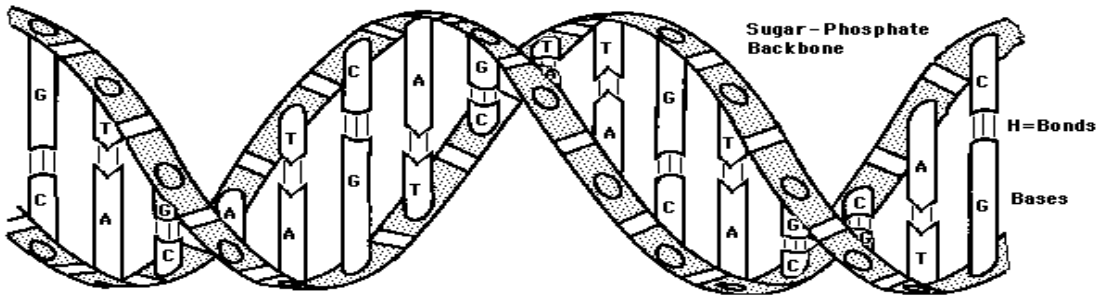
College of the Canyons Introduction to Biotechnology Custom Lab



PCR/ALU Insert Lab

Version 08-18-12

- **Polymerase chain reaction (PCR) is a method that can be used to amplify small amounts of DNA.**
- **You will isolate <u>your</u> cheek cell DNA and amplify a small portion of <u>your</u> genome using PCR.**
- **DNA will be purified using two techniques: A "Chelex" method and a "genomic DNA isolation" kit available from Qiagen will be used. The two DNA isolation techniques will be assessed for success when compared to each other.**
- **Two various PCR cocktails will be prepared *<u>to investigate a very similar piece of DNA</u>*. The cocktail constructs will be compared for success.**
- **The amplified fragments will be electrophoreses and visualized.**
- **Genes inherited in pairs (one from each parent) form the basis of all genetic traits.**
- **Analysis of genes permit 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.**
- **PCR modifications allow for rapid DNA sequencing, detection of viral and bacterial agents (either in patients or as a dispersed bioweapon), creation of DNA libraries and numerous other applications. PCR is one of the most significant new technologies to be used in molecular biology, and simply put has revolutionized many elements of science.**
- **Kary Mullis discovered PCR technology in 1984 and was awarded the Nobel Prize in 1991. This was the shortest period of time between the invention of a technology and its recognition by the Nobel Prize Committee. PCR revolutionized biology like the Xerox machine changed office work!**

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

I. Objectives:

1. Isolate and purified genomic DNA using Chelex system
2. Isolated and purify genomic DNA using a Qiagen genomic purification kit.
3. Create two various cocktails for amplifying "alu fragments" using PCR technology.
4. Assess success of DNA isolation and PCR amplification cocktails by comparing information generated on an agarose gel.
5. Review PCR reagents and process and further investigate success or failure of PCR process and implications of "alu inserts" on human biology.

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 contributes one copy of the "gene" to his or her offspring. 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 and 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, where as most genes exists as a dominate/recessive or other configuration. 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 will be actually be two different "alu inserts". The exact insert will be reviewed later, and its amplification is a function of the primer chosen. The primer is part of the PCR cocktail, and is a small stretch of single stranded DNA that binds to the template DNA providing a site for the DNA polymerase to start copying the DNA. Your text has more information on the alu insert, which actually composes a surprisingly largely amount of the human genome. The presence or absence of this insert apparently has little apparent effect on the health of an individual and is useful to investigate the technique by which "gene" presence is investigated.

Most genetic diversity is much harder to detect. Single nucleotide polymorphisms (SNPs) are single bases that have been changed or are otherwise different from the bulk of the population. As a result, the PCR technology required to reveal these differences is much more sophisticated. As SNPs are much smaller than alu inserts, you need to be able to actually sequence the DNA. By comparison, alu inserts vary by presence or absence. If you position the primer a 50 or so bases on either side of the "alu position", you can amplify the piece in between the primers. So….if an alu insert is present, the person will actually have more DNA. The PCR product will be larger (about 400 bases). If the person lacks an alu insert, the DNA will still be amplified (recall the primers are about 50 bases away from where the alu insert should be), but the fragment will be much smaller (about 100 base pairs). This presence, absence variability is more easily detected, as this size of a piece of DNA can be visualized using a simple agarose / gel system. As you progress through the lab, the results will help to clarify exactly what the alu fragment is and how it is visualized.

