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High yield gDNA purification from blood by the ep*Motion*® M5073 system

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Abstract

To meet the automation demand of low to medium throughput research users, Eppendorf has specifically developed the MagSep Blood gDNA Kit for the ep*Motion* M5073 automated pipetting system. This easy to use combination provides a perfect solution for magnetic bead-based, automated purification of high quality, ready to use genomic DNA from up to 200 µL of fresh and frozen whole blood samples. Due to the flexible design of the ep*Motion* M5073, sample numbers between 1 and 24 can be processed per run. In this study 200 µL fresh human whole blood samples were subjected to automated DNA purification using the Eppendorf MagSep Blood gDNA Kit in combination with the epMotion M5073. Genomic DNA purified with the MagSep Blood gDNA Kit showed yields and qualities that were superior to silica column-based technologies. No cross contamination was detectable and the genomic DNA was directly compatible with downstream real-time PCR amplification. Typical yields of 4-10 µg genomic DNA can be expected from healthy donors and with an overall CV of < 10 %, yields from 24 replicates (a full run) show a very good consistency and reproducibility.

Introduction

The purification of genomic DNA from whole blood samples is routinely performed in a variety of research laboratories with a growing demand for automation, as human blood has to be regarded as potentially hazardous. The Eppendorf MagSep Blood gDNA Kit for the Eppendorf ep*Motion* M5073 is the kit of choice for automated, walk away purification of genomic DNA from fresh or frozen whole blood (EDTA or citrate treated). The instrument and the kit form an automation solution that reproducibly delivers high yield and high quality genomic DNA that is directly compatible with downstream applications. The ep*Motion* M5073 is by default equipped with the proven Thermomixer (TMX) in combination with a magnetic separator. This unique 3D-MagSep Technology (Figure 1) allows the entire process being performed without any labware transport steps.

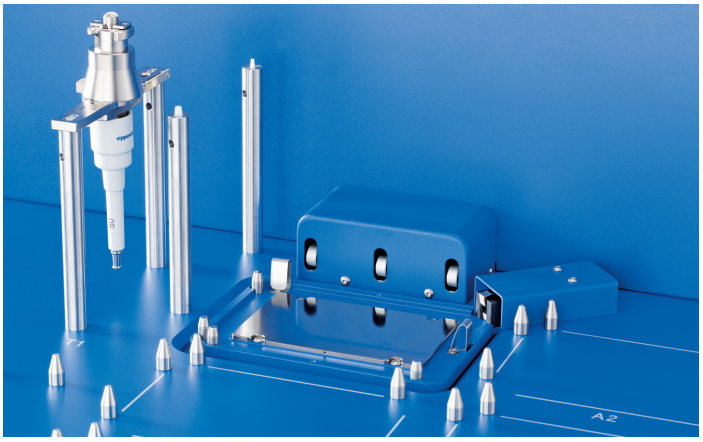

Figure 1: The 3D-MagSep Technology of the epMotion M5073.

Setting up a purification run is extremely user friendly, as the reagents of the kit are provided in a unique tray that can simply be placed in the ReagentRack on the worktable of the ep*Motion* M5073. No buffers have to be decanted manually, reducing overall preparation time and the risk of human error. Once the process is finished, the individual bottles in the tray can be recapped and the remaining buffers stored for future use, adding extra convenience. The user is guided through the entire setup process via a software Assistant (Figure 2), allowing the selection of sample numbers, labware type for the eluates and the adjustment of the elution volume in a range from 25 – 100 µL to get the optimum yield or the maximum concentration of DNA.

Materials and Methods

Materials

Eppendorf ep*Motion* M5073 delivered with

- Dispensing Tool TS 1000
- Dispensing Tool TS 50
- Liquid waste tub
- Waste tub
- ReagentRack
- PrepRack
- Rack 24 Eppendorf Safe-Lock

Eppendorf consumables

- epT.I.P.S. Motion Filter SafeRacks (1000 and 50 µL)
- Eppendorf MagSep Blood gDNA Kit
- 2 mL DNA LoBind Tubes (included in kit)

Methods

If not stated otherwise, 200 µL fresh EDTA or citrate blood were transferred into Eppendorf 2 mL DNA LoBind Tubes (provided with the kit) and placed in an Eppendorf PrepRack on the worktable of the Eppendorf ep*Motion* M5073. All blood replicates were transferred in dispense mode with the Multipette Xstream using Combitips advanced. In the Prep-Assistant it can also be selected to let the epMotion pipet blood directly from a rack with sample vials.

