile **0.2 ml thin-walled microcentrifuge tube** with your DNA sample number (as #2 above). This is your PCR reaction tube.
2. In the ice bucket on your bench you will find a tube labeled **Amp Mix**. Use a P-200 micropipettor to pipet 30 µl of this amplification mixture into your 0.2 ml PCR reaction tube. This mixture contains oligonucleotide primers, the four deoxynucleotides (A, T, C, G), *Taq* polymerase, buffers and tracking dyes for gel electrophoresis.
3. Using a P-20 micropipettor, pipet 10 µl of your extracted DNA sample into the labeled PCR tube. Place the 1.5 ml tube with the rest of your DNA solution on ice.
4. Mix the solutions and spin down your sample in the microfuge.
5. Your DNA is now ready for amplification in the **PCR machine**, or **thermal cycler**. Your instructor will collect your reaction tube, place it in the PCR machine, and start the program.
6. One person should also prepare a negative control (10 µl of water + 30 µl of Amp mix) and a positive control (10 µl of DNA solution from a known heterozygous individual + 30 µl of Amp mix) for the group.
7. The reaction will proceed as follows:

1 cycle	95˚C for 3 minutes (initial denature)
30 cycles	95˚C for 30 seconds (denature) 58˚C for 40 seconds (anneal) 72˚C for 90 seconds (extend)
1 cycle	72˚C for 10 minutes (final extension)

Day 2:

C. Gel Electrophoresis of PCR Product

1. Your instructor will return your PCR reaction tube to you. Your reaction tube now contains your **PCR product**.
2. Centrifuge the tube for 10 seconds before opening the lid.
3. With assistance from your instructor, use a P-200 micropipettor to load the entire 40 µl into one well of a **2% agarose gel.** Record your DNA sample number on the gel diagram sheet next to the appropriate well number for your PCR product. This is so you will know which lane of the gel contains your PCR product.
4. One person should load 10 µl of the 100-bp DNA ladder in Orange G into one well of each row of the gel.
5. The gel will run for ~1 hour at 100V. You will examine the stained gel this afternoon.



100-bp DNA Ladder
(Promega #G2101)

D. Analysis

1. How many people in the class had data (e.g., discernible bands on the gel)?

2. Of these people, how many were homozygous for the TPA-25 allele?

   How did you determine this?

3. How many people were heterozygous for the TPA-25 allele?

   How did you determine this?

4. How many people did not have the TPA-25 allele (e.g., were null for the *Alu* insertion)?

   How did you determine this?

5. What conclusion, if any, can you make about the frequency of TPA-25 allele in the human population?

Instructor's Note: For large class sizes and/or years of cumulative data, the above results can be used to address questions about Hardy-Weinberg equilibrium in your student population.

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