Genomic DNA
Extraction from Buccal Epithelial Cells

The purpose of this lab is to collect a DNA sample from the cells that line the inside of your mouth and to use this sample to explore one of the most powerful techniques in molecular biology—the Polymerase Chain Reaction (PCR). Although PCR has many applications, it is commonly used to produce many copies of a selected gene segment or *locus* of DNA. In criminal forensics, for example, PCR is used to amplify DNA evidence from small samples that may have been left at a crime scene. A skilled technician can even obtain a DNA sample left by the tongue on the back of a postage stamp used to send a letter. DNA samples obtained in this manner have been used for PCR in several high-profile criminal cases.

To obtain your DNA sample, you'll use a toothpick to obtain some buccal epithelial cells. The cells will be transferred to a solution containing Chelex beads. The Chelex beads will bind divalent magnesium ions (Mg^{++}). These ions often serve as cofactors for nucleases that will degrade your DNA sample and may interfere with the enzyme (*Taq* polymerase) used in the reaction. By removing magnesium ions, the degradation of genomic DNA by nucleases is reduced. This mixture will be placed into boiling water to lyse the cells and liberate the DNA.

The mixture of your genomic DNA, cell debris and Chelex beads is then centrifuged to pellet the cell debris and Chelex, while keeping your genomic DNA in the supernatant. This is a quick and easy way to separate genomic DNA from the cell debris. The DNA sample, however, is far from pure as it contains proteins and nucleic acids from organisms that were in your mouth at the time of sampling (mostly bacteria and food). Generally, these contaminants do not inhibit PCR because the process uses specific primers, short segments of DNA about 25 nucleotides in length that can be made to target only human genomic DNA. Therefore, if the supernatant carries some foreign DNA, it should not interfere with the targeting of the human-specific primers. A more detailed description of PCR and the role that primers play will be discussed later in this lab.

Although we are using buccal epithelia as a DNA source, other tissues could have been used. Here are some DNA yields from other human tissues: Blood yields 40 µg/ mL; hair root yields around 250 ng/mL; muscle yields around 3 µg/ mL; and sperm yields 3.3 pg/cell.

The second part of this lab involves the actual PCR. You will use the sample of genomic DNA you just collected as a target for the PCR reaction.

Materials

REAGENTS

0.5 mL of 10% Chelex solution
Master mixes I and II

EQUIPMENT & SUPPLIES

Boiling water bath
Microcentrifuge
Thermal cycler
Microfuge tube
Permanent marker
Sterile toothpicks

Methods

Getting your sample ready...

1. Obtain a Chelex tube. Note that this tube is identified with a number and letter. Record this number and letter in your notebook. Only you will know this anonymous code.

2. To collect buccal epithelial cells, use a sterile toothpick or yellow pipette tip to gently scrape the **inside** of both cheeks. This procedure should be noninvasive, so don't draw blood.

3. Transfer the cells that you have removed from the toothpick to the Chelex tube. Vigorously twirl the toothpick with the Chelex resin to knock off the cells from the toothpick.
   This is important; you want to get as many cells off the toothpick and into the Chelex tube as possible.

4. Close the Chelex tube tightly. Take the tube to the boiling water bath or 100°C hot block. Boil or heat the cells for **10 minutes.** This heating will lyse the cells and help to destroy some of the nucleases, which degrade the DNA

5. Use the high-speed centrifuge to spin down the Chelex and cell debris.

6. Using the P-20 pipette and a clean pipette tip, carefully remove 20 μL of supernatant and place it into a clean 1.5 mL microfuge tube. Avoid aspirating Chelex beads as this will inhibit the downstream PCR procedure. Label this tube with your personal, anonymous code (number).

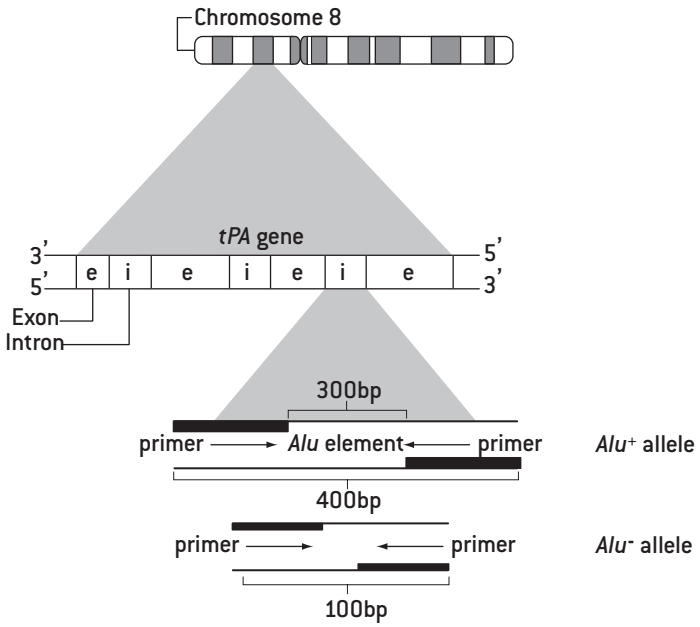
7. If your sample is not used immediately, leave this sample at the front of the room in the rack labeled **"Genomic DNA Samples."** These samples will be placed into the refrigerator overnight and returned to you for the next lab.