The purification run setup was performed using the Prep-Assistant (Figure 2) for the MagSep Blood gDNA Kit and the entire purification process was performed automatically by the ep*Motion* M5073. Final elution of genomic DNA was carried out in 55 µL; subsequently the final eluates were automatically transferred into fresh 2 mL DNA LoBind Tubes, without any detectable carry-over of magnetic beads.

In addition, the automated purification of gDNA from blood samples was also compared to two widely used competitor kits based on silica column technology, following the manufacturer's instructions. The elution volumes for the silica column kits were 200 µL to allow optimal elution efficiency, according to the individual manuals.

The yield and quality of the obtained DNA were evaluated by measuring 3 µL of the eluates using the Eppendorf µCuvette G1.0 with the Eppendorf BioSpectrometer kinetic and by electrophoretically separating the eluates on agarose gels. For cross contamination experiments a real-time PCR for a 146 bp fragment of human GAPDH was performed with 2 µL of 1:10 diluted eluates in a total reaction volume of 20 µL.

Results and Discussion

Tip Usage

A purification process with 24 blood samples with re-use tips function for the wash steps (default setting) requires 54 1000 µL tips, whereas a single use would result in a total consumption of 102 tips.

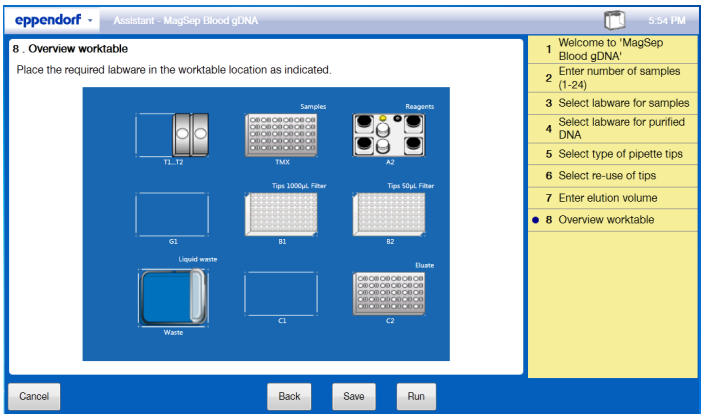

Figure 2: Screenshot from the ep*Motion* Prep-Assistant showing the setup of the ep*Motion* M5073 worktable for use with the Eppendorf MagSep Blood gDNA Kit.

Yield and Purity of gDNA from fresh whole blood

Depending on the donor, DNA yields from 200 µL fresh, whole EDTA blood were in the range of 9.2 – 11.3 µg, reduced blood volumes of 50 µL resulted in average yields of 1.9 – 2.6 µg (Figure 3). DNA purity was high, as indicated by A260/280 ratios between 1.83 – 1.93. A detailed overview of the results is given in Table 1.

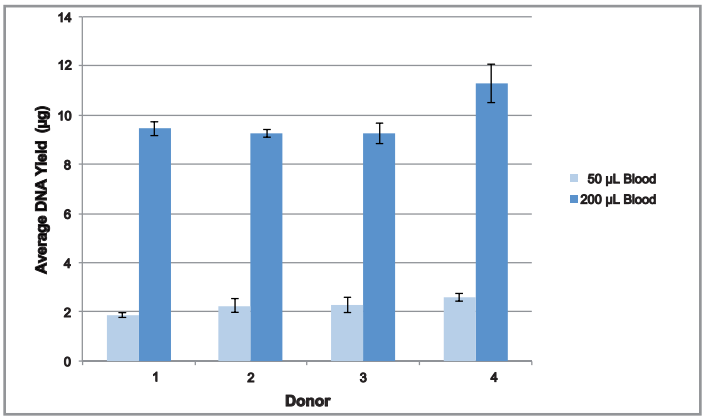

Figure 3: High DNA yields
Genomic DNA was purified automatically on the ep*Motion* M5073 from 50 and 200 µL fresh, whole blood from four donors. DNA yields obtained by the automated method were in the range of 8.5 – 11.3 µg for 200 µL blood. Each bar represents the average yield with SD for 6 replicates in case of 200 µL and 3 replicates for 50 µL (filled up with 150 µL PBS).

