

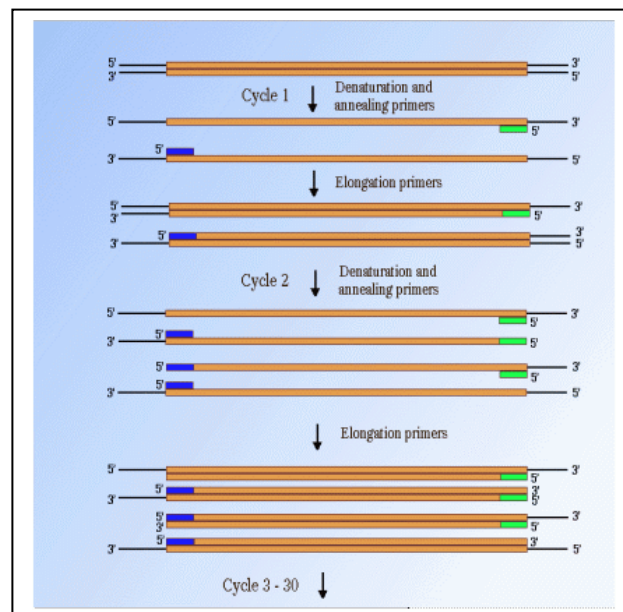
Lab # 9 Polymerase Chain Reaction (PCR) of Alu Sequences

The sequence of nucleotides in the DNA of any 2 humans is greater than 99% similar. However, there are regions (loci) of the human genome that are hypervariable between individuals. These polymorphic (many forms) loci constitute a DNA finger print that can be used to distinguish one person from another. This experiment examines a polymorphism that is caused by the insertion of an Alu transposon. Alu is a member of a family of transposable elements called Short Interspersed Elements (SINEs).

The Alu sequence is only 300 nucleotides long and is not a proper gene because it does not encode a protein. The human genome contains approximately 1 million copies of Alu. The multiplication and distribution of Alu sequences throughout the human genome over the course of time has been facilitated by another type of transposon called Long Interspersed Elements (LINEs). LINEs produce a reverse transcriptase enzyme that converts Alu mRNA into dsDNA. The DNA form of Alu can then “jump” into other sites in the genome. It is predicted that Alu “jumps” once every 200 human births.

Our experiment today involves an Alu sequence that is polymorphic, meaning the sequence may be present or absent on each of 2 homologous chromosomes within any given individual. Therefore the possible genotypes for this Alu sequence are +/+, +/- or -/- . The exact genotype of any individual may be easily determined by amplifying the DNA at the Alu locus by Polymerase Chain Reaction (PCR).

PCR is a method that employs a thermo-stable DNA Polymerase (from *Thermophilus aquaticus*) to synthesize multiple copies of a single DNA sequence. The technique goes as follows: The reaction vessel contains chromosomal DNA, deoxynucleotides, Taq DNA Polymerase, forward and reverse primers. The reaction vessel is placed in a thermocycler. dsDNA is denatured into ssDNA by heating. As the temperature cools, primers anneal (basepair) with the chromosomal DNA. DNA polymerase extends the primers, completing the first cycle. There are now 2 copies of the DNA that lies between the forward and reverse primers. The cycle of denaturation, primer annealing and polymerase extension is repeated for approximately 30 more cycles. A PCR reaction of 30 cycles is predicted to produce more than 1 billion identical copies of a DNA sequence.



Isolate Human DNA from Cheek Cells

Procedure:

1. Every student in the class will isolate their own DNA and analyze it by PCR.
2. Fill a 500 ml beaker half way with tap water and get it boiling on a hot plate.
3. Pour 10 ml of saline solution (found in Dixie cup) into your mouth and vigorously rinse your cheek pockets for 30 seconds.
4. Expel saline solution into the cup.
5. Swirl the cup to resuspend cells that may have settled, then transfer 1500 μ l into a 1.5 ml microcentrifuge tube. Label the tube with your initials.
6. Spin your tube for 90 seconds at 13,000 rpm in a balanced microcentrifuge.
7. Decant the supernatant into the paper cup, but do not disturb the cell pellet. The remaining volume will approximate 0.1 ml.
8. Set the p100 micropipettor to 30 μ l. Resuspend cells by pipetting up and down. Try to minimize formation of bubbles
9. Obtain the tube labeled "chelex". This tube already contains 100 μ l of 10% Chelex. Withdraw 30 μ l of cell suspension and add it to the chelex tube. Label the tube with your initials.
10. Using a pin, poke a hole through the top of the tube containing the cells/chelex mix.
11. Place this tube in a boiling water bath for 10 minutes.
12. Shake the tube vigorously for 5 seconds.
13. Spin your tube in a balanced microcentrifuge for 90 seconds at 13,000 rpm.
14. Using a micropipette, transfer 30 μ l of supernatant to a clean 1.5 ml tube. Be careful to avoid pipetting any cell debris or Chelex beads.
15. Label this tube as DNA and with your initials and then store on ice.

