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Automated Purification of Genomic DNA from Blood with the Macherey-Nagel NucleoMag® Blood 200 µL Kit on the ep*Motion*® 5075

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Abstract

The purification of genomic DNA from whole blood samples is routinely performed in a variety of research laboratories with a growing demand for automation. Automation significantly facilitates the purification, especially for potentially hazardous sample material such as blood.

The NucleoMag 96 Blood Kit from Macherey-Nagel is adapted to the ep*Motion* 5075t/m. The combination of ep*Motion* 5075t/m and the purification kit, allows a walk away purification of DNA from 96 fresh or frozen, EDTA or citrate treated blood samples in less than 100 minutes.

Introduction

The procedure of the NucleoMag 96 Blood kit is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions.
Whole blood, treated with EDTA or citrate is lysed with lysis buffer MBL1 and Proteinase K. After the lysis step magnetic beads and binding buffer MBL2 are added. The contaminants are removed through two washing steps with wash buffer MBL3. Salts are then removed with additional wash step with 80% ethanol. Which replaces the MBL4 wash step. The purified DNA is eluted and can be used directly as a template for qPCR, next generation sequencing, or any kind of enzymatic reactions.
This application note describes the configuration and preparation of the ep*Motion* 5075m/t to automate this kit.

Materials and Methods

Required Labware
Eppendorf ep*Motion* 5075t or 5075m
Dispensing Tool TM 1000-8
Dispensing Tool TM 300-8
Reservoir Rack
Reservoirs 30 mL/ Reservoirs 100 mL
Reservoir 400 mL
NucleoMag Sep (Magnetic separator)
NucleoMag 96 Blood 200 µL Kit

Required Consumables
epT.I.P.S® Motion 1000 µL with filter
epT.I.P.S Motion 300 µL with filter

Samples
Human fresh and frozen blood

Method
This protocol is developed to process up to 96 samples in parallel on the ep*Motion* 5075m or 5075t workstation. This kit is suitable for up to 200 µL blood. The blood samples can be used fresh or frozen, treated with EDTA or citrate.

It is possible to purify human blood and also blood from animals, like pigs and bovines. 200 µL blood are prefilled into each well of the separation plate. All subsequent steps are automated and will be carried out in this plate. This includes dispensing of buffers and beads, removal of the supernatants as well as transport and mixing steps. Whole blood is lysed with lysis buffer MBL1 and Proteinase K. After the lysis step magnetic beads and binding buffer are added. During a mixing and incubation step the DNA is bound to the magnetic beads. Beads are separated on a magnetic plate adapter and the supernatant is removed. Unspecifically bound contaminants are removed through several washing steps with wash buffer MBL3. Remaining salts are removed with additional wash step with 80% ethanol, which replaces the MBL4 buffer. After the last washing step residual ethanol is removed in a drying step of 15 minutes at 70°C. In a last step, the eluate will be transferred to a dedicated elution plate.

A purification process with 96 blood samples with re-use tips for the wash steps requires 240x 1000µL tips and 104x 300 µL tips.

For the method the following positions of the worktable are occupied:

Position	Labware	Comment
A2	300 µL filtertips	
A3	300 µL filtertips	
TMX	Separation Plate (Lysed samples)	
B1	1000 µL filtertips	
B2	1000 µL filtertips	
B3	1000 µL filtertips	
C2	Liquid Waste (400 mL reservoir)	
C3	NucleoMag_Sep	
C4	Reagent reservoirs	
C5	Elution Plate	

Figure 1: ReservoirRack Layout

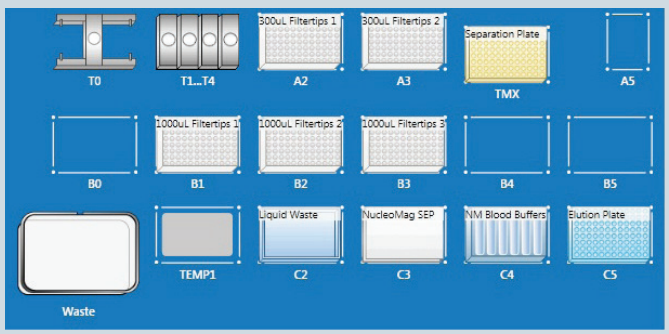


Figure 2: Worktable allocation

Results and Discussion

Purification results from blood: Genomic DNA acquired with the aforementioned method was analyzed by gel electrophoresis of 10 µL eluate via 1% TAE agarose gel; Yield and purity were determined by UV spectroscopy with Synergy HT Multi-detection microplate reader (BioTek®).