Table 1: Yield and Purity of DNA

	Mean DNA Yield				Mean DNA Purity			
	50 µL (n=3)		200 µL (n=6)		50 µL (n=3)		200 µL (n=6)	
	[µg]	%CV	[µg]	%CV	(A260/280)	%CV	(A260/280)	%CV
Donor 1	1.9	4.8	9.5	3.1	1.89	3.29	1.87	0.34
Donor 2	2.3	12.0	9.2	1.6	1.89	1.52	1.91	0.94
Donor 3	2.3	14.1	9.2	4.4	1.83	0.45	1.92	0.40
Donor 4	2.6	5.6	11.3	6.9	1.93	0.24	1.91	0.40

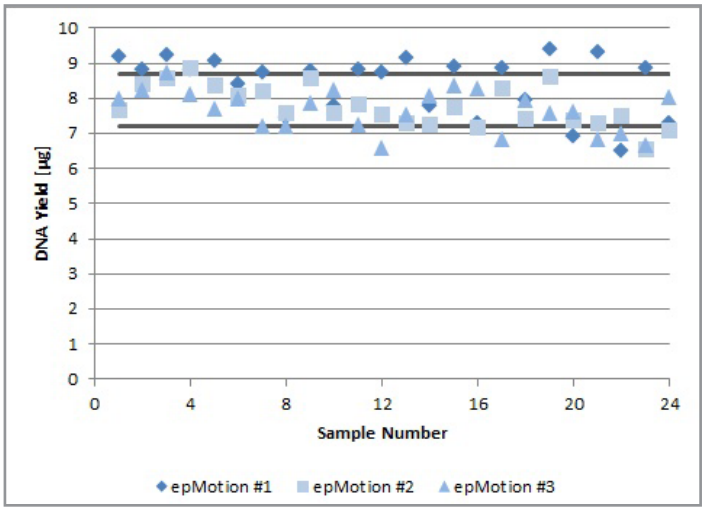

Figure 4a: Reproducible DNA yields
On three ep*Motion* M5073 genomic DNA was purified from 24 200 µL blood replicates each. The overall average yield was 7.95 µg gDNA with a low CV value of 9.34 % showing a high level of consistency between individual instruments. Each symbol type represents the DNA yield from one instrument; grey horizontal bars denote the average yield +/- 1 SD, respectively.

Reproducibility

DNA from one donor (7.9 x 10^6 white blood cells/mL) was isolated on three different ep*Motion* M5073 in 24 replicates each and showed yields from 6.5 – 9.4 µg, with an overall average yield of 7.95 µg and a CV of 9.3 %, giving evidence for a high reproducibility of the automated method (Figure 4a & Table 2). The obtained genomic DNA was of a high molecular weight as indicated by the distinct band and the absence of low molecular weight smear in gel electrophoresis (Figure 4b).

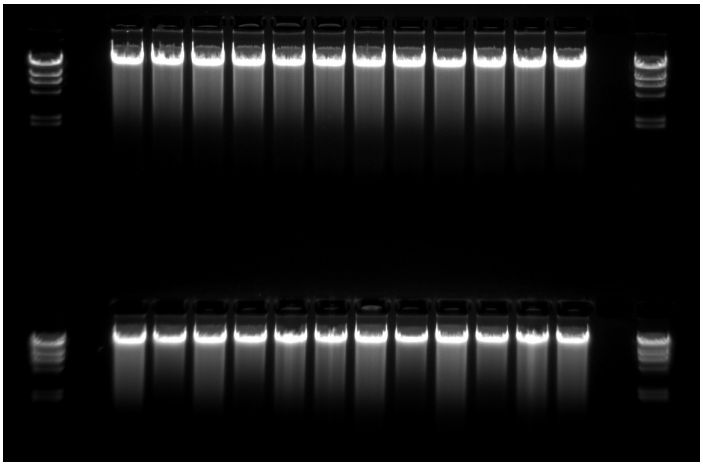

Figure 4b: High quality genomic DNA
From a purification run with 24 replicates, 5 µL out of 50 µL gDNA eluates were separated electrophoretically on a 1 % agarose gel. The obtained DNA displayed a high molecular weight as indicated by the absence of low molecular weight smear, thus being of a high quality and integrity. DNA size standard: Lambda *HindIII* (Fermentas).

