Reducing variability and improving workflow in the collecting, transporting, and processing of blood samples for genomic DNA purification using the PAXgene™ Blood DNA System

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Abstract

The PAXgene Blood DNA System provides an easy-to-implement, standardized method for collecting human whole blood samples and purifying genomic DNA.

Blood samples are collected in PAXgene Blood DNA Tubes containing a unique additive that maintains sample integrity for DNA purification. Collected blood samples can be stored for up to 14 days at room temperature,* or for longer at lower temperatures.

PAXgene Blood DNA Tubes are integrated with the PAXgene Blood DNA Kit, which uses a standardized DNA purification protocol. This protocol was developed to minimize the number of procedural steps and streamline the workflow. The buffer-based protocol minimizes carryover of RNA and protein. The use of a single processing tube per sample reduces the possibility of sample mix-up. The purified DNA is of high yield and quality, and is suitable for use in a range of downstream applications.

* DNA yields may be reduced if tubes are stored at 25°C for 14 days or longer.

Introduction

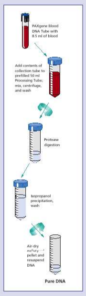
Clinical research studies involving the collection of blood samples from multiple locations around the world are expensive and time-consuming to design and implement. Reducing variability at every step from blood collection to DNA purification ensures consistent results. Prevention of multiple blood draws from the same patient and repeated DNA purification saves time and money.

There are many variables inherent in the collection and shipment of blood samples. The collection tube used can be a source of variability through varying blood draw volumes and the type of anticoagulant used (EDTA, citrate, or heparin). Other sources of variability are temperature fluctuations during transportation and the use of different methods to purify genomic DNA. Salting-out methods can lead to RNA or protein carryover, and the use of organic reagents can result in phenol carryover and poor performance of DNA in downstream applications.

The PAXgene Blood DNA System minimizes many of the variables in the process from blood collection to DNA purification. PAXgene Blood DNA Tubes are calibrated to directly draw 8.5 ml of blood per patient, standardizing the phlebotomy process. PAXgene Blood DNA Tubes use a unique additive to keep blood under optimal conditions during storage or transport. This additive does not interfere with PCR-based assays, which can be a problem with some anticoagulants.

The PAXgene Blood DNA Tubes are integrated with the PAXgene Blood DNA Kit. With this kit, DNA is purified through a streamlined procedure using a single pre-filled processing tube per sample. The possibility of sample mix-up and the processing time required are reduced. The unique buffer-based protocol lyses red blood cells and the outer cell membrane of white blood cells while leaving nuclei intact. This greatly reduces the possibility of RNA carryover in the purified DNA. Nuclei are subsequently lysed together with protease digestion for consistent removal of protein.

Workflow of the PAXgene Blood DNA System



In this poster, we demonstrate storage of blood samples under various conditions and streamlined workflow through a high-throughput purification protocol using the PAXgene Blood DNA System. We also demonstrate that the purified DNA is of high yield and quality, performing well in a range of different downstream applications.

Methods

Human whole blood samples (8.5 ml) from healthy donors (4 x 10^6 – 12 x 10^6 white blood cells per ml) were drawn into PAXgene Blood DNA Tubes. DNA was purified using the PAXgene Blood DNA Kit according to the standard protocol (see handbook supplied with the kit) or the high-throughput protocol (see **www.preanalytix.com/pdf/TN_HT_Protocol_PAX0603_HR.pdf**), depending on the number of samples being processed. Yield and purity of purified DNA were determined by absorbance measurements at 260 nm and 280 nm. Quality of purified DNA was determined by agarose gel electrophoresis.

Results

High-Throughput Purification of DNA

96 blood samples were collected (12 donors, 8 samples per donor), and DNA was purified according to the high-throughput protocol. This protocol allows purification of DNA from 96 blood samples in 3.5 hours.

Average Purity and Yield of DNA Purified from 96 Blood Samples

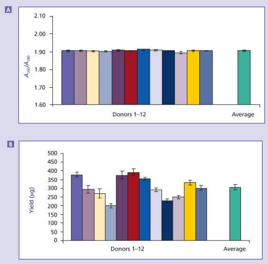


Figure 2. A Average purity and B average yield of 96 DNA samples purified in parallel from blood samples from 12 donors (8 samples per donor).

Purification of High-Molecular-Weight DNA from 96 Blood Samples

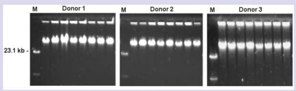


Figure 3. Agarose gel analysis of 400 ng DNA (0.5% agarose gel, 1x TAE buffer, 23 V, 16 h) purified from blood samples from 3 donors (8 samples per donor). **M:** marker.