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 / alu insert. 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 expel your cheek cells into a vial and isolate them. After purification, the DNA from these cells will be amplified by PCR. The PCR purification will take two paths. One

will use a more traditional "Chelex" method, and the other will use a more modern (albeit less apparent) "Qiagen Genomic DNA Kit".

2. The previous samples will be added to two "PCR cocktails" consisting of all of the necessary ingredients for DNA replication and the samples will then be "thermally cycled" to generate many copies of DNA. Both cocktails should amplify alu fragments. The two different reactions will help the student to focus on cocktail composition and how the elements of the cocktail profoundly affect success rates.
3. The samples will be placed into a thermal cycler. Basically it is a very expensive hot plate that cycles the PCR cocktail through all three-temperature regimes. In the PCR cocktail is an enzyme called "TAQ". This is short for *Thermus aquaticus*. This is a bacteria that can live in very hot water (note the name) and thus has a DNA polymerase that can withstand extreme heating. After PCR thermal cycling, the samples will then be loaded into a gel for electrophoresis. The DNA in the gel will then be visualized using "cyber safe" DNA stain, illuminated with a UV light source and photographed using some sort of gel imaging system.
4. The presence or absence of bands on the gel will form the basis of identifying the student's genetic profile. Three profiles are possible: ++, +/--, --/-- (first one for two inserts, next, one insert, one missing, and two missing inserts respectively). This information can then be used to study everything from population studies to pathology, viral vectors, etc.

To test your understanding of the steps needed to complete the lab, please complete the *post-lab QUESTIONS* (at the end of the module) *PRIOR* to coming to class. Also, considering answers the post lab handout on this material. 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 on related quizzes.

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.

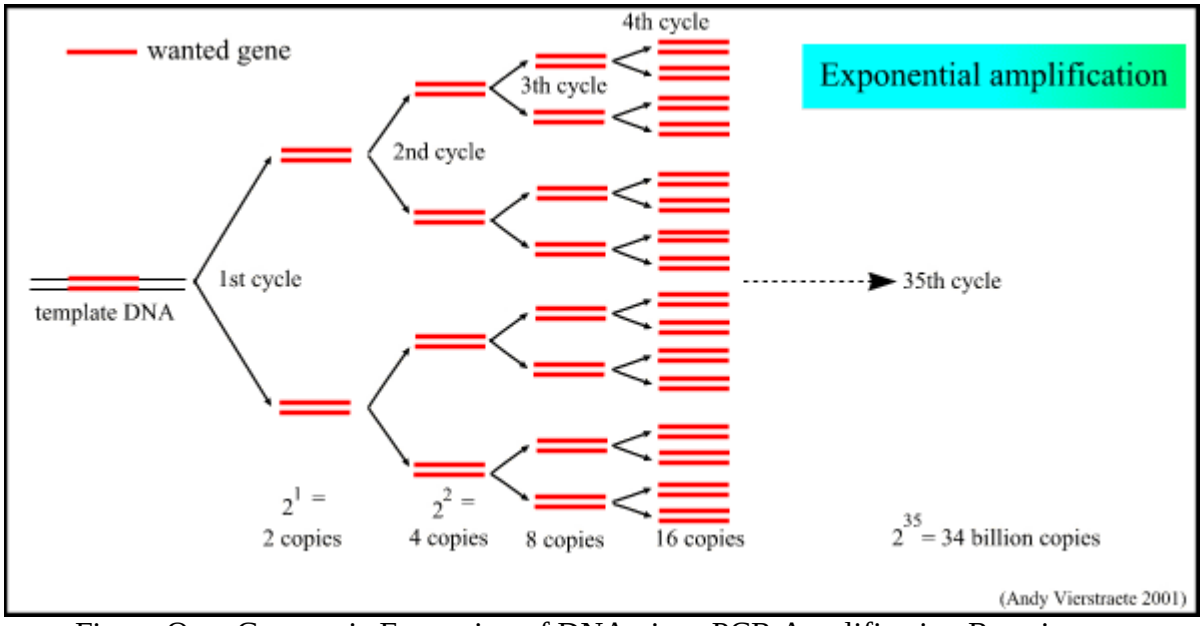


Figure One: Geometric Expansion of DNA via a PCR Amplification Reaction.