Table 2: Yield and purity of DNA from 3 different ep*Motion* M5073

	Mean DNA Yield (n=24)		Mean DNA Purity (n=24)	
	[µg]	%CV	(A260/280)	%CV
epMotion #1	8.4	9.6	1.88	1.12
epMotion #2	7.8	7.4	1.90	1.13
epMotion #3	7.7	7.6	1.86	1.03

Performance comparison

The isolation performance of the automated Eppendorf MagSep Blood gDNA Kit was compared to two widely used competitor silica column-based kits, dedicated for the purification of genomic DNA from 200 µL whole blood samples. Figure 5 clearly shows that the automated method yielded up to twice as much DNA with purities slightly better than obtained with silica column methods. While the reproducibility of the manual procedure is already high (CV values of the average yields 2.5 – 10.2 %), the automated purification process results in even more consistent yields (CV 1.0 – 3.4 %).

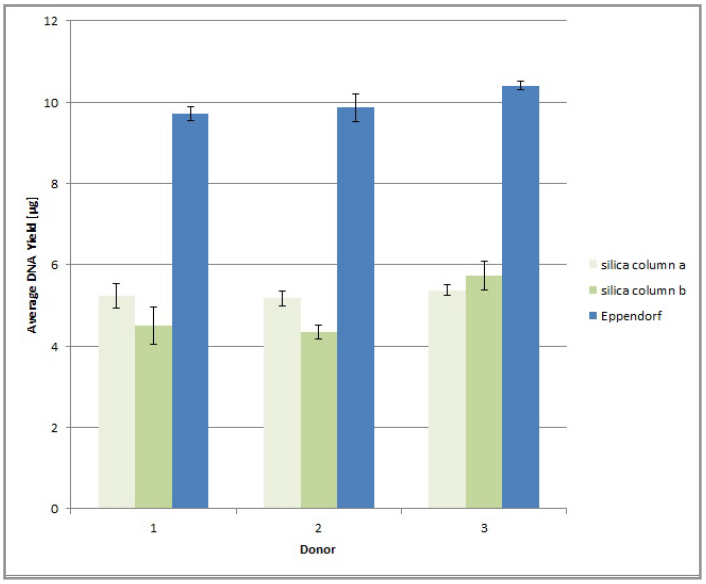

Figure 5: DNA yields obtained with different methods
Genomic DNA was isolated with different methods from 200 µL fresh, whole blood samples from three donors. For each method and sample 5 replicates were processed in parallel. The automated Eppendorf MagSep Blood gDNA Kit was compared to two manual silica column-based kits. DNA yields obtained by the automated method were considerably higher than yields from silica column based methods. Bars represent average yields with SD (n=5).

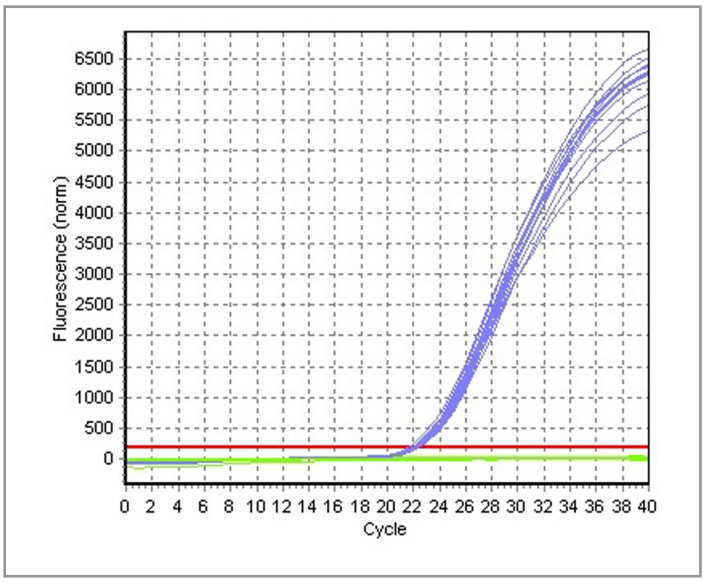

Figure 6a: Cross contamination analysis
Real-time PCR analysis of eluates from blood and PBS purified in a checkerboard pattern. PCR products were only detectable from eluates that originated from blood filled positions. All PBS filled positions gave a negative result. Blue graphs denote blood eluates, green graphs PBS eluates and the threshold is shown in red.

Table 3: Mean yields and purities of genomic DNA from three donors, purified with three methods
For each method and donor, five blood samples of 200 µL were processed.

