SOP 3.4 DNA Extraction from Blood

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Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
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Purpose

This SOP describes the procedure for DNA extraction from whole blood.

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 precautions

- Prior to commencing, read and understand the Material Safety Data Sheets (MSDS) for all hazardous chemicals used in this procedure (for example, isopropanol).

- Protective clothing, including laboratory coat, gloves and protective glasses, must be worn at all times when performing this procedure.

Considerations

- A number of different methods are available for the isolation of DNA from whole blood, including salting out/salt precipitation, phenol/chloroform extraction, silica gel extraction, proteinase K extraction and anion exchange. The choice of method depends on many factors including the required quantity, purity required for downstream application, time, molecular weight of DNA and expense. These guidelines propose the salting out method for DNA extraction from whole blood which appears to be the method of choice for use in molecular biology laboratories and is also used by the majority of biobankers within the P3G Consortium as highlighted in the following link http://www.p3gobservatory.org/dna/comparisonTable.htm. The salting out method proposed is based on the method of Ciulla *et al*, 1988 (30).

- DNA can be isolated from whole blood (EDTA) or a cell pellet following plasma separation from an EDTA sample.
- DNA should be processed as soon as is practicable but a specimen can be stored at 4°C for 48 hours prior to processing or alternatively can be stored directly at -80°C for DNA processing at a later date.

Equipment/reagent requirements

- Red blood cell lysis buffer
- White blood cell lysis buffer
- Protein precipitation solution
- Isopropanol
- Ethanol
- DNA hydration buffer
- A centrifuge capable of generating 3,000g
- A vortex mixer
- -80^{o}C Freezer
- Incubator/water-bath capable of temperatures up to 65^{o}C
- A spectrophotometer capable of reading 260 and 280nm/ Nanodrop

Procedure

Cell Lysis

1. Dispense 30mL of red blood cell lysis buffer (NH_4CL, $NAHCO_3$, EDTA) into a 50mL centrifuge tubes containing 5-10mL whole blood. Incubate at RT for 5 min, inverting occasionally to mix.

2. Centrifuge the samples at 3,000g for 10 min to pellet the white blood cells. Pour the supernatant to waste.

3. Add 10mL white cell lysis buffer (SDS, EDTA) to white blood cell pellet and vortex vigorously for 10 sec (RNAase may be added at this stage to remove RNA from the preparation). Incubate at 37°C for 15min.

Protein Precipitation

4. Add 3.3mL of ammonium acetate protein precipitation solution, and vortex vigorously for 20 sec at high speed.

5. Centrifuge for 5 min at 3,000g. 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.

DNA Precipitation

6. Dispense 10mL isopropanol into a clean 50mL centrifuge 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 for 5 min at 3,000g. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care not to dislodge the pellet.

8. Wash the DNA, by adding 10mL of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge for 5 min at 3,000g and drain to remove ethanol, ensure that the DNA pellet is not disturbed.

DNA Hydration

9. Add 0.3-1.0mL 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.

10. 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.

11. 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 isolation should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.