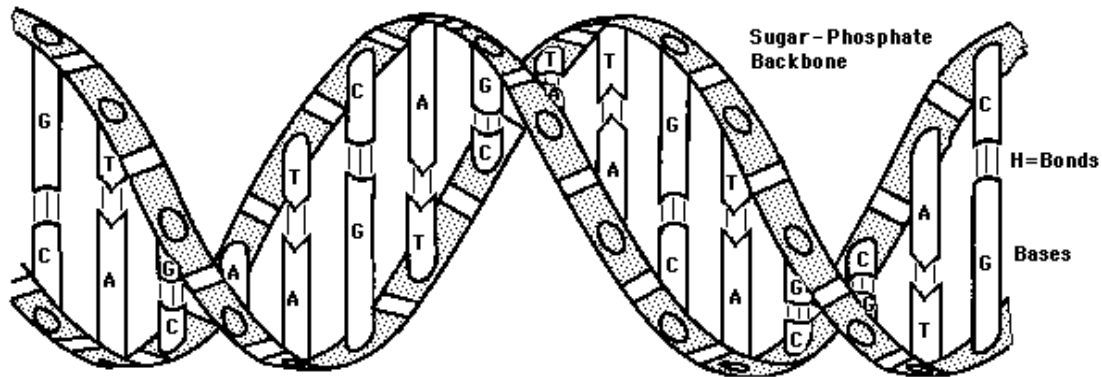


COC Biotechnology Program



PCR/ALU Insert Lab

Version 10-28-03

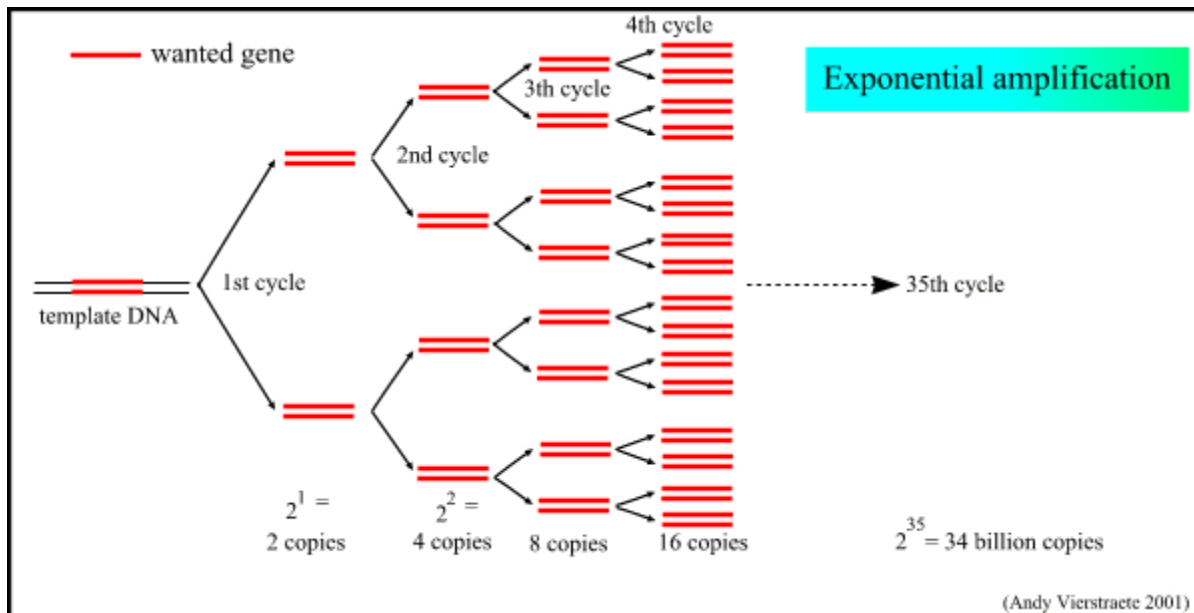
- **Polymerase chain reaction (PCR) is a method to amplify small amounts of DNA.**
- **In this lab you will isolate your cheek cell DNA and amplify a small portion of your genome using PCR**
- **The amplified fragments will be electrophoresed and visualized.**
- **An optional activity for those interested students is to pool the class data and enter it into a national database and compare your findings using various genetic testing tools.**
- **Genes inherited in pairs (one from each parent) form the basis of all genetic traits.**
- **Analysis of these gene pairs permits scientists to investigate diverse topics such as:**
 - **Selective animal and plant breeding.**
 - **Evolutionary relationships among organisms.**
 - **Factors controlling genetic composition of a population.**
 - **Detection of diseases with a genetic link.**

For more information on the College of the Canyons Biotechnology program contact Jim Wolf, Associate Professor of Biology/Biotechnology at (661)362-3092 or email: jim.wolf@canyons.edu

I. Objectives:

1. To take lab experienced students through the steps involved in identification of a genetic trait in humans. Steps include: cell isolation and lysis, PCR, gel electrophoresis, ethidium bromide staining, gel interpretation, and various DNA diagnostic methods.

II. Background:



For this lab you will be analyzing the genetic composition of yourself. Before we get into the specifics of the activity, let's first review the background theory.