III. SOP/Lab Activities:

Important: Always note any addenda that are posted by the instructor. Work in pairs, or if needed groups of three.

A. Isolate Cheek Cell DNA (to be performed by each student) Time Required: 40 min to 1 hr.

NOTE: If you have eaten within the past 2 hours, gently pre-rinse your mouth with tap water (swish and swallow.) Note: Each student should do BOTH the CHELEX and QIAGEN SOPS for isolating genomic DNA. It may be possible to use *one sample* of saliva for both procedures. You may also want to consider doing both procedures in tandem. Have one student work with the CHELEX SOP and the other with the QIAGEN SOP. As needed, be sure to point out to the other student, key steps in the process, and as needed compare the two processes to better understand why each step, reagent is needed. The QIAGEN SOP is provided as a ***separate handout*** and QIAGEN reagents are pre-aliquoted into microfuge tubes available from the professor. The CHELEX is in marked, locking 1.5 ml microfuge tubes and most of the other supplies, etc. you are familiar with their location.

CHELEX SOP: DO QIAGEN SOP IN TANDEM AND CONTACT FACULTY FOR SOP.

If water baths are not already started, please remind the faculty to get a boiling water bath(s) going ASAP.

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* is the beads that collect at the bottom of the tube. More specifically, it is a cat-ion binding bead in a high pH (9) 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 / beads / resin. Before it settles, draw it into the pipette and transfer it 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) into a floating microfuge tube rack and then in a boiling water bath for 10 minutes. Discard the 15 ml tube in biohazard.
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 PELLET OR CHELEX BEADS INTO THE NEW MICROFUGE TUBE!*** ***Save the tube with the saliva sample and only*** discard the tube containing the pellet. If you have complete BOTH the CHELCX and Qiagen DNA isolating procedures on the one sample.

Using the DNA sample (saliva
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. Each cocktail should be prepared by each student, using his or her own DNA sample. Recall you should have two DNA samples (Chelex and Qiagen) and will be doing two cocktails. So…at the end, you should have 4 PCR reactions to cycle. Obviously, you will want to make sure you clearly label **things**. The PCR tubes are REALLY small, so track them by their position in the PCR rack (i.e. B2, C3, etc) and use the below noted "dot " method.

Protocol for PCR procedure to test for PV-92 alu insert: Remember to do two tubes, one from Qiagen DNA and the other from CHELEX DNA. . Label all clearly. Hint..use dots to label origin of DNA. One dot from Qiagen, Two dots for Chelex. As for initial, this can get hard. The PCR tubes are going to be heated and exposed to mineral oil. Both of which can smudge off the supposedly permanent lab marker. The smart idea is to place PCR tubes into rack and again note the location. When you transfer the tubes to the PCR machine, note what samples went where. The PCR machine has a grid with letters and numbers, so record your PCR tube location in you lab notebook. Create a ledger that looks something like this:

PCR sample location:
PV-92 "Chelex" ____ PV-92 Qiagen _____ TPA-25 "Chelex" ____ TPA- 25 Qiagen _____

1. Obtain two, 0.2 ml PCR reaction micro centrifuge tubes (sizes will vary with different thermal cyclers but they all have thin walls and are easily crushed).
2. Label the tube cap with your # of dots (two) and side with initials using a marker on top and side. Remember to also track the samples using their location in the PCR rack.
3. Add:
   7.5 ul PCR **MM1** (contains: dNTP mix, Primer 1, Primer 2, ddH_2O)
4. Use a fresh tip to add:
   10.0 ul buccal cell DNA extract (one dot sample (QIAGEN) and Two dot sample (CHELEX).
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, ddH_2O, and "***Expand***" an enzyme mix containing Taq DNA Polymerase)

TECHINCAL NOTE: MM2 contains the enzyme TAQ at the concentration of 4ul /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 50 ul 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.

