Microprep DNA extraction from whole-blood employing a modified Drabek method.

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DNA extraction represents a crucial step for downstream nucleic acid based molecular methods used to characterize the genetic features of human tissue samples as well as of viral, prokaryote, fungal and parasitic agents. Molecular epidemiology and genomic characterisation studies usually involve the processing and genetic testing of large numbers of samples. Although many commercial DNA extraction methods offer convenient and high-throughput capabilities, their use for the processing of large specimen volumes (though not numbers) becomes very expensive. The Viral & Human Genomics Laboratory developed an adaptation of a previously published method using sugar, laundry detergent, and salt for DNA extraction from blood. This method allows for the processing of different sample volumes for use in different downstream applications. This low-cost, laundry detergent based DNA extraction technique maximizes DNA yield and quality which avoids the use of toxic reagents or of molecular-grade reagent requirements. Four different processing modalities have been optimized (maxiprep, midiprep, miniprep and microprep) for use with two different bio-specimens (blood-bank discarded leukocyte concentrates as well as whole blood). The optimised protocol produces 4.5 mg of genomic human DNA from 15 ml of leukocyte concentrate with spectrophotometric quality and genomic DNA integrity that has proved amenable to downstream applications such as endpoint PCR human genetic testing, study of human polymorphism, detection of blood-borne viral nucleic acids; real-time detection of SNPs and allele assignment, as well as blood-borne virus quantitation. The long-term stability and quality of extracted DNA samples rivals that of phenol–chloroform extracted samples at 10 years follow-up.

Procedure

For the processing of 750 µL of EDTA or ACD anti-coagulated whole-blood.

1. Transfer 750 µL of whole blood to a 1.5 mL micro-centrifuge tube and add 750 µL of lysis buffer. Mix vigorously to disrupt cells, first by inversion followed by vortex. This step breaks cell membranes but leaves cell nuclei intact. Use of vortex mixing IS allowed in modalities other than maxiprep.

2. Spin at 3,000 G for 5 minutes, discard the supernatant containing cell membranes, cytoplasm and organelles by transferring to a 0.1% NaOCl decontamination solution without disturbing the nuclear pellet.

3. Add 500 µL of cell lysis buffer to nuclear pellet. Mix vigorously by inversion and vortex until nuclear pellet is completely resuspended. This step further eliminates contaminating erythrocytes and haemoglobin.

4. Spin at 3,000 G for 5 minutes, discard the supernatant by transferring to a 0.1% NaOCl decontamination solution.

5. Repeat steps #3 and #4 as many times as needed until nuclear pellet is free of red colouring.

6. Add 37.5 µL of 10 mM pH 8.0 Tris-HCl to the nuclear pellet, vortex vigorously until completely resuspended.

7. Add 37.5 µL of the 20 mg/mL laundry detergent solution and vortex vigorously until nuclear pellet is completely disrupted. This step disrupts the nuclear membrane and viral capsids freeing nucleic acids along with proteins.

8. Add 32.5 µL of 5M NaCl and vortex vigorously until a cloudy solution is produced. This step precipitates both human chromatinic proteins and the nuclear membrane.

9. Transfer cloudy solution to a new 1.5 mL micro-centrifuge tube.

10. Spin at 16,000 G for 10 minutes using any micro-centrifuge with fixed angle rotor. This step sediments the previously precipitated proteins and membranes leaving a nucleic acid containing supernatant (containing human, mitochondrial and viral DNA and potentially both host and viral RNAs.

11. Transfer the supernatant to a new 1.5 mL micro-centrifuge tube without disturbing the sediment. Discard the tube having the sediment into a 1.0% NaOCl decontamination solution. This supernatant contains the nucleic acids which will now be purified and enriched to further eliminate contaminants.

12. Add 500 µL of 96% ethanol previously chilled to -20°C, mix gently. This step precipitates all nucleic acids present in the supernatant.

13. Retrieve the precipitated DNA using a flame-sealed microcapillary tube or Pasteur pipette (use of non-sealed tubes or pipettes will lead to sequestration of nucleic acids and lower nucleic acid yields). Discard supernatant, keep 1.5 mL micro-centrifuge tube.

14. Return DNA containing microcapillary to the previous 1.5 mL conical tube and add 300 µL 70% ethanol, wash with up and down movements of capillary in ethanol for 20 seconds. This step decreases salt concentration in DNA.

15. Repeat steps #13 and #14 ONE more time.

16. Retrieve the washed DNA with the microcapillary tube and allow to air dry for 10 to 15 minutes by placing microcapillary tube in open 1.5 mL micro-centrifuge tube or the Pasteur pipette in a test tube rack in the thermomixer. Extreme sharps caution should be taken!