The presence or absence of genetic information is complicated by the fact that each parent inherits one copy of the "gene". The resulting combination could be ++, +-, or -- denoting an individual that has both copies of the "gene", an individual with one copy of the "gene" or an individual with no copies respectively. It is interesting to note that the "gene" we will be looking at exists in a present/not present state, whereas most genes exist as a dominant/recessive configuration, and many pieces of genetic information are not genes at all but represent "non essential" nucleic acids. (The reason for this unique feature will be discussed later). The specific "gene" we will investigate is the tissue Plasminogen Activator (tPA) ALU insert. The presence or absence of this insert apparently has no effect on the health of an individual and is useful to investigate the technique by which "gene" presence is investigated.

The lab has four distinct steps to it. These steps are typical of the steps that a technician would conduct to check for the presence or absence of gene. Following a review of the four steps will be some review exercises to test your awareness of why the lab steps are taken so consider each step carefully.

1. After a saline wash, you will spit cheek cells into a vial and isolate them. After purification the DNA from these cells will be amplified by PCR.
2. The previous samples will be added to a "PCR cocktail" consisting of all of the necessary ingredients for DNA replication and the samples will then be thermally cycled to generate many copies of DNA.
3. After PCR the samples will then be loaded onto a gel for electrophoresis. The gel will then be visualized using ethidium bromide and an UV light source.

4. The presence or absence of bands on the gel will form the basis of identifying the student's genetic profile.

To test your understanding of the steps needed to complete the lab, please complete the *pre-lab QUESTIONS* (at the end of the module) PRIOR to coming to class. These questions will be discussed, and to get the most out of the lab you should try to answer them on your own and be prepared to discuss them as a group.

Points to Consider Prior to Conducting the Lab.

As a class you should try to complete each step together and occasionally stop to assess your progress. As you go through these steps try to work as a team (a common plan in a professional lab setting.) Watch out for your fellow students as some of the steps they are taking can influence the entire classes' success.

III. SOP/Lab Activities:

Important: Always note any addenda that are posted by the instructor.

A. Isolate Cheek Cell DNA (to be performed by each student) Time Required: 40 min

NOTE: If you have eaten within the past 2 hours, gently pre-rinse your mouth with tap water (swish and swallow.)

1. Obtain a 15 ml tube w/ 10 mls of 0.9% NaCl solution, and a paper cup.
 2. Label this tube with your initials using a marker (no tape)
 3. If you have just eaten, rinse you mouth out a few times with tap water. Pour all of the saline solution into your mouth. Save the tube! Swish vigorously for 1 minute. Pump the saline over your teeth, gums, tongue and cheeks. Best results are obtained if you actively scrape your cheek linings with your teeth (chew your cheek linings with your back teeth).
 4. Expel the saline/cell extract into the paper cup.
 5. Carefully pour the solution (over the sink) back into the 15 ml labeled tube. Discard the paper cup.
- STOP POINT Sample may be refrigerated overnight or frozen indefinitely. If time permits, proceed to DNA isolation.**
6. Place your tube into the swinging bucket centrifuge. Coordinate this step with the other students in lab to ensure that everyone is using the centrifuge at the same time. Ensure that it is balanced and **DO NOT LEAVE UNATTENDED.** Spin at 2000 rpm for 10 minutes to pellet cells.
 7. Being careful not to lose the pellet (which appears a small light lump at the bottom of the tube), pour off the supernatant into the sink. Place the tube with the cell pellet on ice. "Ropey" saliva may cling to the pellet and draw it out of the tube, so use a poly transfer pipette to remove the supernatant fluid if necessary.
 8. Obtain a 1.5 ml microfuge tube containing Chelex solution (*Chelex* are clear resin cation binding beads in a high pH solution). Obtain a clean poly transfer pipette.
 9. Label this tube on the side and the cap with your initials using a marker (no tape).
 10. Using the transfer pipette, pump up and down to resuspend the Chelex resin. Before it settles, draw it into the pipette and transfer to the pellet tube. Save the tube!
 11. Pump the suspension of resin beads and cells vigorously with the transfer pipette until all cell clumps are dispersed (look carefully!). Before things settle, draw up the entire volume and transfer back to the labeled 1.5 ml locking microfuge tube (that initially held the *Chelex*).

12. Place the 1.5 ml microfuge tube (ensure cap is securely locked) in a boiling water bath for 10 minutes. Discard the 15 ml tube.
13. Ice the 1.5 ml tube for at least 1 minute.
14. Centrifuge the 1.5 ml tube @ 10-15,000 rpm for 30 seconds (remember to balance the tubes in the centrifuge and check to see if other students are ready to load their samples into the centrifuge. Do not leave centrifuge unattended).
15. Obtain a clean 1.5 ml microfuge tube and a clean transfer pipette.
16. Label this tube with your name and sample # using a waterproof marker.
17. Using a clean poly transfer pipette, transfer the supernatant to the recently labeled tube: this is your buccal cell DNA extract. Try to avoid disturbing the pellet during transfer. IT IS VERY IMPORTANT TO NOT GET ANY OF THE PELLETS OR CHELEX BEADS INTO THE NEW MICROFUGE TUBE! Discard the tube containing the pellet.