Furthermore a qPCR with SensiFast®Probe Lo-Rox Kit (Bioline®) on an Applied Biosystems® 7500 was used to check for the absence of inhibitors.

Blood Pool from one donor

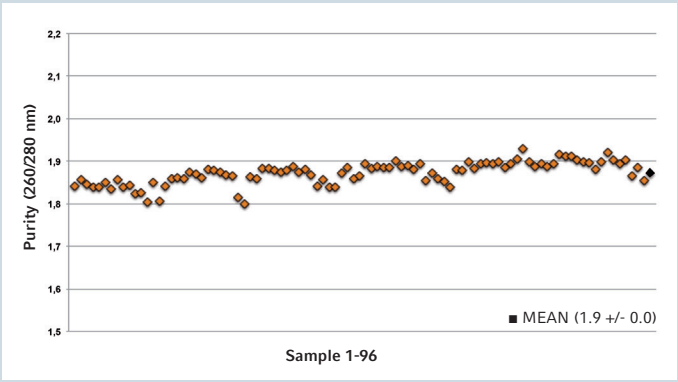


Figure 3: Purity of gDNA from 96 whole blood samples.

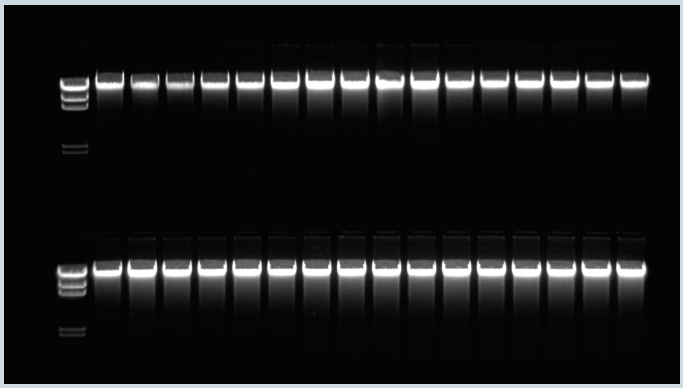


Figure 4: 32 samples randomly selected.10 µL eluate per lane of the agarose gel (1 %), running time 30 minutes at 100 V.

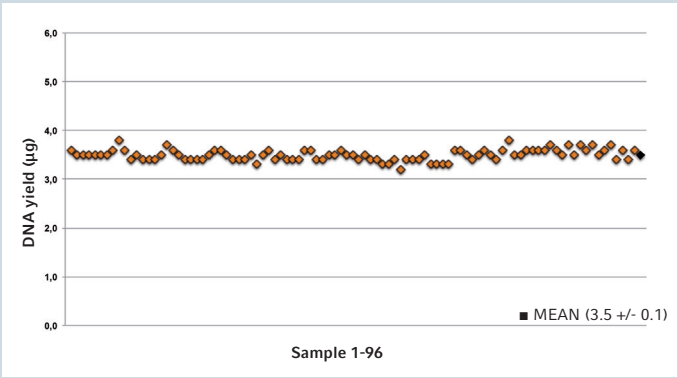


Figure 5: gDNA yields from 96 whole blood samples.

