Applications

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Genomic DNA isolation of variable sample numbers using the MACHEREY-NAGEL NucleoSpin® 8 Blood kit on the ep*Motion*® 5075 from Eppendorf

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Abstract

The automated vacuum processing of variable sample numbers in DNA purification is often inconvenient since most of the commercially available kits include 96-well filterplates. When lower numbers than 96 samples are frequently processed the 96-well filterplates may be reused several times, however the risk of introducing contamination or DNase increases. Here we present a convenient way of automated processing of samples in multiples of eight: the integration of the MACHEREY-NAGEL NucleoSpin® 8 Blood kit into the automated liquid handling system ep*Motion*® 5075 VAC. The procedure is very easy to perform, user friendly and yields DNA suitable for various downstream applications, e.g. for PCR analysis. In contrast to other procedures, heat incubation steps for lysis or elution are not required. Fresh or frozen human blood samples were used as starting material. Up to 48 samples can easily be processed in approximately 90 minutes (including sample transfer from primary blood tubes) or 75 min (with samples supplied in 96-well plate).

Introduction

Analysis of DNA with PCR-based downstream applications, e.g. genotyping, has become more important over the last years. DNA purification of increasing numbers of samples represents a bottleneck in sample processing. Many laboratories changing from manual to automated processing of samples wish to have flexible method scripts to adapt variable sample numbers. With the integration of the MACHEREY-NAGEL NucleoSpin 8 Blood kit into the automated liquid handling system ep*Motion* 5075 VAC an easy to use, flexible and fully automated DNA isolation method was developed. The vacuum based silica filter method of the NucleoSpin 8 Blood kit replaces time consuming and tedious methods involving salt precipitation steps or methods using toxic substances such as phenol/chloroform.

The NucleoSpin 8 Blood kit is designed for low to medium throughput using a flexible system of 8-well silica filter strips. In contrast to 96-well plate based systems sealing of unused plate positions is not required. Use of 8-well filter strips avoid the reuse of partially used 96-well filter plates, a source of potential contamination. The NucleoSpin Blood procedure allows complete sample processing at room temperature including the sample lysis step. Therefore, heat incubations which usually interrupt the automated processing can be avoided. The NucleoSpin Blood procedure is applicable for fresh or frozen samples of human or animal blood. The purified DNA is of high quality and can be used in any downstream application including: PCR, real-time PCR, genotyping, HLA typing.

Materials and Methods

Eppendorf ep*Motion* 5075 VAC
Collection Plate Adapter for MN Tube Strips
Channeling Plate
Vac Frame 2
MACHEREY-NAGEL NucleoSpin 8 Blood kit
MACHEREY-NAGEL Starter Set A
Blood samples

Product use limitations and safety information

Please read the MACHEREY-NAGEL NucleoSpin 8 Blood Kit manual before performing the method for the first time.

Blood samples

Blood samples were taken from healthy donors. Samples were collected using Sarstedt Monovette® EDTA KE/9ml with EDTA as anticoagulant. Samples were stored at 4 °C for up to 5 days or frozen at -20 °C for long time storage. Before starting the DNA extraction samples were incubated for 10 min at room temperature with moderate agitation.

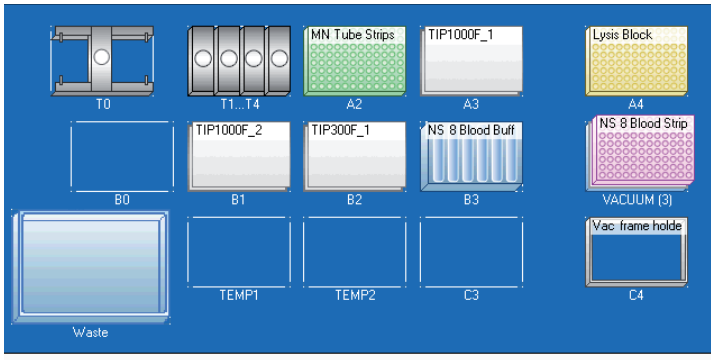

Figure 1: Screenshoot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 Vac worktable for use with the NucleoSpin 8 Blood kit

Table 1: ep*Motion* 5075 VAC worktable details for the NucleoSpin 8 Blood protocol.

