FRED HUTCHINSON CANCER RESEARCH CENTER
SCIENCE EDUCATION PARTNERSHIP

ISOLATION OF BUCCAL CELL DNA

(Adapted from David Fankhauser, U of Cincinnati Clermont College)

1. Aliquot 10 ml of sterile 0.9% NaCl into a labeled 15 ml sterile conical tube.

2. Pour 10 ml of the NaCl into your moth and swish against your cheeks for 30 seconds.

3. Place a funnel in the mouth of the 15 ml tube and expel the saline back into the conical tube.

4. Pellet the epithelial cells by centrifugation at 2000 g for 10 minutes in a clinical centrifuge.

5. Remove and discard the liquid by carefully pouring the NaCl into a waste container (beaker). Remove the last bit of NaCl by aspiration with a micropipet, taking care not to dislodge or aspirate the cell pellet.

6. Resuspend the Chelex beads (10% in sterile H_2O), and add 500 μl of the Chelex suspension to the cell pellet.

7. Resuspend the cell pellet and mix with the Chelex beads by aspirating and expelling the components until no visible clumps of cell remain.

8. Transfer 500 μl of the cell/Chelex to a labeled 1.7 ml microfuge tube.

9. Place the tube in a boiling water bath, or temp-block set at 100^{o}C and incubate for 10 minutes. (If using a boiling water bath, poke a hole in the top of the tube with a hot needle to allow gas to escape.)

10. Transfer the tube containing the buccal cells to ice and chill for 2 minutes.

11. Pellet the Chelex beads by centrifugation in a microcentrifuge at maximum speed for 1 minute.

12. Transfer 200 μl of the supernatant to a clean 1.7 ml microcentrifuge tube and add 10 μl of 5 M Nacl (final is 0.25 M) and 400 μl 100% EtOH. The high molecular weight DNA will be visible when it precipitates. The DNA can be stored in EtOH at -20^{o}C indefinitely.

13. Pellet the DNA by cetrifugation in a microcentrifuge for 10 minutes at maximum speed.

14. Remove the EtOH and resuspend the DNA in 150 μl of TE (10.1) pH8.0.

15. Store the DNA at -20^{o}C.