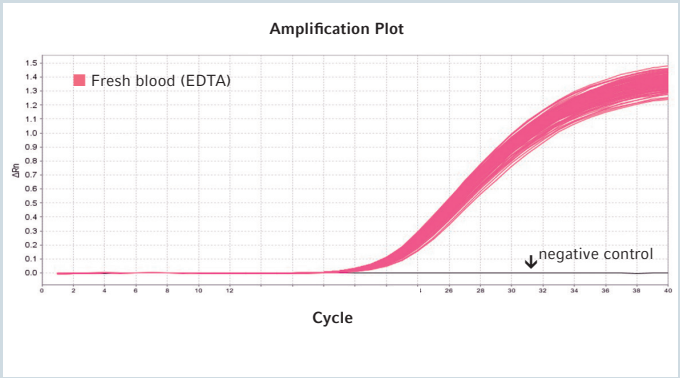


Figure 6: 4 µL of selected eluates were assayed in a quantitative PCR with a Taqman® probe for a 250 bp beta-Actin amplicon using the SensiFast Probe Lo-Rox Kit (Bioline) on an Applied Biosystems® 7500.

Individual donors

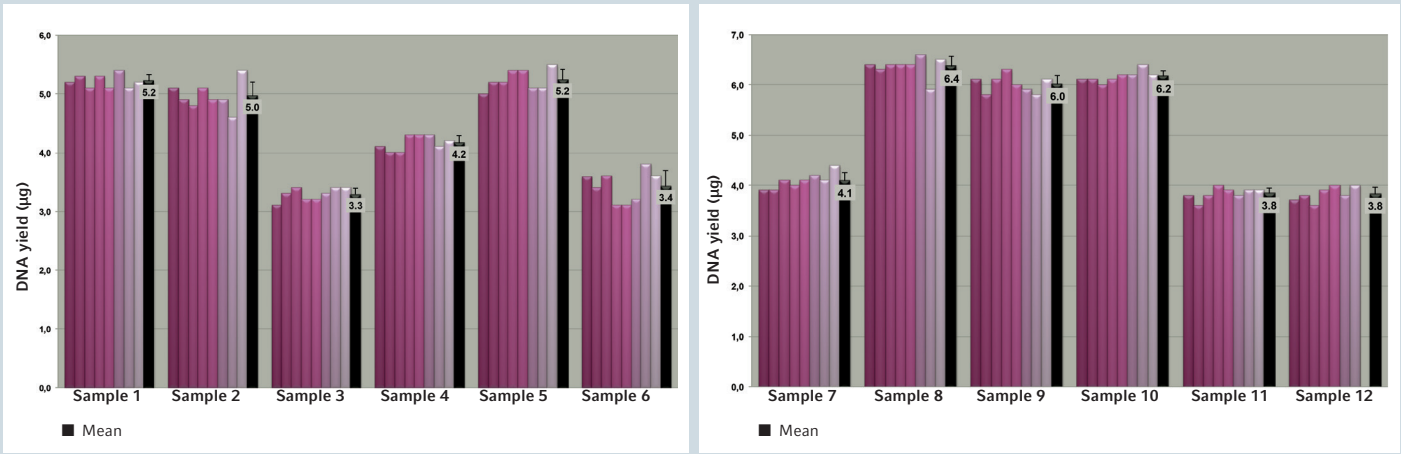


Figure 7-8: Results of 12 individual donors. Sample material was 200 µL fresh blood. DNA yield from 96 purified samples were determined with Synergy HT Multi-detection microplate reader, Biotek

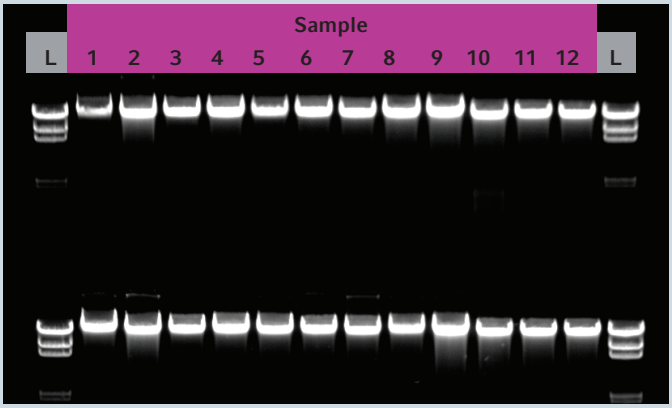


Figure 9: 2 samples randomly selected. 10 µL eluate respectively per lane of the agarose gel (1 %), running time 30 minutes at 100 V.