Position	Labware	Comment
A2	MN Tube Strips (MN_TP_1200_48)	elution tubes* (***)
A3	epT.I.P.S Motion Filter 1000 µL	
A4	Blood sample lysis block (MN_DWP_2000LB_deep)	96-well plate with blood samples
B1	epT.I.P.S Motion Filter 1000 µL	
B2	epT.I.P.S Motion Filter 300 µL	for addition of proteinase K
B3	Reagent Reservoirs Position 1: Buffer BE Position 2: Buffer BQ1 Position 3: 96 % Ethanol Position 4: Buffer B5 Position 5: empty Position 6: Buffer BW Position 7: proteinase K	 30 mL reservoir 30 mL reservoir 30 mL reservoir 100 mL reservoir empty 100 mL reservoir 30 mL reservoir
Vacuum	NucleoSpin Blood Binding Strips (MN_FP_8_1400) in Column Holder A** Vacuum Frame 2 Reservoir 400 mL with channeling plate	DNA binding strips collar for vacuum manifold collects waste
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting tool
T2	TM 300-8	300 µL 8-channel pipetting tool

*) require Collection Plate Adapter for MN tube strips, see ordering information
**) 8-well strips are inserted into MACHEREY-NAGEL Column Holder A which is part of the Starter Set A, see ordering information
***) 96 well MTP can be used optionally

Table 2A: Reaction conditions for beta-actin PCR

Component	Volume
Water	28.75 µL
dNTP mix (10 mM each), Invitrogen	1.0 µL
primer forward (10 pmol/µL)	1.0 µL
primer reverse (10 pmol/µL)	1.0 µL
reaction buffer (10fold conc.)	4.0 µL
$MgCl_2$ (50 mM), Invitrogen	2.0 µL
Taq polymerase (5U/µl) Invitrogen	0.25 µL
Template DNA	2 µL

Table 2B: PCR program for beta-actin PCR

Step	Temp	Duration	Cycles
Intitial template denaturation	95 °C	5 min	1 x
Denaturation Primer annealing Elongation	95 °C 62 °C 72 °C	30 sec 20 sec 20 sec	35 x
Final elongation	72 °C	5 min	1 x
Cooling	4 °C		

Results

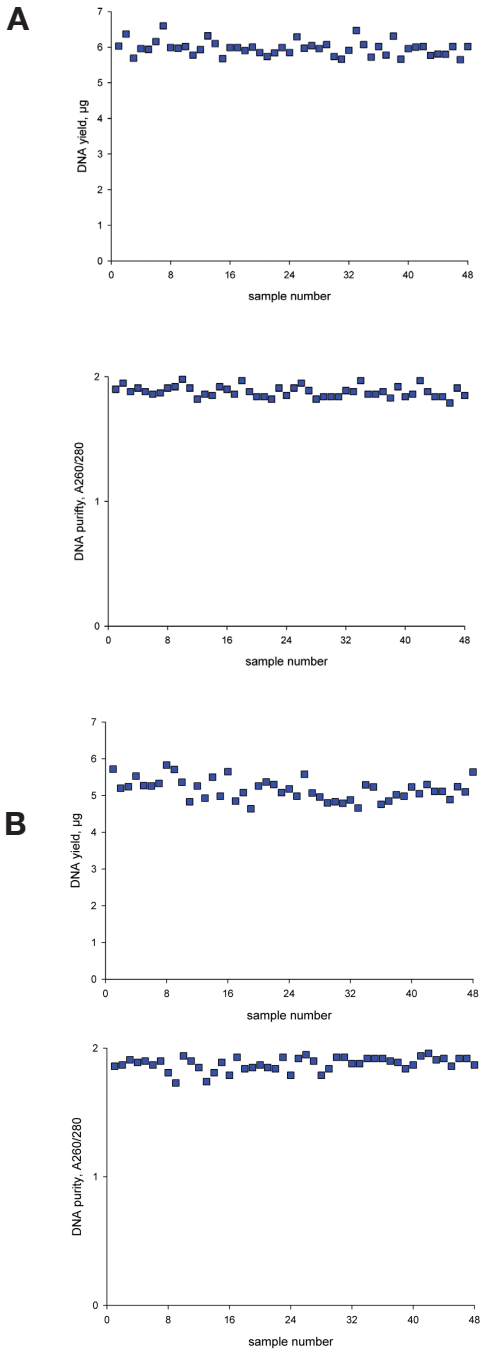

Figure 2: Reproducibility of DNA purification
DNA was isolated from a pool of human blood samples stored at 4 °C (A) or -20 °C (B), respectively. 200 µL of the human blood samples were used for DNA purification. DNA yield and purity was determined by UV measurement.

Yield and purity of DNA

Yield of DNA may depend on the sample donor (leukocyte cell number), storage condition of the blood sample and storage time of the sample. Typically, from a 200 µL sample a DNA yield of approx. 4-6 µg can be expected. The average yield and purity, standard deviation, minimum and maximum yield and purity are summarized in table 3.

Table 3: Yield and purity of DNA

	DNA yield (µg)		DNA purity (A260/280)	
Blood samples stored at	4 °C	-20 °C	4 °C	-20 °C
average yield / purity	5.97 µg	5.16 µg	1.88	1.88
standard deviation	0.21 µg	0.29 µg	0.04	0.05
min. yield / purity	5.65 µg	4.64 µg	1.79	1.73
max. yield / purity	6.60 µg	5.83 µg	1.98	1.96

Quality of DNA

High molecular weight DNA was obtained as shown by analysis of purified samples by agarose gel electrophoresis. A distinct high molecular weight band was obtained. No degradation of DNA was observed (figure 3).

