Applications

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High throughput DNA isolation using the MACHEREY-NAGEL NucleoSpin® 96 Blood kit on the ep*Motion*® 5075 from Eppendorf

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Abstract

The DNA purification of hundreds of blood samples often represents a bottleneck in sample processing for genotyping or general screening projects. Automated DNA isolation can help to streamline this process. Here, we present the integration of the MACHEREY-NAGEL NucleoSpin® 96 Blood kit into the ep*Motion*® 5075 VAC automated pipetting system. 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 kit procedures, heat incubation steps for lysis or elution are not required. Fresh human blood samples were used as starting material. 96 samples could easily be processed in approximately 90 minutes. The absence of cross-contamination was demonstrated using a checkerboard PCR assay.

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 frequently represents a bottleneck in sample processing. With the integration of the MACHEREY-NAGEL NucleoSpin 96 Blood kit into the ep*Motion* 5075 VAC automated pipetting system an easy to use, fully automated DNA isolation protocol was developed. The vacuum based silica filter method of the NucleoSpin 96 Blood kit replaces time consuming and tedious methods involving salt precipitation steps or methods using toxic substances such as phenol/chloroform. 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 many downstream applications including PCR, real-time PCR, genotyping, and HLA typing.

Materials and Methods

- Eppendorf ep*Motion* 5075 VAC [1]
- Reservoir 400 mL
- Collection Plate Adapter for MN Tube Strips
- Channeling Plate
- Vac Frame 2
- MACHEREY-NAGEL NucleoSpin 96 Blood kit
- Blood samples
- Reservoir Rack with Reagent Reservoirs
- Vac Holder

Product use limitations and safety information

Please read the MACHEREY-NAGEL NucleoSpin 96 Blood Kit manual [2] before performing the method for the first time.

Blood samples

Blood samples were taken from healthy donors. Samples were collected using Sarstedt S-Monovette® with EDTA as anticoagulant. Samples were stored at 4 °C for up to 5 days. For long term storage samples should be kept frozen at -20°C. Before starting the DNA extraction, samples were incubated for 10 min at room temperature with moderate agitation on a SRT1 tube roller mixer (Stuart Scientific).

Materials and Methods

Table 1: ep*Motion* 5075 VAC worktable details for the NucleoSpin 96 Blood protocol.

Position	Labware	Comment
A2	MN Tube Strips (MN_TP_1200)	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: Buffer B5 Position 6: Buffer BW Position 7: Proteinase K	 30 mL reservoir 30 mL reservoir 30 mL reservoir 100 mL reservoir 100 mL reservoir 100 mL reservoir 30 mL reservoir
Vacuum	NucleoSpin Blood Binding Plate (MN_FP_96_1500) Vacuum Frame 2 Reservoir 400 ml with channeling plate	DNA binding plate 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

Table 2: Reaction conditions for β-actin PCR

Component	Volume /µL
Template DNA	2
Water	30
10 X Reaction Buffer	4
50 mM Magnesiumchlorid Solution	2
dNTP Master Mix (10 mM each)	1
Forward Primer (10 pmol/µL) 5´-CTGTGGCCATCTCCTGCTC-3´	1
Reverse Primer (10 pmol/µL) 5´-TATGCCTCTGGTCGTACCAC-3´	1
***Taq* DNA Polymerase (5 U/µL)**	0,25

Step	Temperature	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	Hold	

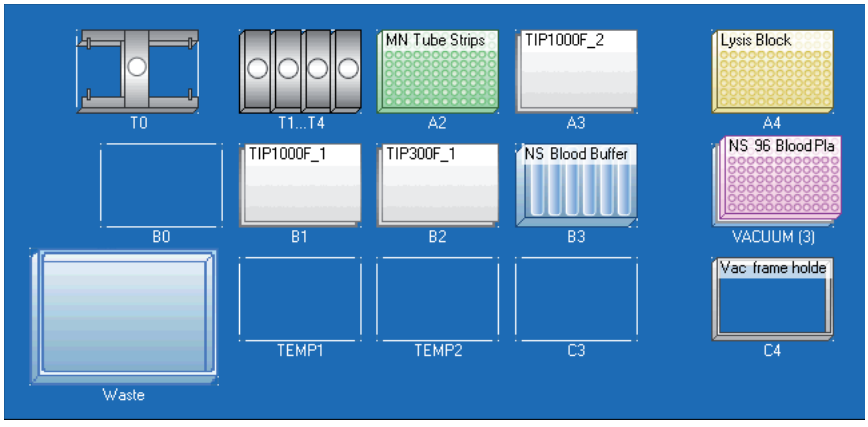

Figure 1: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for use with the NucleoSpin 96 Blood kit

Results

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. 6 µg can be expected (Fig. 2). The average yield and purity, standard deviation, minimum and maximum yield and purity are summarized in table 3.