Cross contamination

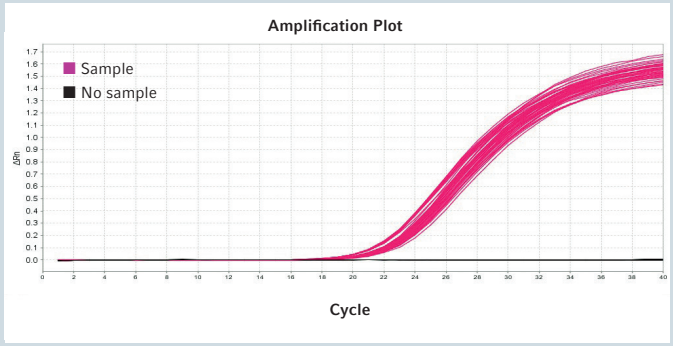
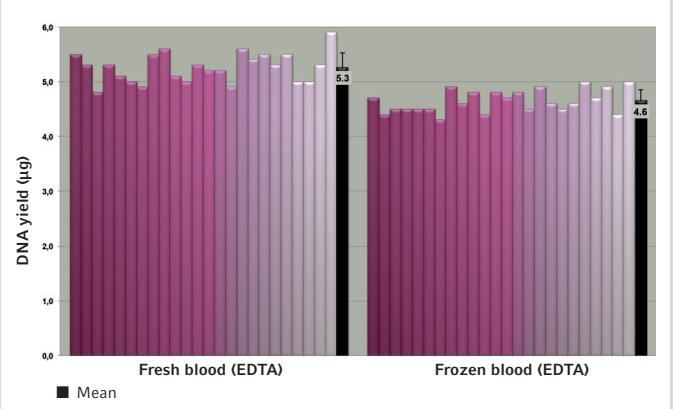


Figure 10: No PCR amplification could be observed in eluates from negative controls



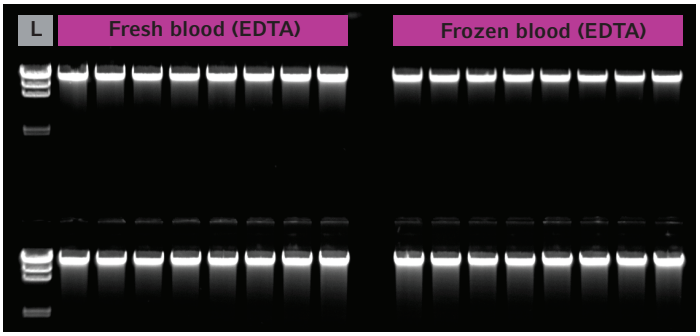


Figure 11-12: : The graphics and the gel picture show the comparison between fresh and frozen blood.

Conclusion

The above results show that the combination of the NucleoMag Blood kit and the epMotion 5075m/t reliably delivers high yields and of high quality DNA - from up to 200 µL blood. No cross contamination is detectable. The purified DNA is suitable for a full range of downstream methods.

The results from the electrophoresis analysis, qPCR, UV spectroscopy as well show the performance of the described procedure. The total time to process 96 samples is 110 minutes. The use of Eppendorf SafeRack tips along with the re-use function, have a direct impact on the cost per sample.

Ordering information

Description	Order no. international
ep*Motion*® 5075t	5075 000.302
ep*Motion*® 5075m	5075 000.305
ReservoirRack	5075 754.002
TM 1000-8 Dispensing tool	5280 000.258
TM 300-8 Dispensing tool	5280 000.231
epT.I.P.S® Motion 1000 µL SafeRack with filter	0030 014.650
epT.I.P.S® Motion 300 µL with filter	0030 014.456
Reservoir 30 mL	0030 126.505
Reservoir 100 mL	0030 126.513
Reservoir 400 mL	5075 751.364

Macherey-Nagel	
NucleoMag® 96 Blood 200 µL	REF 744501
NucleoMag® SEP	REF 744900
Square well block	REF 740481

Your local distributor: www.eppendorf.com/contact
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