	Donor 1				Donor 2				Donor 3			
	[µg]	%CV	(A260/280)	%CV	[µg]	%CV	(A260/280)	%CV	[µg]	%CV	(A260/280)	%CV
Silica 1	5.2	5.6	1.84	1.76	5.2	3.6	1.79	1.50	5.4	2.5	1.83	1.38
Silica 2	4.5	10.2	1.69	6.10	4.3	3.7	1.71	1.65	5.7	6.1	1.74	1.29
MagSep Blood gDNA	9.7	1.8	1.86	0.23	9.9	3.4	1.87	0.15	10.4	1.0	1.86	0.80

Cross contamination analysis

The ep*Motion* M5073 comes with a new feature that allows re-using tips to reduce cost and waste plus freeing up worktable space. To evaluate the risk of cross contamination when re-using tips during a purification process, 12 blood and 12 PBS buffer samples were processed in a checkerboard pattern and the eluates were subjected to real-time PCR analysis. Figure 6a shows the absence of a PCR product in the positions that were initially filled with PBS buffer, whereas all blood-filled positions gave a clear product. This was also verified by agarose gel electrophoresis of 4 µL of the PCR products (Figure 6b).

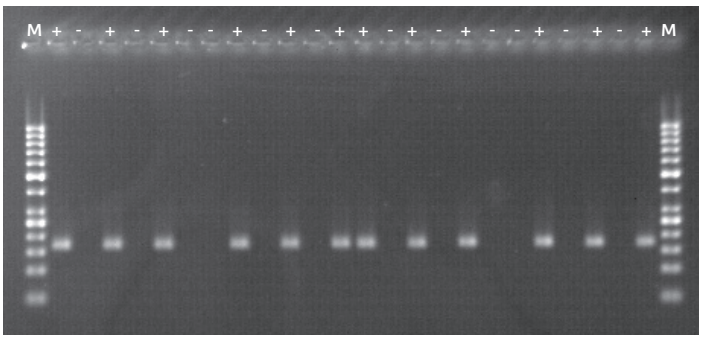

Figure 6b: Cross contamination analysis
Blood and PBS buffer were purified in a checkerboard pattern and resulting eluates were analysed by real-time PCR. Only the eluates from blood samples gave a 146 bp human GAPDH product. DNA size standard (M): 50 bp ladder (Fermentas), + denotes blood sample, - denotes PBS.

Conclusion

Automation is regarded as ideal approach for everyday laboratory routine – especially when highly reproducible and high quality results are required. This study clearly shows that the Eppendorf MagSep Blood gDNA Kit in combination with the Eppendorf ep*Motion* M5073 reliably delivers high yields of high quality genomic DNA from up to 200 µL human whole blood. No cross contamination is observed during the process and the obtained DNA is compatible with downstream applications such as real-time PCR. The combination of kit and pipetting instrument can be regarded as an ideal system, delivering the highest possible degree of automation with hands on time reduced to a minimum.

Overall tip consumption is safely reduced by the implementation of the Eppendorf SafeRack along with the re-use tip function, having a direct impact on the cost per sample.

Due to the unique flexibility and usability of the system – 1 to 24 samples can be processed; reagents are supplied ready to use in a tray and recappable, assistant guided run setup - the ep*Motion* M5073 and the Eppendorf MagSep Blood gDNA Kit form an attractive automation bundle for low and medium throughput DNA purification requirements in an everyday routine.

Ordering information

Description	International Order no.	North America Order no.
Eppendorf ep*Motion*® M5073 for automated nucleic acid preparation of 1–24 samples with Eppendorf MagSep Kits, 100–240 V/50–60 Hz, with Control-Panel, Software epBlue and Prep-Assistants, TS 50, TS 1000, PrepRack, ReagentRack, Rack 24 Eppendorf Safe-Lock Tubes	5073 000.205	5073 000.205
MagSep Blood gDNA Kit	0030 451.007	0030 451.007
epT.I.P.S.® Motion Filter SafeRacks 1000 µL	0030 014.650	0030 014.650
epT.I.P.S.® Motion Filter SafeRacks 50 µL	0030 014.618	0030 014.618
Eppendorf BioSpectrometer® kinetic	6136 000.002	6136000010
Eppendorf µCuvette G1.0	6138 000.018	6138000018
Multipette Xstream®	4986 000.025	022460811
2 mL DNA LoBind® Tubes	0030 108.078	022431048
10 mL Combitips advanced®	0030 089.464	0030089464

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