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| [Home](http://docs.google.com/home.html)  [Goals](http://docs.google.com/goals.html)  [Abstract](http://docs.google.com/abstract.html)  [Introduction](http://docs.google.com/intro.html)  [Literature Review](http://docs.google.com/review.html)  [Hypothesis/Prediction](http://docs.google.com/hypo.html)  [Materials](http://docs.google.com/material.html)  [Data](http://docs.google.com/data.html)  [Images](http://docs.google.com/images.html)  [Conclusions](http://docs.google.com/conc.html)  [Recommendations](http://docs.google.com/recom.html)  [Bibliography](http://docs.google.com/biblio.html)  [Journal](http://docs.google.com/journal.html)  [Web Resources](http://docs.google.com/resource.html)  [Acknowledgements](http://docs.google.com/acknow.html)  [Project Creek Watch](http://www.pleasanton.k12.ca.us/amador/creek/index.html) | **Procedure**  **DNA Preparation Using a Saline Mouthwash**  1. Swirl 10 *u*L of 0.9% saline in mouth for 30 seconds.  2. Expel saline into a cup and swirl to mix the cells.  3. Transfer 1000 *u*L of the liquid into a 1.5 *u*L microfuge tube, labeled with a pin #.  4. In a balanced centrifuge, spin sample for 1 minute.  5. Observe cell pellet at the bottom of tube. Pour off the supernatant, being careful not to lose cell pellet. Note: it is okay if some supernatant if left in the tube.  6. Resuspend cell pellet in 30 *u*L of saline. Make sure the entire cell pellet is thoroughly mixed by vortexing, pipeting up and down several times, or "racking" the tube.  7. Withdraw 30 *u*L of the cell suspension and add it to a 1.5 *u*L tube containing 200 *u*L of 5% Chelex.  8. Secure tube with a cap lock and place it in a 99 degree C heat block or boiling water bath for 10 minutes.  9. Shake tube well or briefly vortex it and then place it in a balanced centrifuge. Spin for 1 minute.  10. Withdraw 60 *u*L of supernatant (no Chelex beads) to a clean tube, labeled with the same pin number.  11. Place DNA tube in a microfuge rack to refrigerate isolated DNA until ready to prepare PCR amplification.  **Polymerase Chain Reaction**  1. Label a 200 *u*L PCR tube with pin number.  2. Change pipette tip and dispense 20 *u*L of Master Mix into PCR tube.  3. Change pipette tip and add 20 *u*L of Primer Mix into PCR tube.  4. With a new pipette tip, add 10 *u*L of purified DNA into PCR tube.  5. Place reaction into the thermal cycler and record the location of tube on a grid.  6. The cycling protocol for amplification of this Alu region is :  95oC, 10 minutes;  94oC, 30 seconds;  52.5oC, 30 seconds; X 35 cycles  65oC, 2 minutes;  72oC, 10 minutes;  4oC, hold  **Agarose Gel**  Will need 2% agarose gel. If agarose gel casting tray holds 50 mL, then calculate the amount of agarose needed as follows:  (Ci) (Mi) = (Cf) (Mf)  C = concentration (100%) (Mi) = (2%) (50 g)  M = Mass 100 (Mi) = 100 g  i = initial Mi = 1 g agarose powder  f = final 50 g � 1 g = 49 g = 49 mL buffer  **Electrophoresis of Amplified DNA**  1. Retrieve PCR tube and spin it briefly to bring the liquid to the bottom of the reaction tube.  2. Add 5 *u*L of loading dye to PCR tube. Slowly pipette the mixture up and down until the contents in the tube are uniformly colored.  3. Carefully load 15 � 20 *u*L of reaction into a well in the agarose gel. Avoid poking the pipette tip through the bottom of the gel or spilling sample over the sides of the well. Use a new tip for each sample.  4. Load 5 *u*L of the 100 bp ladder (molecular weight marker) into one of the wells in each gel.  5. When all samples are loaded, attach the electrodes from the gel box to the power supply. Electrophorese samples at 125 Volts for 45 � 50 minutes.  6. Gels may be stored in the 70% ethanol for one day in the refrigerator before staining and photographing.  **Staining and Photographing Agarose Gels**  1. Place the agarose gel in a staining tray.  2. Pour enough ethidium bromide (0.5 *u*g/mL) to cover the gel. Wait for 15 minutes. **CAUTION:** Ethidium bromide is a carcinogen. Always wear gloves and safety glasses when handling.  3. Pour the ethidium bromide solution back into its storage bottle. Pour enough water into the staining tray to cover the gel. Wait 5 minutes.  4. Pour the water out of the staining tray into a hazardous waste container and place the stained gel on an UV light box. **CAUTION:** Ultraviolet light can damage your eyes and skin. Always wear protective clothing and UV safety glasses when using an UV light box.  5. Place the camera over the gel and take a photograph. |

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