NAME: _____

PERIOD: _____

DNA Extraction from Cheek Cells

Laboratory Protocol

MATERIALS

15 mL conical tubes
Gatorade® (clear, yellow or green)
5M NaCl
Small paper cups
Lysis buffer
95 percent ethanol (cold)

Note: Reagent formulations are in Appendix A of this manual

EQUIPMENT

Centrifuge
Hot water bath (65 - 70°C)

LABORATORY PROTOCOL

1. Vigorously swish 15 mL of Gatorade® in your mouth for 30 seconds. Chew on your cheeks while swishing so you get as many cells as possible.

2. Spit the Gatorade® back into your cup and pour it into a clean 15 mL conical tube. Mark your tube with your initials.

3. Centrifuge the conical tubes at high speed for three to five minutes to collect the cells at the bottom of the tube. Decant the supernatant (pour off the liquid) into a waste container.

4. Repeat steps 1 through 3 again with fresh Gatorade®.

5. Add 2.0 mL of Lysis Buffer to the cell pellet. Mix by flicking the tube several times (this may require several minutes of vigorous mixing). Do not vortex. The cell pellet must be completely resuspended and broken to ensure a good cellular lysis.

6. Incubate the cells at 65 - 70°C for a minimum of one hour to overnight (longer incubation times ensure better cellular lysis). Before proceeding, the solution should be clear and the cells should not be visible.

7. Add 1.0 mL of 5M NaCl to the clear solution in the conical tube. Mix well by flicking the tube and then centrifuge for 10 minutes at high speed.

8. Carefully decant the supernatant into a new 15 mL conical tube; avoid transferring any of the solids at the bottom of the tube to the new tube. If necessary, use a clean pipet to transfer only the liquid. Dispose of the first tube (containing the cellular debris).

9. Centrifuge the supernatant again for 10 minutes at high speed. Carefully decant the supernatant into a new 15 mL conical tube; avoid transferring any of the solids at the bottom of the tube to the new tube. If necessary, use a clean pipet to transfer only the liquid.

PROTOCOL NOTE
If the supernatant is still cloudy a third spin may be required. Repeat step nine.

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10. Add 4 mL of cold 95 percent ethanol to the liquid (supernatant). Mix well by rocking the tube gently back and forth until the DNA becomes visible. It should look like fine white fibers or lint.

> **PROTOCOL NOTE**
> Check with your instructor before continuing onto step 11.

11. Centrifuge the conical tube at high speed for 20 ± 5 minutes. Carefully decant the supernatant and try not to disturb the white pellet at the bottom of the tube. If no pellet is visible, repeat the centrifugation.

14. Allow the tube to air dry (with the cap off) until there is no trace of ethanol in the tube. This will take 15 – 30 minutes.

12. After the DNA has dried, add 100 μL (or one small drop) of water to the DNA. Gently flick the tube to mix.

OBSERVATIONS

14. Briefly describe what each of the following does during the DNA extraction:

    Gatorade

    Lysis Buffer

    Cold Ethanol

    Centrifuge

15. If DNA was in the sample, what did the DNA look like?

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Polymerase Chain Reaction

Laboratory Protocol

MATERIALS

PCR reaction tube (and caps)
PCR Reaction Mix
Forward Primer (GGATCTCAGGGTGGGTGGCAATGCT)
Reverse Primer (GAAAGGCAAGCTACCAGAAGCCCCAA)
Sterile water

Note: *Reagent formulations are in Appendix A of this manual*

EQUIPMENT

Thermalcycler
Thermalcycler base/retainer

LABORATORY PROTOCOL

1. Place a clean 200 μL PCR reaction tube in an appropriate thermalcycler base and place the retainer over it. This will properly hold your tube in place for reaction set-up and may be used in the appropriate Applied Biosystems thermalcycler (the instructor will give you the appropriate tray/ retainer and base to use).

2. To the reaction tube, add one *Amersham Biosciences* Ready-To-Go™ PCR Beads. Then, add the following:

Sterile water	16.5 μL
Your DNA	4
Primer Mix	6
TOTAL VOLULME	**25 μL**

3. Cover all tubes in the tray/retainer with strip caps and use the strip cap tool to ensure the tops are tight.

4. Tap the tray/retainer gently on the top of a table to collect the contents of the tubes at the bottom. Any liquid left on the side of the tube may inhibit the PCR reaction.

