APPLICATION NOTE

Reproducible yields of high-quality DNA from buffy coat samples using the BioRobot® M48 workstation with MagAttract® technology

This study shows that reproducible, high yields of high-quality DNA can be obtained from buffy coat samples using efficient MagAttract® technology in combination with the BioRobot® M48 workstation.

The leukocyte concentration in buffy coat samples may vary considerably depending on the number of leukocytes in the donor blood sample and the efficiency of leukocyte harvesting in the buffy coat preparation. These factors make a robust and reliable purification method critical for the success of downstream applications.

Materials and methods

DNA was purified from buffy coat samples prepared from donors with varying white blood cell (WBC) counts. Buffy coat samples were prepared by centrifuging samples at 300 x *g* for 10 minutes; the intermediate layer containing white cells was harvested. Automated DNA purification was performed on 75–100 µl highly enriched buffy coat samples and on 150–300 µl buffy coat samples with normal to low leukocyte count. The 75 µl, 150 µl, and 300 µl Buffy Coat protocols were used in combination with the MagAttract® DNA Blood Midi M48 Kit. Replicate purifications from each sample donor are shown (Table 1). Purified DNA was eluted in 200 µl water. DNA yield was quantified by absorbance (A_{260}/A_{280}) corrected for background.

Results

Average DNA purity was 1.87 ± 0.02. DNA yields were highly reproducible (Table 1, Figure 1) and PCR of single-copy genes was consistently successful (Figure 2).

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Table 1. DNA yields obtained from different buffy coat sample volumes using the BioRobot M48 System and the appropriate protocol

Sample	Donor	Buffy coat WBC count (10^6/ml)	Number of replicates	Sample volume	DNA yield (µg)
Highly enriched buffy coat*	A	-	12	75 µl	7.6 ± 0.3
	B	-	36	75 µl	11.8 ± 0.4
	C	-	6	75 µl	12.6 ± 1.3
Enriched buffy coat†	D	27.1	3	150 µl	13.3 ± 0.4
	E	21.7	3	150 µl	12.9 ± 0.1
	F	21.3	3	150 µl	12.9 ± 0.2
	G	20.3	3	150 µl	12.0 ± 0.1
	H	17.9	3	150 µl	11.2 ± 0.3
Buffy coat with low WBC concentration	I	10.7	3	300 µl	13.2 ± 0.2
	J	9.8	3	300 µl	11.8 ± 0.3

* *Prepared from blood bag, 10x enrichment. This type of buffy coat preparation tends to result in very efficient leukocyte enrichment.*

† *Prepared by centrifuging 10 ml EDTA-blood tubes at 300 x g for 10 minutes, and harvesting the intermediate layer of cells.*

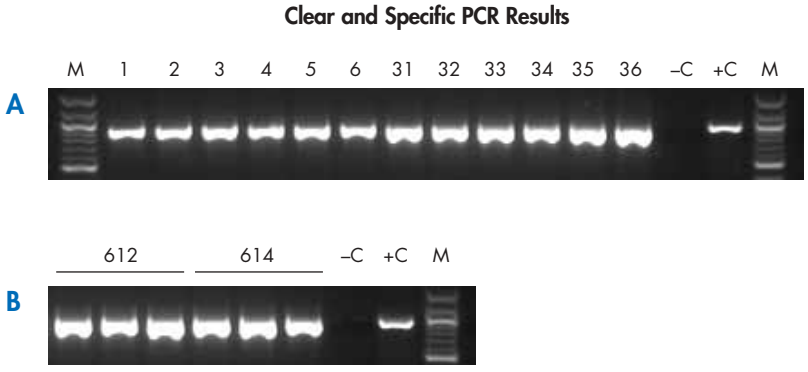

Figure 1. *Amplification of a single-copy gene (MECL-1) using 5 µl template DNA purified from buffy coat, in 50 µl PCR. A 5 µl aliquot (10%) of each PCR was visualized on a 1.5% agarose gel.*
A*: PCR of DNA purified from Donor B, using the first 6 and last 6 replicates in a 36-replicate run.*
B*: PCR of DNA purified from 2 different buffy coat samples (Donors I and J) with low WBC concentration.* ***M****: 100 bp marker;* ***+C****: positive control;* ***–C****: negative control.*

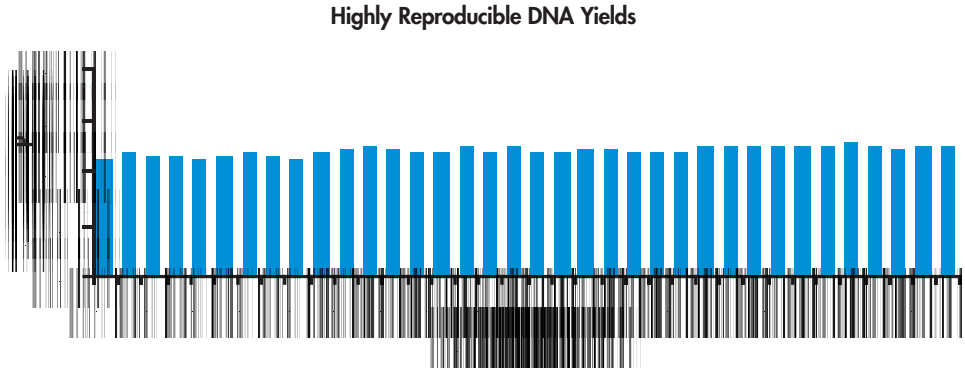

Figure 2. *DNA yields from 36 buffy coat samples (75 µl) from Donor B.*

Conclusions

High-quality DNA can be reproducibly purified from buffy coat samples using MagAttract technology in combination with the BioRobot M48 workstation.

- Protocols can be selected to suit the concentration of most buffy coat samples
- DNA yields within the same processing run are highly reproducible (CV <3%)
- Purified DNA performs consistently well in PCR of a single-copy gene

Contact QIAGEN today and discover how automating buffy coat DNA preps can benefit your lab!

Ordering Information

Product	Contents	Cat. No.
BioRobot M48 workstation	Robotic workstation for automation of magnetic-particle purification technology	9000708
MagAttract DNA Blood Midi M48 Kit (192)*	MagAttract Suspension and reagents for purification of genomic DNA from 192 whole blood samples of up to 350 µl using the BioRobot M48 workstation	951356

The BioRobot M48 is intended as a microtiter diluting and dispensing device. No claim or representation is intended for its use in identifying any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of the BioRobot M48 for any particular use, since its performance characteristics have not been validated for any specific organism. The BioRobot M48 may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

* MagAttract Kits are intended as general-purpose devices that may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

BioRobot Systems are not available in all countries; please inquire.

The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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