Figure 3: Agarose gel analysis. DNA was isolated from a pool of human blood samples stored at either 4 °C or -20 °C. 200 µL of human blood was used for DNA purification for each sample. DNA was eluted in two steps with 100 µL elution buffer in each step. Recovered volume of DNA was approx. 170 µL. 10 µL each of 19 eluted DNA samples were loaded on a 1% TAE-agarose gel (ethidium bromide stain).
Left panel: DNA isolated from blood samples stored at 4 °C, right panel: DNA isolated from blood samples stored at -20 °C. The size marker is Lambda/*Hind* III (MBI Fermentas).

Cross contamination analysis

No PCR fragment was obtained in the wells which were initially pre-filled with PBS buffer indicating the absence of cross contamination. All DNA samples obtained from blood samples showed the expected 200 bp PCR fragment (figure 4).

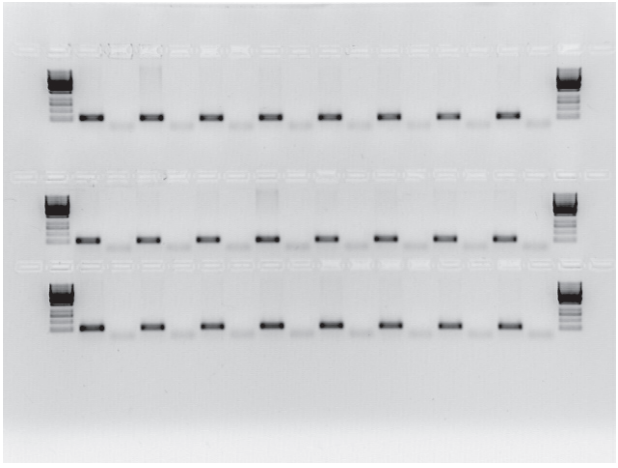

Fig. 4: Cross contamination analysis. Human blood samples and PBS buffer were loaded in a checker board pattern into the sample lysis plate. DNA isolation was performed with the NucleoSpin 8 Blood protocol. Following elution step 2 µL of all eluted samples were analyzed with a 35 cycle PCR for presence of a PCR product. The size marker is a 100 bp ladder (MBI Fermentas).

Conclusion

The integration of the MACHEREY-NAGEL NucleoSpin 8 Blood kit into the ep*Motion* 5075 VAC resulted in a complete system for automated purification of high quality total DNA from blood samples. The flexible method is designed for low or medium throughput and allows processing of 8 to 48 samples (in multiples of eight samples) in one run. All steps including transfer of blood samples from sample tubes are included in this method. The procedure is very easy to perform, user friendly and yields DNA suitable for various downstream application, e.g. for PCR analysis. Furthermore, in contrast to many other comparable procedures, heat incubation steps for lysis or elution are not required. From samples of 200 µL human blood an average yield of 5.97 µg with an average A260/280 ratio of 1.88 was obtained. The DNA was of high molecular weight and the absence of cross-contamination was clearly demonstrated by PCR analysis. For higher throughput needs the ep*Motion* 5075 VAC can also be equipped with the Nucleospin 96 Blood kit and can then easily process one 96-well plate pre-loaded with blood samples in approximately 90 minutes [1]. Taken together the NucleoSpin Technology and the ep*Motion* 5075 VAC form an attractive and versatile system for the automated isolation of genomic DNA from blood samples in every throughput range.

References

[1] Eppendorf Application Note 161 (www.eppendorf.com)
Operating Manual Eppendorf ep*Motion* 5075
User Manual MACHEREY-NAGEL NucleoSpin 8 Blood kit

Eppendorf Ordering Information

Product	Order no. International	Order no. North America
Collection Plate Adapter MN	5075 785.064	960002571
Channeling Plate	5075 794.004	960002540
Vac Frame 2	5075 785.005	960002261
ep*Motion*® 5075 VAC 230 V (vacuum chamber included)	5075 000.164	n/a
ep*Motion*® 5075 VAC 120 V (vacuum chamber included)	n/a	960020014
Dispensing tool TM 1000-8	5280 000.258	960001061
Dispensing tool TM 300-8	5280 000.231	960001052
Reservoir Rack	5075 754.002	960002148
Reservoirs 100 ml (10 x 5 reservoirs in bags/case, PCR clean)	0030 126.513	960051017
Reservoirs 30 ml (10 x 5 reservoirs in bags/case, PCR clean)	0030 003.993	960050100

Macherey-Nagel Ordering Information

Product	Order no.
NucleoSpin® 8 Blood kit	
12x8 preps	740 664
60x8 preps	740 665.5
Starter Set A	740 682

NucleoSpin® is a trademark of MACHERY-NAGEL GmbH & Co. KG, Dueren, Germany
Monovette is a registered trademark of Sarstedt AG, Nümbrecht, Germany

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