5. Place the tray/retainer in the thermalcycler and cycle using the *Alu* PCR program.

6. To store the PCR reaction after cycling for up to 24 hours, place at 2 - 8°C. For longer than 24 hours, PCR reactions should be stored at –15 to -25°C. Long term or incorrect storage of PCR reaction may affect results.

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OBSERVATIONS

7. Briefly describe what each of the following does during the PCR reaction:

   DNA Polymerase

   Primers

   Thermalcyclers

8. What are some uses for PCR?

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Agarose Gel Electrophoresis

Laboratory Protocol

MATERIALS

Agarose
Loading Dye
Zip lock bag
123 bp DNA Ladder *(> 0.25 μg/μL with loading dye added)*
1X TBE buffer
0.025 % methylene blue

Note: *Reagent formulations are in Appendix A of this manual*

EQUIPMENT

Gel electrophoresis box
Plastic staining tray
Microwave oven
Power supply
Erlenmeyer flask

LABORATORY PROTOCOL

1. Follow the guidelines from the instructor for setting up your agarose gel.

2. After the gel has solidified, place it in the electrophoresis chamber and carefully remove the comb. Add a sufficient volume of the 1X buffer used in step 1 to the electrophoresis chamber to cover the gel by 1 – 2 mm. Ensure that the wells of the gel are closest to the black electrode of the electrophoresis chamber.

3. Add 9 μL of 1X gel-loading dye to the amplified PCR product and pipet up and down to mix. The gel-loading dye will both stop the PCR reaction and prepare the sample for electrophoresis. The gel-loading dye contains glycerol, which will make your DNA denser so that it will sink into the wells of the gel. It also contains dye, which will travel through the gel to provide a visual tracking method so you know how far the DNA has traveled through the gel.

4. Pipet 5 μL of the reference sample (provided by the instructor) into the first well of the gel – this reference is a DNA ladder that contains multiple DNA fragments of different size to serve as a reference on the gel.

5. Repeat step four until all of the participants' samples and controls have been loaded into the gel. Remember to record which lanes contain which sample or control.

6. After all of the samples have been loaded, close the lid of the gel box. Attach the electrode, placing the negative electrode at the end with the sample wells. Apply voltage from a direct-current power supply. Run the gel at between 80 –120 volts until the loading dye has traveled a quarter to a half of the way down the gel (about 30 –45 minutes).

7. Once the gel has run far enough (you can tell by the distance the loading dye has traveled from the wells), turn off the power and disconnect the cables from the electrophoresis box. Follow your instructor's guidelines for viewing the results of your gel electrophoresis. Observe the banding patterns on your gel and record the results in step eight.

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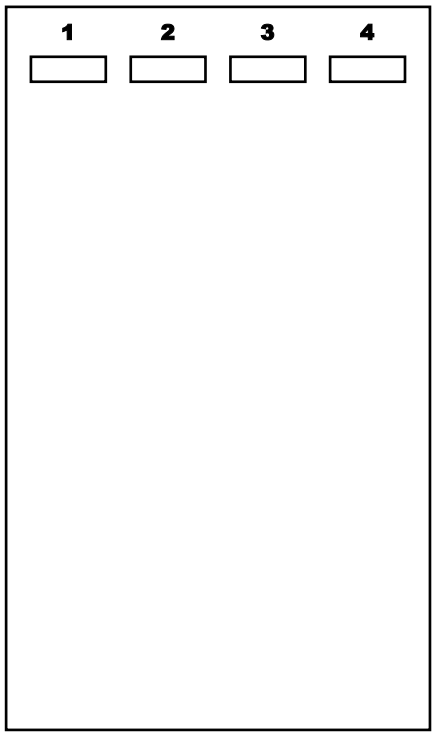
OBSERVATIONS

8. Use this diagram to record where you loaded each sample and the results you observe:

Lane 1 _____ **Lane 3** _____

Lane 2 _____ **Lane 4** _____



9. What is the function of the agarose gel?

10. Why is the gel in electrophoresis buffer?

11. Describe what happened in the gel when the electric current was applied.

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Agarose Gel Electrophoresis Analysis