17. Resuspend DNA in 50 µL of 10:1 Tris-EDTA buffer to prepare *stock DNA solution.*

18. Incubate *stock DNA solution* in thermomixer or water bath for 30 minutes at 70ºC.

19. Prepare *working DNA solutions* at 100 to 200 ng/µL and store at -20ºC or -70ºC until future use.

20. Prepare long-term storage strategy (see notes below) for remaining DNA and note freezing date on lab log.

Notes

1. Multiple freeze-thaw cycles can degrade DNA and compromise the obtention of genetic data. make multiple aliquots that will only be thawed when they are needed. DNA material used in a short time frame may be stored at -20C. DNA stored long term should be in ultra-low freezers, typically at or below -80C to prevent the degradation of nucleic acids in the DNA. To ensure high quality nucleic acid based methods, the following DNA storage strategies are recommended:

    - Short-term storage (weeks) at 4°C in 10:1 M Tris-EDTA
    - Medium-term storage (months) at –80°C in 10:1 M Tris-EDTA
    - Long-term storage (years) at as –80°C as ethanol precipitate or FTA card immobilized
    - Long-terms storage (decades) at –164°C in 10:1 M Tris-EDTA

2. To prepare 1 litre of **Cell Lysis Buffer** add 10 gr of Triton X-100 to a flask having 350 mL of dH_2O and mix using a magnetic stirrer until completely dissolved. Add 102.69 gr of sucrose and 1.016 gr of hexahydrated Magnesium Chloride and 10 mL of 1M pH 7.6. Tris-HCl and allow all reagents to dissolve. Transfer the mix to a graduated cylinder and top up to 1 litre using dH_2O. Dispense 50 mL aliquots and keep refrigerated between 0 and +4ºC.

3. Prepare a saturated **Laundry Detergent Solution** by dissolving 80 grs of commercial household powdered laundry detergent* in 2 litres of dH_2O and mix in magnetic stirrer for at least 12 hours. Allow saturated solution to sit and precipitate for 12 hours. Carefully retrieve saturated solution supernatant, filter through a grade 1 Whatman filter paper and prepare the corresponding working dilutions by adding either 1 volume of dH_2O for the 1:2 dilution used in the maxiprep modality (to process leukocyte concentrates) or by adding 3 volumes of dH_2O for the 1:4 dilution used in the remaining modalities (for the processing of whole blood). Leukocyte concentrates require less laundry detergent solution as they do not normally have as much contaminating erythrocytes as whole blood samples.

4. Once the DNA has been precipitated in 96% Ethanol do not disturb the sediment nor centrifuge as experience has shown that such procedures (common to other DNA extraction methods) convey the risk of contaminating the DNA solution.

5. Working DNA solutions for short term use are best kept stored in 0.2 mL PCR tubes for ease of use.

6. Evaluate working DNA solution quality and yield as recommended in the corresponding protocol (see "Spectrophotometric evaluation of DNA using a Nanodrop ND-1000 multimedia protocol available in https://www.youtube.com/watch?v=k2tZTDDyxaw&feature=youtu.be.

7. Evaluate genomic DNA integrity in 1% agarose gel electrophoresis by loading 5 µL per well of a 1 µL working DNA solution + 4 µL of dH_2O + 3 µL of 6x O orange loading buffer running at 6 volts per cm og gel length for 50 minutes per inch of travel. Good integrity DNA should exhibit a single high molecular smear above the 10 kb marker, degraded DNA will show lower weight molecular smears whose size will vary depending on degree of degradation. DNA obtained from old blood samples or those not stored in refrigeration will normally exhibit 200 bp apoptotic banding patterns.

8. Functional applicability of extracted DNA should be assessed depending on downstream procedures. For endpoint PCR applications, samples should be screened for the presence of housekeeping genes or suitable conserved genes such as KIR3DL2 or KIR3DL3 (see corresponding protocol "Killer-cell Immunoglobulin-like Receptor (KIR) genotyping" available from http://lgvh.hostingerapp.com/Protocolos/Hum_KIR.pdf.

***Important development note**

The most economic, simple laundry detergent formulas (those lacking fabric softeners and whitening additives) have, in our experience, resulted in better results when compared to high-end commercial formulas. We have optimized our protocol for the use of powdered Foca detergent (Fábrica de Jabón la Corona, Mexico). Nonetheless, this can and must be optimized for locally available detergents. Feel free to contact us for further support at lgvh.uaslp@gmail.com.

References

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Revision history

1.0 Original document.

1.2 Nov 01, 2013. Final DNA resuspension volume reduced to 100 µL for microprep and 500 µL for midiprep modalities. The DNA precipitation step at -20ºC for 20 min was eliminated. The 96% Ethanol volume was reduced from 1500 to 1000 µL in the midiprep modality.

1.3 Nov 29, 2019. Protocol layout modified and split into individual protocols for micro, mini, midi and maxiprep modalities. Modifications to recommended DNA solution storage strategies and laundry detergent solution preparation. Midiprep and miniprep DNA resuspension volume reduced to 500 µL and that of microprep modality to 50 µL.