SOP 3.9 Processing of Buccal Swabs for DNA Extraction

SOP Number: 3.9
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for DNA extraction from buccal swabs.

Responsibility

It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Cell Lysis Solution
- Proteinase K solution
- Protein precipitation solution
- DNA hydration solution
- Ethanol
- Isopropanol
- A refrigerated centrifuge capable of 2,000g
- A vortex mixer
- -80^{o}C Freezer
- An incubator/water-bath capable of generating a temperature of 65°C
- A spectrophotometer capable of reading 260 and 280nm/Nanodrop

Considerations

RNA and protein extraction is not recommended from cheek cells because of the small number of cells.

Procedure

1. Aliquot 450µL of cell lysis Solution to a labeled microcentrifuge tube.

2. Carefully unwrap cheek swab and place cotton tip into the cell lysis solution and add 20µL of proteinase K (100µg/mL stock) solution.

3. Incubate samples for 30 min at 65°C.
4. Add 100µL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.

5. Centrifuge at 12,000g for 5 min. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step.

6. Dispense 300µL isopropanol into a clean 1.5mL eppendorf tube and add the supernatant from the previous step. It is important to ensure that the protein pellet is not dislodged during pouring.

7. Mix by inverting gently 50 times until the DNA is visible as threads or a clump. Centrifuge at 12,000g for 3 min. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.

17. Wash the DNA, by adding 300µL of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge at 12,000g at 4°C for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.

18. Add 50µl of DNA hydration buffer (TE buffer) and vortex for 5 sec at medium speed to mix. Incubate at 65°C for 1 hour to dissolve DNA. Incubate at RT overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Centrifuge samples briefly.

19. The absorbance of the DNA at 260nm and 280nm should be measured using quartz cuvets or the nanodroplet method to assess purity. A 260/280 ratio between 1.8 and 2.0 is desirable. A 260/280 ratio greater than 2.0 may indicate solvent contamination and a ratio less than 1.8 may indicate protein contamination. DNA concentration can be measured using absorbance at 260nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50ug/mL / nanodrop. The DNA sample is

aliquoted into cryostorage tubes and stored at -80^{o}C.

20. An aliquot of the DNA from a representative sample from each batch may be analysed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100kbp and preferably exceeds 200kbp.

Note: There are a number of commercially available kits based on the salting out methods from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/ package insert included with the kit. The method used for DNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.