SOP 3.11.1 DNA Extraction from Frozen tissue

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Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for DNA extraction from frozen tissue.

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.

General considerations

- There is a number of tissue types including paraffin-embedded, formalin fixed and frozen tissue and protocols for extraction of DNA, RNA and protein will vary accordingly to tissue type as outlined in the following SOPs.
- Lysis time will vary from sample to sample depending on the type and amount of tissue processed.
- Yields will depend both on the size and the age of the sample processed. Reduced yields compared fresh or frozen tissue, are expected. Therefore eluting DNA in 50-100μL TE (10mM TRIS-HCL PH 8.0, 1mM EDTA) buffer is recommended.

Equipment/reagent requirements

- Phosphate Buffered Saline
- DNA extraction buffer
- Proteinase K
- Protein precipitation solution
- Isopropanol
- Ethanol
- DNA hydration buffer
- An incubator/water-bath capable of 65^{o}C
- A vortex mixer
- A refrigerated centrifuge capable of 14,000g

- A spectrophotometer capable of reading absorbance at 260 and 280nm/Nanodrop

Procedure

1. Homogenise 3-5mg of tissue in sterile PBS using a mechanical homogeniser according to the manufacturer's instructions for use and centrifuge at 14,000g for 10min at 4°C. Discard supernatant and resuspend the tissue pellet in 180µL of DNA extraction buffer (0.1M EDTA, 0.2M NaCl, 0.05M Tris-HCl, pH 8.0, 0.5% SDS, 50µg/mL DNAse free RNAse).

2. Add proteinase K to a final concentration of 100µg/mL and gently swirl the eppendorf tube to mix the components. Incubate the beaker at 37°C for at least 3 hours, preferably overnight, with gentle agitation. This can be achieved using a shaking water bath or by occasional swirling by hand. The solution should be reasonably clear and viscous at the end of the incubation.

3. Add 100µL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.

4. 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.

5. 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.

6. 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.

7. 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 for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.

8. 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 at 12,000g for 5 min.

9. 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 cooled cryostorage tubes and stored at -80^{o}C

10. An aliquot of the DNA from a representative sample from each batch may be analyzed 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.