Human Buccal Cell DNA Isolation
With a Qiagen QIAamp Mini Kit

I. Introduction:

A small quantity human cheek (buccal) cells are obtained with an oral swab with a soft bristled, sterile cytobrush. After dislodging the buccal cells by vortexing in PBS, they are lysed in the presence of a lysis buffer containing a chaotropic salt (guanidine hydrochloride) to disrupt the cell and nuclear membranes and to denature proteins. Additionally, the solution is subjected to further protein digestion by proteinase K and RNA digestion with RNase A. The resultant solution is then added to silica-gel membrane spin columns that selectively bind DNA based on pH and salt concentrations. The DNA is eluted after the spin column matrix is washed to remove protein and other impurities that can inhibit PCR and other downstream enzymatic reactions. The DNA is then eluted with 10 mM Tris-Cl, 0.10mM EDTA; pH 8.0. The resulting solution is then subjected to UV spectroscopy to determine the concentration of the isolated DNA and its purity. The [DNA] in, mg/mL (or µg/µL) is based on the following:

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[DNA] = A_{260} \times 0.050 \text{ mg/mL} \times \text{dilution factor}
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A relatively pure DNA preparation for downstream applications is indicated by \(A_{260}/A_{280} = 1.7-2.0\), where lower values are indicative of protein contamination and higher values for RNA contamination. Some of the sample will be concentrated for later use in PCR experiments.

II. Materials:

1. Epicentre Catch-All\textsuperscript{TM} Sample Collection Swabs
2. PBS (phosphate buffered saline) buffer; pH 7.4.
3. Proteinase K, 500 mAU/mL.
4. Lysis buffer; QIAamp DNA Mini Kit Buffer AL.
5. RNase A, 100 mg/mL.
6. Ethanol

7. QIAamp Spin column.

8. Wash buffer 1: QIAamp DNA Mini Kit Buffer AW1

9. Wash buffer 1: QIAamp DNA Mini Kit Buffer AW2

10. Centrifuge: Eppendorf Model 5415 C

11. Elution buffer: T.E (10mM Tris, 0.10mM EDTA, pH 8.0).

12. Microfuge tubes: 1.5 mL sterile capped tubes.

13. Eppendorf Model 5415C table top centrifuge.


15. 56°C water bath.


III. Methods: (Note: refrain from consuming food or drink at least 30 min. prior to lab.)

1. Wear gloves for the entire procedure. Keep all reagents on ice until needed.

2. Obtain some cheek cells from the inside of your mouth using a soft-bristled, sterile cytobrush. Twirl the brush in between your upper and lower gums and cheeks for a total of 1 min. Air-dry the cytobrush. Place the cytobrush into a 1.5 mL microfuge tube and clip off the brush handle with a pair of scissors. Add 0.50 mL (500 µL) of PBS buffer and label it.

3. Vortex mix the tube for 1 min. Scrape the bristle against the walls of the tube. Remove the brush with a pair of forceps and discard. Place your sample tube into the Eppendorf table top centrifuge and centrifuge for 5 min at 8,000 RPM. Discard the supernatant of these centrifuged samples. Add 200 µL of the PBS buffer and resuspend the cell pellet by Vortex mixing vigorously for 1 min.

4. Add 20 µL of the Proteinase K solution and 4 µL of the RNase solution. Vortex mix for 15 sec. Add 200 µL of Buffer AL to the sample and mix by pulse-vortexing for 15 sec.

5. Incubate at 56°C for 10 min. Every two minutes, remove the sample tube and vortex mix, returning them to the water bath.
6. After the 10 min. digest incubation, cool your incubation tubes to room temperature and centrifuge at 8,500 RPM for 10 sec to remove drops from inside the lid.

7. Add 225 µL of 100% ethanol to the sample. Vortex mix for 15 sec.

8. Carefully apply all of the sample (including any precipitate) to the QIAamp Spin column placed in a 2 mL collection tube. Be sure to label your column. Avoid wetting the rim and touching the membrane with the pipet tip. Close the cap, and centrifuge at 8,500 RPM for 1 min. Discard the filtrate and the collection tube. If the solution has not completely passed through the membrane, centrifuge again at a higher speed.

9. Place the QIAamp Spin Column into a clean 2 mL collection tube. Add 500 µL of Buffer AW1 without wetting the rim. Close the cap and centrifuge at 8000 RPM for 1 min. Discard the filtrate and re-use the 2 mL collection tube.

10. Carefully open the QIAamp Spin Column and add 500 µL of Buffer AW2 without wetting the rim. Close the cap, and centrifuge at full speed (14,000 RPM) for 3 min. Examine the column to ensure that there is no residual liquid adhering to the silica-gel membrane. If there is liquid present, discard the filtrate and centrifuge again. There must be no Buffer AW2 present as its presence may cause problems in downstream applications.

11. Discard the filtrate and collection tube. Place the QIAamp Spin Column into a clean, sterile, labeled 1.5 mL microfuge tube. Carefully open the column and add 150 µL T.E elution buffer. Incubate at room temperature for 2 min. Centrifuge at 8500 RPM for 2 min.

12. Add 70 µL of your sample to a clean UV micro-cuvette and take the absorbance readings at 260 nm and 280 nm using the T.E elution buffer as the blank. Calculate the $A_{260}/A_{280}$ ratio and the [DNA] based on the $A_{260}$ reading. Consult with your instructor before continuing. Recover all of your sample from the cuvette and add back to your labeled 1.5 mL microfuge tube.

13. Clean up this smaller second sample with Zymogen DNA cleanup kit as described following:

   a. Add 2 volumes (300 µL) of DNA Binding Buffer to your 150 µL DNA sample. Mix briefly.

   b. Load entire sample into a Zymo-Spin column and place column into a 2 mL collection tube.

   c. Centrifuge at full speed for 10 sec. Discard flow through.

   d. Add 200 µL of Wash Buffer to the column. Centrifuge at top speed for 10 sec. Add another 200 µL of Wash Buffer and centrifuge at top speed for 30 sec.
e. Add 8 µL of molecular biology grade sterile water directly to the column matrix. Place column into a clean, sterile, labeled 1.5 mL microfuge tube. Incubate at room temperature for 1 min. Centrifuge at top speed for 15 sec. Using the same microfuge tube add 7 µL of molecular biology grade sterile water to the column, incubate at room temperature for 1 min., and centrifuge at top speed for 15 sec. Assuming 100% recovery and 15 µL of total eluate, calculate the [DNA] in ng/µL for this solution.

14. Store your samples at -80°C until needed for the PCR experiments.

IV. Points for Discussion:

What does your A$_{260}$/A$_{280}$ ratio suggest for the purity of your isolated DNA sample? What are [DNA] concentrations for the isolated and concentrated solutions. If 10 ng of DNA was required for as a template for a PCR reaction, what volume would be required for the isolated solution and for the concentrated solution?

V. References:


