Introduction

Detection of TPA-25 Alu by PCR

A Human DNA Fingerprinting Lab Protocol
1994 Cold Spring Harbor Laboratory DNA Learning Center

In this exercise, the polymerase chain reaction (PCR) is used to amplify a nucleotide sequence from chromosome 8 to look for an insertion of a short DNA sequence called ALU within the tissue plasminogen activator (TPA) gene. 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) and provide the basis for genetic disease diagnosis, forensic identification, and paternity testing.

The Alu family of short interspersed repeated DNA elements are distributed throughout primate genomes. Over the past 65 million years, the Alu sequence has amplified via an RNA-mediated transposition process to a copy number of about 500,000, comprising an estimated 5% of the human genome. Alu sequences are thought to be derived from the 7SL RNA gene which encodes the RNA component of the signal recognition particle that functions in protein synthesis. Alu elements are approximately 300-bp in length and derive their name from a single recognition site for the endonuclease AluI located near the middle of the Alu sequence.

An estimated 500-2,000 Alu elements are mostly restricted to the human genome. A few of these have inserted recently, within the last one million years, and are not fixed in the human species. One such Alu element, called TPA-25, is found within an intron of the tissue plasminogen activator 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 exercise, oligonucleotide primers, flanking the insertion site, are used to amplify 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 the presence of TPA-25 (400-bp fragment only), homozygous for the absence of TPA-25 (100-bp fragment only), and heterozygous (both 400-bp and 100-bp fragments) are distinguished following electrophoresis in agarose gels.
The source of template DNA is a sample of several thousand cells obtained by saline mouthwash (bloodless and noninvasive). The cells are collected by centrifugation and resuspended in a solution containing the resin "Chelex", which binds metal ions that inhibit the PCR reaction. The cells are lysed by boiling and centrifuged to remove cell debris. A sample of the supernatant containing genomic DNA is mixed with Taq polymerase, oligonucleotide primers, the four deoxynucleotides, and the cofactor magnesium chloride. Temperature cycling is used to denature the target DNA, anneal the primers, and extend a complementary DNA strand. The "upstream" primer, 5'- GTA AGA GTT CCG TAA CAG GAC AGC T -3', brackets one side of the TPA locus, while the downstream primer, 5' CCC CAC CCT AGG AGA ACT TCT CTT T -3', brackets the other side. The size of the amplification products(s) depends on the presence or absence of the Alu insertion at the TPA-25 locus on each copy of chromosome 8.

In order to compare the genotypes from a number of different individuals, aliquots of the amplified sample and those of other experimenters are loaded into wells of an agarose gel, along with the DNA size markers and an unamplified control. Following electrophoresis and staining, amplification products appear as distinct bands in the gel—the distance moved from the well is inversely proportional to the presence or absence of TPA-25 insertion. One or two bands are visible in each lane, indicating that an individual is either homozygous or heterozygous for the Alu insertion.

**PCR Lab Activity**

**TPA-25 Alu Sequence**

**Part 1**

1. Label the 1.5 ml tube containing 10% chelex solution and marked with a blue dot on the lid with your name/number. It’s best if you label the lid of the tube to help keep your name/number from rubbing off.
2. Take cotton swab and “swab”/scrap the inside of inside of mouth for 30 seconds. During that time swab both inside cheeks of the mouth.
3. After 30 seconds transfer the swab to a 1.5 ml blue dot tube containing 1 ml of 10% chelex solution and vigorously stir/swirl the swab in the solution. The solution should have a “milky” appearance.
4. Smash the swab tip against the side of the tube to squeeze out the solution back into the tube. Try to return as much of the 10% chelex-cheek cell solution as possible to the tube. You can either dispose of the entire swab safely or cut off the swab end and leave it in the tube.
5. Place the tube in a boiling water bath or 100°C block heater for 10 minutes. Boiling also eliminates human contamination.
6. Return to your lab seats to sign up for an experiment number and/or presentation on PCR and the TPA 25 Alu sequence.
7. After boiling, carefully remove your tube from the bath or heater and allow the solution to cool for several minutes.
8. Place the tube in the microcentrifuge for two minutes at maximum RPMs to pellet the Chelex and cellular debris to the bottom of the tube.
9. Using a small clean needle point transfer pipet or P200 micropipettor to carefully collect and transfer a small amount (two to three drops) of the supernatant (top layer of solution) to a fresh 1.5 ml tube labeled with your number. AVOID
TRANSFERING ANY OF THE CHELEX/DEBRIS PELLET AT THE BOTTOM OF THE TUBE. You only need a very small amount (5 ul) of the supernatant for the PCR reaction. This is your TEMPLATE DNA for part 2.

10. Place the tube in freezer until the next class/session or continue to part 2.

Part 2

1. Each member of your group will receive a 0.2 ml tube containing a PCR bead, and enough reagents to complete the reaction matrix below.

2. Everyone should be assigned an individual number. Label the upper side of the 0.2 ml PCR bead tube with your number.

3. Use a 2-20 ul micropipettors to add the following reagents to the PCR bead tube according to the matrix below.

4. Reaction Matrix- add in reagents order to 0.2 ml tube with PCR reaction bead

   1. Sterile water 16.0 ul
   2. Upper primer (5 pmol/ul) 2.0 ul
   3. Lower primer (5 pmol/ul) 2.0 ul
   4. Cheek cell solution (template, from Part 1) 5.0 ul

   Total 25.0 ul

5. Place the tubes in the microcentrifuge and spin for 30 seconds.

6. Give the tubes to your instructor for thermocycling.

Part 3 Gel Analysis

1. Prepare a 1X TBE solution by adding 70 ml of 10X TBE to 630 ml of distilled water.

2. Prepare a 2% agarose gel by melting (microwave to boil) 1.8 g of agarose mixed with 90 ml of 1X TBE buffer.

3. Allow the solution to cool 5 mins.

4. Pour the molten agarose into the gel tray with comb set in the casting position in the gel chamber and let harden. 30-45 mins

5. Add 3 ul of loading/migration dye to the PCR product.

6. Pull the comb from the casting tray and turn the gel tray 90⁰. Set the wells’ side of the gel at the negative (black) electrode portion of the gel chamber. Remember- “DNA runs toward RED.”

7. Load 20 ul of the PCR product into each well. Adjust the sample size according to your gel’s well capacity.

8. Carefully add the 1X TBE until the gel is completely covered.

9. Electrophores for 90 mins at 85 volts or until the first bands of the migration reaches the middle of the gel.

10. Stain and view the gel results.

Cycling times and temperatures:
<table>
<thead>
<tr>
<th>Cycle</th>
<th>Denature</th>
<th>Anneal</th>
<th>Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle #1</td>
<td>94° C, 5 mins</td>
<td>54° C, 15 sec</td>
<td>72° C, 1 min</td>
</tr>
<tr>
<td>Cycles #2-34</td>
<td>94° C, 15 sec</td>
<td>54° C, 15 sec</td>
<td>72° C, 1 min</td>
</tr>
<tr>
<td>Cycle #35</td>
<td>94° C, 5 mins</td>
<td>54° C, 15 sec</td>
<td>72° C, 10 mins</td>
</tr>
</tbody>
</table>