PCR Cocktail TPA-25 Alu Insert:

1. Obtain a 0.2 ml PCR reaction micro centrifuge tubs (sizes will vary with different thermal cyclers but they all have thin walls and are easily crushed).
2. Label the tube cap with your dot and initials as noted above (no tape). An RECORD position in the rack as wells..
3. Add 10 ul of Qiagen DNA to tube with one dot, 10 ul of DNA into tube with two dots.
4. To each tube add 2 ul of upstream TPA 25 primer and 2 ul of downstream TPA 25 primer.
5. From instructor, get 25 ul of super mix. The super mix has 6 ul of TAQ added to 300 ul of super mix. The TAQ is really viscous and should be mixed very will to ensure it gets into suspension.
6. Using pipet, aspirate and expel a few times to fully mix the cocktail.
7. Centrifuge briefly if all ingredients **are not** in a single drop on the tube bottom.
8. Add 50 ul 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.

Take a minute to reflect on tubes. Make sure each student gets their 4 tubes and their location recorded in such a way so that every student has access (so write it down in EVERYONE's lab notebook!).

To be performed by the instructor / lab technician.
Program and start thermal cycler with a step file (likely under the heading "alu insert").

94^{o} - 0:15-0:30 sec (the denaturation or melting step)

65^{o} - 0:30-0:45 sec (the primer-annealing step)

72^{o} - 0:45 sec for 10 cycles, then increasing by 20 sec per cycle for cycles 11 to 30 (the extension or build-out step)
This PCR regime is preprogrammed as program 1 in the PCR machine's menu
Total run: 30 cycles, about 3 hours.

C. Cast and run a 2% agarose gel to visualize PCR products: 1-hour time limit

Please note. In a PCR reaction, it is very important that you get the entire PCR product (all of the PCR cocktail and loading dye). The amount of DNA in the cocktail (after thermal cycling) is not exactly known. It is assumed that the reaction was successful, but the exact amount of DNA is hard to verify. So, you want to put all of the PCR product into the gel as the amount of DNA in the well will impact whether or not the DNA product will even be visible. So, we make the wells for the PCR gels larger (to help hold the sample). The TPA samples should have about 47 ul and the PV-92 sample should have 31 ul. So, when you add the loading dye, the new volumes will be… *FINISH THIS THOUGHT*.

So, the gel for the PV-92 will have 12 lanes in it. Each well can hold about 40 ul and the gel for the TPA -25 can hold about 50 ul in tis wells as it has 10 wells on the comb. One 10 comb gel can hold 8 samples as we leave the edge wells open to place a DNA ladder (a sample worth DNA of known mass and amount for easy comparison. Lastly you do not want to make the gel wells *too* big, as the larger wells will spread out the DNA, possible make the band less visible and/or distinct. So, in closing, complete the exercise with an eye to keep all of the PCR product. Make sure they go into the right gel and be careful when loading to ensure that everything gets into the well.

Gel Pouring: We will have a maximum of 96 samples, and need to keep two wells per lane free for the marker. The TPA gel has ____ wells in it per lane and the PV-92 gel has ____ wells in it per lane. AS a result, each gel can hole ____ PV 92 samples or _____ TPA samples. So, cite this part of your lab and any addenda from the instructor to help you naviagate this part of the lab. We will likely adjust the number of samples, and therefore address the number of gels we will pour. If you are assigned a gel, you will want to pour it according to earlier noted procedure (2 %, cyber safe dye and 2 combs per gel ___ number of teeth per comb), and place a legend near the gel to assist your fellow lab partners in understanding what samples have gone where.