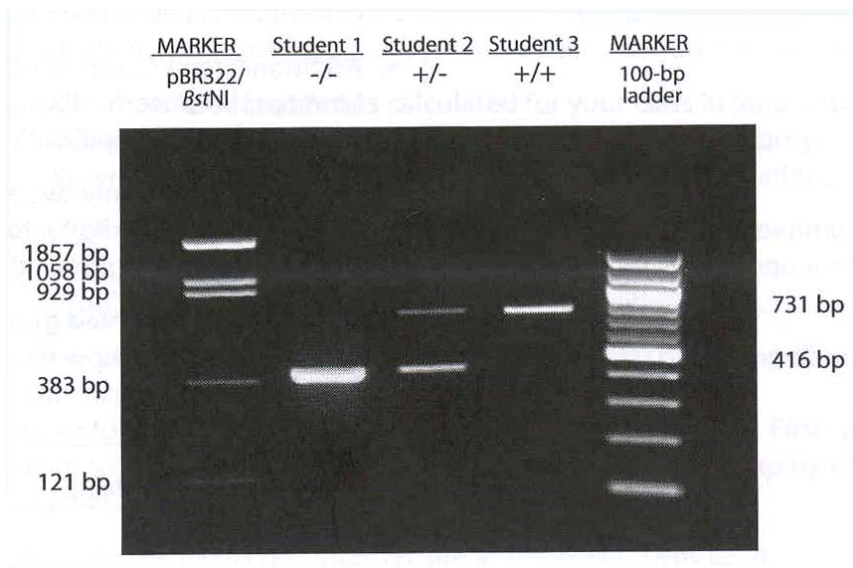
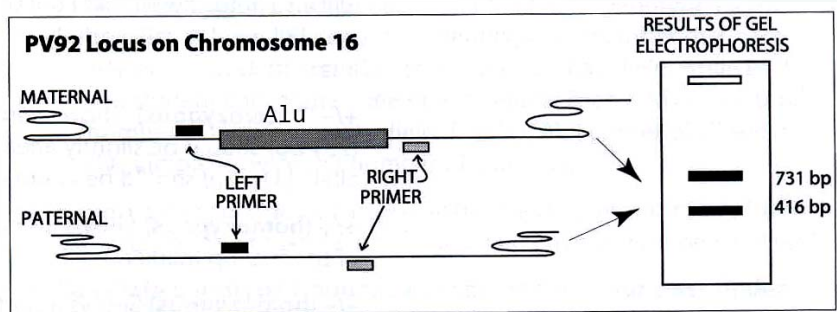
PCR of Alu Sequences

Procedure:

1. Obtain a 0.2 ml PCR tube. Label it with your initials.
2. Add to the tube.
 - 7.5 μ l dH₂O
 - 2.5 μ l of your own chromosomal DNA
 - 1.25 μ l of forward primer (5 μ M)
 - 1.25 μ l of reverse primer (5 μ M)
 - 12.5 μ l Taq polymerase/dNTP/buffer Mix
 - Mix the reagents well. Be certain all reagents are at the bottom of the tube.
3. Place your tube in the Thermocycler Machine that has been programmed for 30 cycles with the following parameters:
 - Denaturing step 94⁰C 30 seconds
 - Annealing step 68⁰C 30 seconds
 - Extending step 72⁰C 30 seconds

Electrophoresis of Amplified DNA

1. Pour the minimum number of gels needed to fit all class samples plus size marker.
Communicate with the other groups to ensure that this happens. It is best to have many samples on one gel so we can see clear differences in genotypes between classmates.
2. Pour a 1.0% agarose gel that contains EtBr, using an 8-well comb. Wear gloves.
3. Allow the gel to solidify completely
4. Place the gel in the electrophoresis chamber and cover the gel with 1X TBE.
5. Load 10 μ l of DNA ladder into an outside lane (avoid lanes 1 and 8).
6. Add 2 μ l of loading dye to your personal sample and load 10 μ l.
7. Load student samples left to right in a pre-determined order. Be certain you document whose samples went into what lane.
8. Run the gel at 120 Volts for approximately 30 minutes.
9. Put the gel on the UV light box and snap a picture of that Bad Boy!
10. Make Xerox copies of the photograph for your lab mates and your mother (after all you inherited your Alu genotype from her and your Dad).
11. Staple a copy of the photograph to your lab.
12. Refer to the figures below when you analyze your data. What is your genotype? _____



Questions

1. What is a transposon?
2. How common are transposons in the human genome?
3. How does the Alu transposon “jump” from one site in the genome to another?
4. What is a polymorphism?
5. How can PCR be used to determine the Alu genotype of an individual?
6. How is Taq DNA polymerase different from DNA polymerase found in *E. coli*?