STOP POINT--Cheek cell DNA can be stored frozen indefinitely without degradation.

B. Prepare DNA Samples for PCR Amplification (to be performed by each individual) Time Required: 40 min.

1. Obtain a PCR reaction microcentrifuge tube (sizes will vary with different thermal cyclers but they all have thin walls and are easily crushed).
2. Label the tube cap with your initials using a marker on top and side (no tape.)
3. Add:
 - 7.5 ul PCR **MM1** (contains: dNTP mix, Primer 1, Primer 2, ddH₂O)
4. Use a fresh tip to add:
 - 10.0 ul buccal cell DNA extract
5. Centrifuge briefly, if needed, to spin all ingredients to the tube bottom. Use inserts made of *decapitated* 1.5 ml and/or 0.5 ml microfuge tubes to support your PCR tube in the centrifuge.
6. Open your PCR tube and add Master Mix 2.
 - 7.5 ul **MM2** mix (contains: buffer, ddH₂O, and "**Expand**" an enzyme mix containing Taq DNA Polymerase)

TECHINCAL NOTE: MM2 contains the enzyme TAQ at the concentration of 1.25 ul/75 ul of MM2. After addition of MM2 to the cocktail, ensure that the samples are kept away from extreme heat or cold.

25.0 ul total reaction vessel volume should now be present

7. Centrifuge briefly if all ingredients **are not** in a single drop on the tube bottom.
8. Add 1 drop PCR mineral oil **gently just above the surface** of the PCR mixture.
9. Carefully cap your PCR tube - **it must be seated all around**, but the tube will be crushed if you force it. Gently bend the cap hinge down.

To be performed by the instructor / lab technician

Program and start thermal cycler with a step file:

94° - 0:15-0:30 sec (the denaturation or melting step)

65° - 0:30-0:45 sec (the primer-annealing step)

72° - 0:45 sec for 10 cycles, then increasing by 20 sec per cycle for cycles 11 to 30 (the extension or build-out step)

ALU is a DNA sequence thought to have arisen in early vertebrates from a retrovirus. From this origin the DNA has copied and inserted itself in our DNA many times, until today humans have about 300,000 copies of the ALU insertion in our genome. Most of the ALU insertions are common to many mammals but the tPA-25-ALU is unique to humans, absent even from our closest primate ancestors. By studying the geographic distribution of those who have this insertion, Dr. Mark Batzer at the Lawrence Berkeley Lab hopes to discover patterns of human evolution, migration and breeding.

A successful PCR amplification of your tPA-25-ALU segment will appear on the gel as a band pattern: Since you have two parents you may inherit 0, 1, or 2 copies of the insertion. A 400 bp segment is amplified if the ALU insertion is present (ALU = 300bp, two primers = 59bp, included extra bases = 30bp). If this ALU segment is absent from your genome, a band of about 100bp should appear. Include gel image and table of percent of both genotypes and allele frequency in your lab notebook. Recall that computer animations of the process of PCR are available at <http://cshl.org> under the heading of DNA Learning Center, Resources.

IV. Post-Lab Questions/Activities: The following post lab questions are for your benefit. The questions will help you to address a range of topics relating to the lab activity. Along with the post lab handouts, these questions will help to ensure that you have both correct information regarding the lab data and crucial lab processes. Complete the post lab questions at the end of the lab and post lab handouts (keys for both of these are available from your instructor) before making any lab-notebook entries.

Why are cheek cells a good cell to use to investigate a patient's DNA? Can you think of other cells that may be good as well?

Consider the following list of "PCR Cocktail" ingredients: What is the role of EACH in the process of PCR? (Template DNA, primers, nucleotides, MgCl₂, buffer).

What occurs at the three different temperature steps of the PCR cycle?

Why did we look at "junk" DNA as opposed to say, real genes? List three reasons that help to support your rationale for using junk DNA.

Finish discussion with a few thoughts on using alleles for studying populations.

V. Notebook Entries: Data from the lab should be the focus of this section and if there are any incorrect results, you should discuss this as well as expected results. Section V will contain both your results and discussion. Your data should drive the discussion. An informed discussion is dependent on understanding the post lab questions/activities.

Your intro should:

- Define PCR and role in forensics.
- Alu as a marker of individuals and populations.
- Visualization via Et-Br staining and comparison to standards.

Results should be:

- Gel image with ladder mass legend.
- Ensure that the gel has all three genotypes visible.

Discussion should consider the following:

- Define alu and possible impact on cell.
- Range of genotypes (and yours).
- Possible explanation for lanes with no PCR product and why and why –alu still gives a result.

The previous lab protocol can be reproduced for educational purposes only. It has been developed by Jim Wolf, and/or those individuals or agencies mentioned in the references.

References:

- California Lutheran University Enriched Science Program: www.clunet.edu
- Professor Martin Ikkanda, Pierce Community College, Woodland Hills, CA.
- DNA Science; Bloom, M.V.; Freyer, G.A.; Micklos, D.A.; Benjamin/Cummings Publishing Company, Menlo Park Ca, 1996.