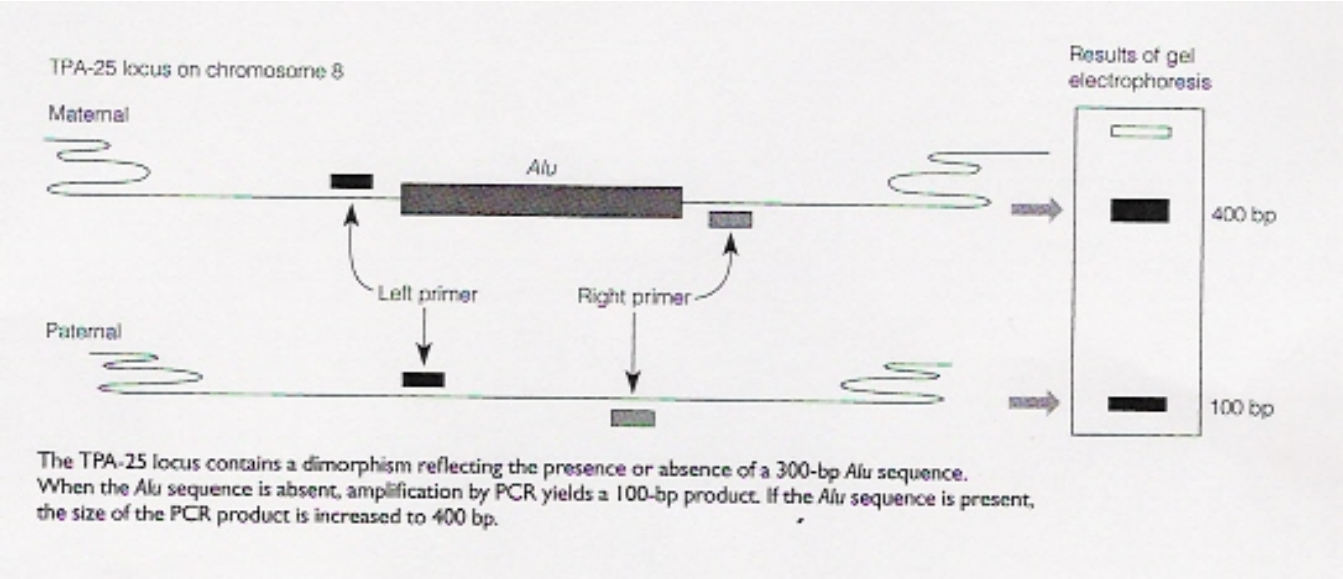
At the maximum, we my pour ____ number of gels for the PV-92 system and ____ number of gels for the TPA system. Since we re using a double comb system and each gel lane has the first and last wells unoccupied, the 10 tooth gels can hold 8 samples and the 12 tooth comb can hold 10 samples. So….It stands to reason that assuming a full class of 24 students, 4 samples per student and half of the samples are TPA and the other half PV-92, there are 48 wells per sample. So we will need 3 gels with the 10 tooth comb, 2 combs (20 x 3) and 4 gels for ladder equals 3 x 16 or 48 wells. For the 12 tooth variety, each gel can hold 16 samples, 3 gels equals 48. In a nutshell, pour 7 gels, 3 will have 12 tooth double combs and the other 4 will have 10 tooth double combs. All of the gels are 2 % agarose with cyber green added and prepared using TAE buffer to generate the liquid agarose (upon heating).

As a preparation for this phase, some 2 % agarose aliquots have been prepared for you. 4 ____ ml samples and 3 ____ ml samples have been prepared and are waiting in the 65 degree water bath! Recall the ____ gels with ___ teeth have to be poured with ____ mls to ensure that the wells are deep enough to handle the _____ ul of PCR sample to be loaded into each gel well. By comparison, the _____ gels with _____ teeth will need to be poured with ____ mls of agarose as the ___ PCR samples will have to hold _____ ul of PCR sample.

1. After noting the above and any addenda from your instructor, figure out a gel-and-well in which to load your PCR products, be sure to record the well and gel you choose (both on the legend on the gel box and in your notebooks (again, make sure everyone has everything). Record your gel number and well number (reading from left to right) here: _____ and….
2. Obtain: a micropipette, a box of tips, two squares of Parowax (AKA Parafilm,) a microfuge tube with 5X gel loading buffer (yellow), and your PCR reaction tube.
3. Remove the paper from the Parowax square. Tape down one edge and be sure to remember which is which. You will want to repeat this process for each PCR sample and to use a new piece of Parafilm for each PCR product.
4. Using a fresh tip add 6 µl 5 X gel loading buffer (yellow) to the bottom portion of your PCR reaction tube (**under** the mineral oil, so push the tip through the oil. Remember this is for the PV TPA sample92 sample, ____ dots.