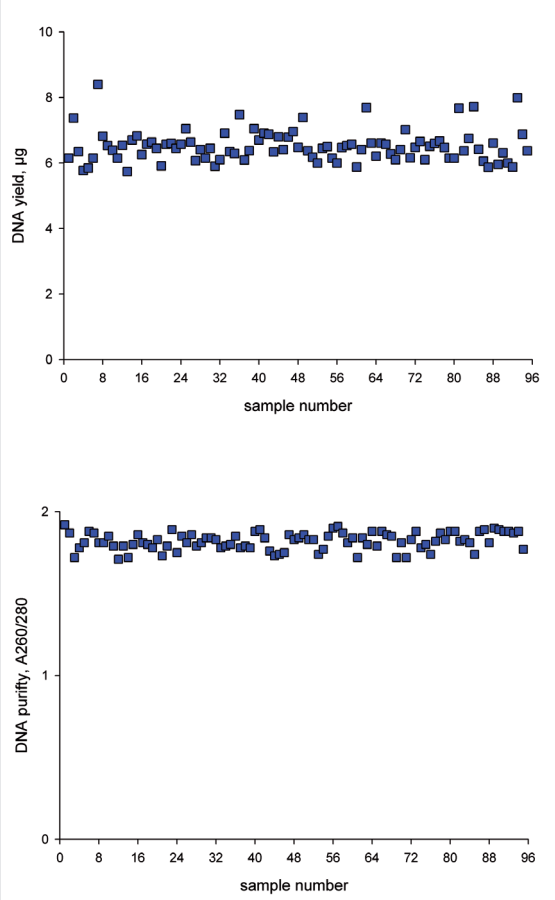

Figure 2: Reproducibility of DNA purification.
gDNA was isolated from a pool of human blood samples stored at 4 °C. 200 µL of human blood were used for DNA purification. DNA yield and purity were determined by UV measurement

Table 3: Yield and purity of DNA

	DNA yield (µg)	DNA purity (A260/280)
average yield / purity	6.51 µg	1.82
standard deviation	0.48 µg	0.05
min. yield / purity	5.74 µg	1.71
max. yield / purity	8.40 µg	1.92

Figure 3: Agarose gel analysis
DNA was isolated from a pool of human blood samples stored at 4 °C. 200 µL of human blood were used for DNA purification. DNA was eluted in two steps with 100 µL elution buffer 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). A λ/Hind III size marker (MBI Fermentas) was used as a standard.

Quality of DNA (Fig. 3)
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.

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

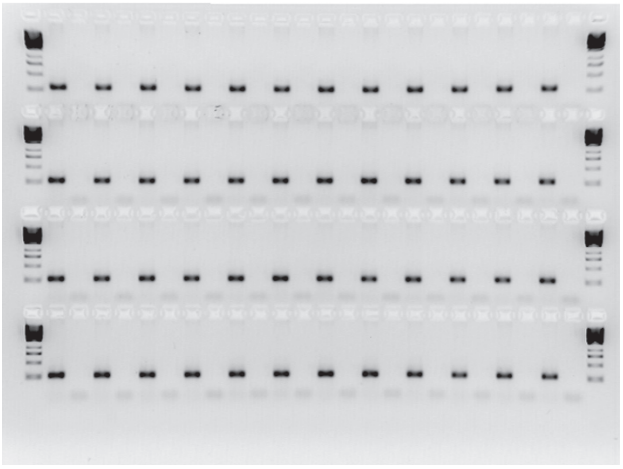

Figure 4: Cross contamination analysis
Human blood samples and PBS buffer were loaded in a checker board pattern into the sample lysis plate. DNA isolation was performed with the NucleoSpin 96 Blood protocol. Following the elution step, 2 µL of all eluted samples were subjected to a 35 cycle PCR and analyzed for presence of a PCR product. A 100 bp ladder (MBI Fermentas) was used as a size marker.

Conclusion

The integration of the MACHEREY-NAGEL NucleoSpin 96 Blood kit into the epMotion 5075 VAC resulted in a complete system for automated purification of high quality total genomic DNA from blood samples. The procedure is very easy to perform, user friendly and yields DNA suitable for various downstream applications, e.g. for PCR analysis. Furthermore, in contrast to many other comparable procedures, heat incubation steps for lysis or elution are not required. Therefore, no thermal modules inside the epMotion are needed.

From samples of 200 µL human blood, an average yield of 6.51 µg with an average A260/280 ratio of 1.82 was obtained. The DNA was of high molecular weight and the absence of cross-contamination was clearly demonstrated by PCR analysis. The epMotion 5075 VAC can easily process one 96-well plate pre-loaded with blood samples in approximately 90 minutes. For lower throughput needs, MACHEREY-NAGEL offers the NucleoSpin 8 Blood kit, where 8 to 48 samples can be processed in individual and convenient 8-well filterstrips. This protocol can also be used on the epMotion 5075 VAC and will be described in a separate application note. Taken together, the NucleoSpin Technology and the epMotion 5075 VAC automated pipetting system form an attractive and versatile system for the automated isolation of genomic DNA from blood samples.

References

[1] Eppendorf, Operating manual epMotion 5075
[2] MACHEREY-NAGEL, NucleoSpin 96 Blood kit user manual

Eppendorf Ordering information

Description	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

Ordering information MACHEREY-NAGEL

Description	Order no.
NucleoSpin® 96 Blood kit	
1x96 preps	740 665.1
4x96 preps	740 665.4
24x96 preps	740 665.24

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S-Monovette® is a registered trademark of Sarstedt AG & Co., Nürmbrecht, Germany

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