Extraction of Genomic DNA from Whole Blood

1 Purpose / Introduction

1.1 This procedure is intended to purify high molecular weight DNA from 5-10mL of fresh or frozen whole blood, and was originally written by Laura-Lee Boodram.

2 Equipment/Reagents/Materials

Refrigerated benchtop centrifuge, RT microfuge

Shaking water bath at 55°C

Vortex mixer

Buffer A Red blood cell lysis buffer

final concs	**1 litre**	**500ml**
0.32 M sucrose	109.536g	54.768g
10 mM Tris HCl	10mls of 1M	5mls of 1M
5 mM $MgCl_2$ ($6H_2O$)	1.017g	0.508g

Adjust pH to 7.6
Autoclave or Filter sterilise 0.22u prior to addition of Triton-X-100

0.75% Triton-X-100	7.5mls	3.75mls

Buffer B Proteinase K buffer

final concs	**1 litre**	**500mls**
20 mM Tris-HCl	20mls of 1M	10mls of 1M
4 mM Na_2EDTA	8mls of 0.5M	4mls of 0.5M
100 mM NaCl	20mls of 5M	10mls of 5M

Adjust pH to 7.4 autoclave or Filter sterilise 0.22u

10% SDS

Proteinase K 20mg/ml (defrost -20°C stock solution) read COSHH MSDS

Sterile water (chilled overnight)

Isopropanol (-20°C) read COSHH MSDS

70% Ethanol read COSHH MSDS

Haz-Tabs prepare 2.5% bleach solution read COSHH MSDS

3 Principle

3.1 To extract high molecular weight DNA from fresh or frozen blood, red cells are lysed and white cells are digested overnight in buffer with SDS and proteinase K. Salt is then added to precipitate digested protein and cell debris, leaving DNA in solution. DNA is precipitated with isopropanol then washed in 70% ethanol to remove salt and contaminants. The DNA is dried to remove ethanol and re-suspended as required for downstream use.

4 Procedure

1. Add 1 volume of buffer A to 1 volume of blood and 2 volumes of cold, sterile, distilled, deionised water in a sterile, conical, 50ml tube. Vortex gently or invert tube 6-8 times and leave to incubate on ice for 2-3 minutes.

2. Spin at 3500 rpm for 15 minutes at 4ºC. Discard supernatant into 2.5% bleach solution and re-suspend pellet (vortex for 30 seconds at medium speed) in 2 ml of buffer A and 6

ml of water. Spin at 3500 rpm for 15 minutes at 4ºC. The pellet should be white to cream in colour. If pellet is significantly red, repeat washing step again.

3. Add 5 ml of Buffer B and 500 µl of 10% SDS to pellet. Re-suspend pellet by vortexing vigorously for 30 seconds. Then add 50 µl of Proteinase K solution (20mg/ml).

4. Leave to incubate for at least 2hrs or up to overnight at 55ºC in a shaking water bath. Remove samples and leave to cool to room temperature (or leave for 2-3 minutes on ice). Add 4 ml of 5.3M NaCl solution. Vortex gently for 15 seconds.

5. Spin at 4000 rpm for 20 minutes at 4ºC. Pour off supernatant containing DNA into a fresh 50 ml tube. Take care not to dislodge pellet. Add an equal volume of cold isopropanol (stored at -20ºC). Invert gently 5-6 times to precipitate DNA.

6. Remove DNA with a wide bore tip and transfer to 5 ml 70% EtOH in a 15ml tube. Wash pellet by inverting several times.

7. Transfer pellet with a wide bore tip to 1 ml 70% EtOH in a microfuge tube and wash by inverting tube.

8. Spin at 10,000g 5mins in RT microfuge. Remove EtOH and leave DNA to air dry for 15-20 minutes at RT.

9. Re-suspend in 300-400 µl of Tris-HCl or TE pH 8.0 as required. Leave to re-dissolve for a few hours at RT. Transfer to 4ºC. Mix by inversion before quantitation.

5 References

Protocol online, Extraction of genomic DNA from whole blood, author Laura-Lee Boodram 2004.

Helms, C. Salting out Procedure for Human DNA extraction. In The Donis-Keller Lab - Lab Manual Homepage [online]. 24 April 1990. [cited 19 November 2002; 11:09 EST]. Available from: http://hdklab.wustl.edu/lab_manual/dna/dna2.html.

Epplen, J.E., and T. Lubjuhn. 1999. DNA profiling and DNA fingerprinting. Birhkhauser Verlag, Berlin. p.55.