PreAnalytiX Supplementary Protocol

Purification of genomic DNA from the PAXgene® Blood ccfDNA Tube using the QIAamp® DNA Blood Mini Kit

This protocol uses the QIAamp DNA Blood Mini Kit to purify genomic DNA from the buffy coat fraction generated after centrifugation of whole blood collected into PAXgene Blood ccfDNA Tubes.

IMPORTANT: Whole blood samples must be collected into PAXgene Blood ccfDNA Tubes. See the *PAXgene Blood ccfDNA Tube Product Circular* for information about sample collection, blood stabilization and plasma processing.

For details about the preparation of genomic DNA, read the *QIAamp DNA Mini and Blood Mini Handbook* before beginning this procedure, paying close attention to the "Safety Information" and "Important Notes" sections.

For Research Use Only. Not for use in diagnostic procedures. The performance characteristics of this product have not been fully established.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Ethanol (96–100%, purity grade p.a.)*
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Microcentrifuge with rotor for 2 ml tubes†
- Vortexer†
- Water bath or heating block at 56°C†
- Optional: RNase A (100 mg/ml)

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

† Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

For vacuum protocols

- QIAvac 24 Plus (QIAGEN cat. no. 19413) or equivalent*
- VacConnectors (cat. no. 19407)
- Vacuum Regulator (cat. no. 19530) for easy monitoring and release of vacuum pressure*
- Vacuum Pump (cat. no. 84010 [USA and Canada], 84000 [Japan] or 84020 [rest of world]) or equivalent pump capable of producing a vacuum of –800 to –900 mbar*
- Optional: VacValves (cat. no. 19408)
- Optional: QIAvac Connecting System (cat. no. 19419)

Starting material

Starting material for genomic DNA purification is whole blood collected into a PAXgene Blood ccfDNA Tube (see the *PAXgene Blood ccfDNA Tube Product Circular* for information about sample collection, blood stabilization and plasma processing).

Important points before starting

- Make sure that kit boxes are intact and undamaged, and that buffers have not leaked. Do not use a kit that has been damaged.
- When using a pipet, make sure that it is set to the correct volume and that liquid is carefully and completely aspirated and dispensed.
- To avoid transferring samples to the wrong tube or spin column, make sure that all tubes and spin columns are properly labeled. Label the lid and the body of each tube. For spin columns, label the body of its processing tube.
- Close each tube or spin column after liquid is transferred into it. Spilling samples and buffers during the procedure may reduce the yield and purity of DNA.
- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature (15–25°C).

* Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Things to do before starting

- Preheat a water bath or heating block to 56°C.
- Equilibrate Buffer AE to room temperature for elution.
- Prepare Buffer AW1, Buffer AW2 and QIAGEN Protease according to instructions in the *QIAamp DNA Mini and Blood Mini Handbook*.
- Dissolve any precipitate that forms in Buffer AL by incubating the buffer at 56°C.

Procedure: Purification of genomic DNA from buffy coat generated from whole blood collected into PAXgene Blood ccfDNA Tubes

1. **Collect whole blood into PAXgene Blood ccfDNA Tubes as described in the *PAXgene Blood ccfDNA Tube Product Circular*.**
2. **Pipet 20 µl QIAGEN Protease into the bottom of a 1.5 ml microcentrifuge tube.**
3. **Prepare plasma following the instructions in the *PAXgene Blood ccfDNA Tube Product Circular*.**

   Note: After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat and contains concentrated leukocytes; the bottom layer contains concentrated erythrocytes (red blood cells).
4. **Carefully open the PAXgene Blood ccfDNA Tube following the instructions in the *PAXgene Blood ccfDNA Tube Product Circular* and remove the plasma fraction.**

   Note: For purification of circulating, cell-free DNA (ccfDNA), process the plasma following instructions in the *QIAamp Circulating Nucleic Acid Handbook* or the *QIAsymphony PAXgene Blood ccfDNA Kit Handbook*, or store the plasma in a suitable tube at −20°C or −80°C.
5. **Transfer 200 µl buffy coat (intermediate layer) into the microcentrifuge tube containing QIAGEN Protease.**

   Note: If there is no visible buffy coat, collect 200 µl of plasma nearest to the red blood cell fraction, which contains buffy coat cells.
6. **Follow instructions of the protocol "DNA Purification from Blood or Body Fluids (Spin Protocol)" or the protocol "DNA Purification from Blood or Body Fluids (Vacuum Protocol)" in the *QIAamp DNA Mini and Blood Mini Handbook*, starting with step 3, where 200 µl Buffer AL are added to the sample.**

   Note: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) to the sample before adding Buffer AL.

For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX or QIAGEN kit handbook or user manual. Handbooks and user manuals are available at **www.qiagen.com** and **www.preanalytix.com** or can be requested from QIAGEN Technical Services or your local distributor.

Safety data sheets (SDS) for any QIAGEN or PreAnalytiX product can be downloaded from **www.qiagen.com/safety**.

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