5. Obtain a fresh tip. Using a s-l-o-w thumb and careful technique, draw ALL µl of the PCR products from under oil drop covering your reaction. Reflect on the total amount of PCR reaction under the oil: PCR mix, genomic DNA, primers and loading dye. You may have noticed that only the PCR mix is colored, as the polar loading dye will not dissolve in the non-polar mineral oil (recall HPLC lab). It's essential that you depress the plunger (to the first stop) before inserting the tip under the oil, and that you don't force the pipette tip against the reaction tube bottom. Slowly aspirate up the entire volume and try to stop if you see mineral oil going into the tip. Deposit this PCR product onto the Parowax square. Remember to not what PCR sample is on what piece of Parafilm. Perhaps you should use apiece of tape and label it Qiagen or CHELEX, PV-92 or TPA.
6. Use the pipette tip to "push" the yellow drop around the Para film. Any mineral oil, which is present in the sample, will stick to the parafilm as you move the drop. Keep moving the drop around the Para film until you no longer see a trail of mineral oil following the drop. You now have a relatively pure sample free of mineral oil. Repeat for all 4 samples (changing tips and noting sample volumes and type as needed. Transfer the drop to a labeled microfuge tube.
7. With all four samples in the microfuge tubes, double check the volume. Adjust the volume as needed on the micropipette and use the pipet to determine the volume (recall micro-pipetting lab).
8. Changing tips draw up and deposit all of the PCR product / yellow drop into the gel well. MAKE SURE you put your samples into the correct gel. The PV-92 samples will have ____ wells per lane and the TPA will have ____ wells per lane. Also, remember to leave the first and last wells empty, as this will used for DNA ladders. You will want to change the volume on the pipet so that you suck up the entire volume and leave no gap of air at the tip. Any gap of air in the tip will result in a bubble coming out of the tip when it is in the gel well. This bubble will rise and take with it some of your PCR product. You want to "dribble" this syrupy liquid into the well, so remember the s-l-o-w thumb!! In a nutshell, get ALL of you PCR sample. Verify your gel #, Type and lane number: _____. You have 4 samples, so record all of this information in yor lab notebook as needed.
8. Save the PCR reaction tube; discard the Parafilm.
9. **NOTE**: Two wells in each gel should be reserved for marker DNA: B-M Marker VIII is provided, premixed: load 5-6 µl in at least one lane in each gel. If your are the last student to load a PCR sample into a gel (i.e. there are 4 empty wells (two in each lane), then load these markers as noted above. The marker is available from the instructor.
10. When all PCR gels are loaded, run @ 90-100 v for 20-25 minutes min. Observe with appropriate gel imaging system using U.V. light and then photograph. Make sure you make electronic copies and hard copies for ALL people using the gel image. Make sure you provide a electronic copy for the instructor and label ALL copies with relevant information: gel %, run time, voltage, lane by lane ID and relevant masses on the DNA fragments in the DNA ladder.

D. Interpreting the gel results: An open-ended experiment:



The TPA-25 locus contains a dimorphism reflecting the presence or absence of a 300-bp *Alu* sequence. When the *Alu* sequence is absent, amplification by PCR yields a 100-bp product. If the *Alu* sequence is present, the size of the PCR product is increased to 400 bp.

The primers provided permit amplification of a non-coding region located in an intron of the human tissue Plasminogen Activator gene. All Humans, and most vertebrates have the TPA gene but only some of us have this inserted segment, called the tPA-25-ALU segment.

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 these "post lab questions" and post lab handouts (answers for both of these are available from your instructor) before making any lab-notebook entries (and ideally for

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, TAQ).

What occurs at the three different temperature steps of the PCR cycle (98 ^{o}C, 54^{o}C, 72^{o}C).

Why did we look at "junk" DNA as opposed to say, real genes? List three reasons that help to support your rational 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 its role in forensics, medicince, etc (briefly mentio
- Alu as a marker of individuals and populations. Describe any information relevant to the TPA and PV 92 inserts.
- Visualization via cyber safe 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 a double negative –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.