The yield, purity, and quality of the purified DNA were determined:

- Average DNA yield from 96 samples was 305 µg
- Coefficient of variation (CV) with regard to DNA yield was calculated for each donor: the range of values obtained was 2.3–10.1%
- Average A_{260}/A_{280} ratio from 96 samples was 1.91, indicating high purity with no protein contamination
- All DNA samples ran quantitatively above a 23 kb marker

Purification of DNA with No RNA Carryover

Agarose gel analysis of genomic DNA purified with a salting-out method from whole blood samples from 8 donors revealed RNA contamination. DNA purified with the PAXgene Blood DNA Kit was free of detectable RNA contamination.

DNA Purification with No RNA Carryover

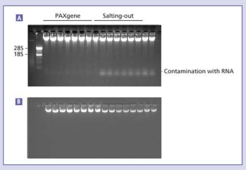


Figure 4. Agarose gel analysis of 10 µg DNA purified from 8 donors using the PAXgene Blood DNA Kit or a salting-out method. A Purified DNA before treatment with RNase. B Purified DNA after treatment with RNase at room temperature for 24 h.

DNA Purification after Storing Blood under Different Conditions

Blood samples from various donors were collected and stored at different temperatures and for different lengths of time:

Storage time	Storage temperature	Number of samples
DNA purified on day of collection		70 donors, 2 samples per donor
14 days	25°C	70 donors, 1 sample per donor
7 days	30°C	20 donors, 1 sample per donor
28 days	8°C	30 donors, 1 sample per donor
10 weeks	–20°C	30 donors, 1 sample per donor
10 months	–80°C	29 donors, 1 sample per donor

Average Purity and Yield of DNA Purified from Blood Stored Under Different Conditions

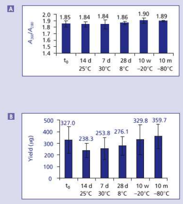


Figure 5. A Average purity and B average yield of DNA purified from blood samples stored at different temperatures and for different lengths of time. **Note:** high inter-donor variation in the number of nucleated cells leads to high standard deviations of DNA yield.

After storage, DNA was purified according to the standard protocol. The average yield and purity of the purified DNA were determined for each storage condition:

- Average DNA yields were in the range 238–360 µg
- Average A_{260}/A_{280} ratios were over 1.84, indicating high purity

Use of Purified DNA in Downstream Applications

DNA purified from blood using the PAXgene Blood DNA System performed well in the following downstream applications:

- Multiplex PCR
- Allelic discrimination
- Southern blotting

Multiplex PCR of 8 Single-Copy Gene Fragments

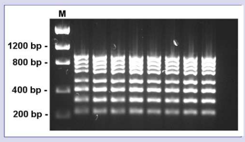


Figure 6. Multiplex PCR of fragments from 8 single-copy genes (955 bp from CD19, 845 bp from CD59, 756 bp from CD40, 662 bp from CD14, 523 bp from AGTR, 414 bp from cKit, 310 bp from B29 E4, 222 bp from PRP) using 250 ng DNA purified from blood from 8 donors. **M:** marker.

SNP Genotyping at the Cytochrome P450 Gene CYP2C19

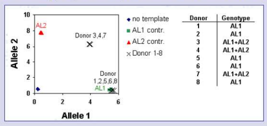


Figure 7. Allelic discrimination assay using the QuantiTect® PCR Kit and a TaqMan® assay with DNA purified from blood from 8 donors. **Allele 1:** CYP2C19*1; **Allele 2:** CYP2C19*2.

Restriction Digestion and Southern Blotting of Purified DNA

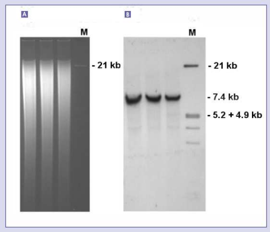


Figure 8. A Agarose gel analysis of 5 µg DNA samples restriction-digested with EcoRI (0.8% agarose gel, 1x TAE buffer, 23 V, 16 h). B Southern blotting of the restriction-digested DNA samples with a digoxygenin-labeled 922 bp fragment of the mitochondrial tRNAlys/ATPase gene. **M:** marker.

Summary

The PAXgene Blood DNA System reduces variability and improves workflow for genomic DNA purification from whole blood, providing:

- A standardized and streamlined method for collection of large volume blood samples and subsequent purification of DNA
- Stable storage of blood for up to 14 days at 25°C or longer at lower temperatures, enabling transport from sites of blood collection to a central location for DNA purification
- DNA purification using a unique buffer-based method that minimizes protein and RNA carryover
- DNA purification using a single processing tube per sample, reducing the risk of sample mix up.
- High-throughput purification of DNA from large numbers of blood samples (96 samples processed in 3.5 hours)
- Purified DNA that performs well in a wide range of downstream applications, including multiplex PCR, SNP genotyping, restriction digestion, and Southern blotting