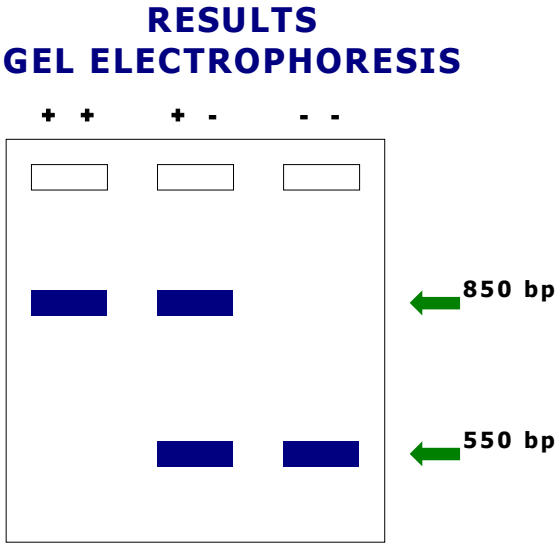
Laboratory Protocol

The results of the analysis will be scored based on the number and size of bands on the agarose gel. Three possible combinations can occur:

+/+ 850 bp band — Individual is homozygous for the *Alu* Insertion Polymorphism; both copies of chromosome 16 contain the *Alu* gene.

+/- 850, 550 bp band — Individual is heterozygous for the *Alu* Insertion Polymorphism; one copy of chromosome 16 contains the *Alu* gene and one copy does not.

-/- 550 bp band — Individual is homozygous for the lack of the *Alu* Insertion Polymorphism; neither copy of chromosome 16 contains the *Alu* gene.



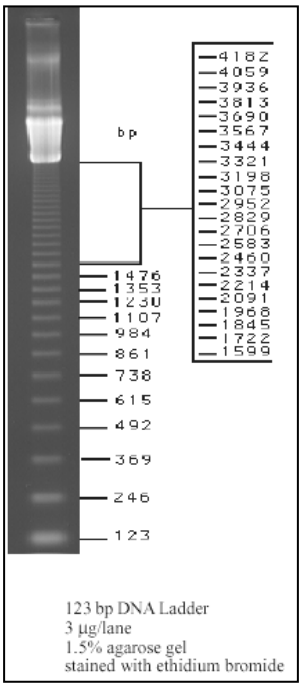
LABORATORY PROTOCOL

1. Observe the results of the gel electrophoresis. Orient the photograph with the sample wells at the top. Interpret the band(s) in each lane of the gel. Scan across the photograph to get an impression of what you see in each lane. You should notice that virtually all lanes contain one to three prominent bands.

2. Locate the lane containing the size markers. An image of the 123 bp DNA Ladder is on the right. Locate your lane and count the number of bands visible. The following combinations are possible:

   One band visible Compare the migration of the band to the 984 bp and 369 bp bands in the lane containing the size markers. If the PCR product migrates slightly ahead of the 929 bp band, then the person is homozygous for the PV92 *Alu* insertion (would be scored as +/+). If the PCR product migrates slightly behind the 383 bp band, then the person is homozygous for the absence of the PV92 *Alu* insertion (would be scored as -/-).

   Two bands visible Compare the migration of each band to the 984 bp and 369 bp bands in the lane containing the size markers. Confirm that one PCR product corresponds to a size of about 850 bp and the other to 550 bp. The person is heterozygous for the PV92 *Alu* insertion (would be scored as +/-).



123 bp DNA Ladder
3 µg/lane
1.5% agarose gel
stained with ethidium bromide

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Note: *It is common to see an additional band lower on the gel representing an artifact of the PCR reaction that results from the primers overlapping one another and amplifying themselves. The primer dimer is approximately 50 bp and should be in a position ahead of the 121 bp marker.*

OBSERVATIONS

3. Which bands, if any, were present in your sample?

4. Make a determination if you are homozygous for the *Alu* Insertion Polymorphism, heterozygous for the *Alu* Insertion Polymorphism or homozygous for the lack of the *Alu* Insertion Polymorphism. Explain how you made this determination.