Your genomic DNA sample can be kept in the refrigerator at 4°C or freezer at _–20°C until you are ready to run the PCR reaction.

Amplification of the tPA Locus using the Polymerase Chain Reaction

The polymerase chain reaction, PCR, is a molecular biology technique that was discovered by Kary Mullis during the early 1980s. The technique uses some elegant chemistry and precise thermal cycling of reactants to target and amplify a specific location (locus) along the DNA molecule. From a single DNA target, PCR can produce more than one billion copies of the target in about 1.5 hours. This powerful chemistry proved to be so significant that Mullis was awarded the Nobel Prize for Chemistry in 1993. Today, PCR is considered to be a standard protocol in molecular biology and hundreds of scientific papers using this technique are published each year.

The locus we will amplify is located in the **t**issue **P**lasminogen **A**ctivator (*tPA*) gene. This gene is carried on chromosome 8, the gene codes for a protein that is involved with dissolving blood clots. tPA is a protein administered to heart attack victims to reduce the incidence of strokes. The region we will be amplifying, however, is located in an *intron* (nontranslated region) of the *tPA* gene.



The intron that we will be targeting for amplification is *dimorphic*, which means the locus has two forms. One form carries a 300 bp DNA fragment known as an ***Alu* element** and the second form of the locus does not carry this fragment. Therefore, when we examine this locus, we find that it may or may not carry an *Alu* element. The figure below indicates the intron we will be targeting for PCR.

Alu elements are short, around 300 bp, DNA fragments that are distributed throughout our genome. It has been estimated that we may carry more than 1,000,000 copies of this fragment. The *Alu* element appears to be a part of the DNA coding for an RNA molecule that aids in the secretion of newly formed polypeptides from the cell. Unless it happens to become inserted into an exon or coding region, it has little if any effect on protein function.

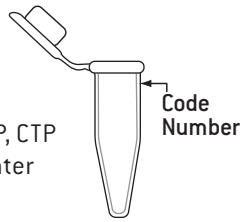
Amplification of DNA by PCR is dependent upon primers that target specific loci. The two primers that we will be using have unique nucleotide sequences that are complementary to only one locus in the human genome. The primer sequences are:

Forward primer: 5' G T A A G A G T T C C G T A A C G G A C A G C T 3'
Reverse primer: 5' C C C C A C C C T A G G A G A A C T T C T C T T 3'

Methods

1. Obtain the genomic DNA sample with your number and the PCR tube labeled with your anonymous code number.

2. The PCR tube **already** contains **Master mix I.** Master mix I contains the two **primers** that target the tPA locus, dNTP's (**d**eoxy**n**ucleotide **t**riphosphates: ATP, TTP, CTP and GTP), PCR buffer, molecular grade water (very pure) and *Taq* polymerase.



3. Using a clean pipette tip, add 5 μL of **your genomic DNA** to this PCR tube. Carefully add your DNA sample directly into the 10μL of Master mix I. Do this without creating bubbles.

4. Carefully cap the PCR tube. This is a very thin walled tube so avoid crushing it, but make sure that the cap is firmly seated over the opening of the tube.

5. Place your PCR tube into the ice bucket by the thermal cycler.

6. The instructor will add 10μL of **Master mix II,** containing $MgCl_2$, just before placing your samples into the thermal cycler. *Taq* polymerase, an enzyme from the bacterium *Thermus aquaticus,* requires Mg^{++} ions as cofactors to activate it.

7. Discard your genomic DNA sample.

8. Your instructor will run the PCR reaction at another time.

9. After the PCR run, 15 μL of your PCR product will be loaded into a 2% agarose gel. The gel will be stained and photo-documented. These steps will be done by your instructor.

Conclusions

These questions should be answered after you have seen the PCR results.

1 What is your genotype with respect to the *tPA* gene?

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2 With respect to the *tPA* gene, how many genotypes are possible?

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3 Complete the following table using the class information.
Begin by determining the number of each genotype present in your class.

Genotypes	*Alu*$^+$ alleles*	*Alu*$^-$ alleles
Alu$^+$ *Alu*$^+$		
Alu$^+$ *Alu*$^-$		
Alu$^-$ *Alu*$^-$		
	Total *Alu*$^+$ alleles	Total *Alu*$^-$ alleles

* The term "alleles" refer to *forms* of a gene or DNA sequence.

4 Calculate the frequency (percentage) of each genotype present.

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5 Calculate the frequency of each allele present in your class.

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6 Compare your results with other classes.
Is there a difference in relative frequencies of genotypes and alleles?

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