

Automated gDNA Purification from whole blood samples using the NucleoMag[®] Blood 200µL kit on the epMotion[®] 5073m

Introduction

This protocol describes the automated process on the liquid handling system epMotion 5073m using the NucleoMag Blood 200µL Kit from MACHEREY-NAGEL. We show the configuration and pre-programmed method for automated genomic DNA purification from

up to 24 fresh human blood samples. The complete procedure according to the user manual of this kit including lysis and proteinase K treatment is performed automatically on the deck by the epMotion system.

Configuration and method procedure

Method name: NucleoMag_Blood200_5073m.export

This protocol is programmed to process up to 24 samples in parallel on epMotion 5073m. The NucleoMag Blood 200 µL kit is based on reversible adsorption of nucleic acids to magnetic beads under appropriate buffer conditions. The epMotion 5073m is by default equipped with a Eppendorf ThermoMixer[®] (TMX) in combination with a magnetic separator, allowing the entire process being performed without the need for labware transports. This protocol can be transferred to the bigger liquid handling system epMotion 5075m as well.

The blood samples from one blood pool are applied in volumes of 200 µL into 2.0 ml tubes in the PrepRack and placed on the TMX (Fig. 1). To maximize the efficiency of DNA recovery, it is recommended to use Eppendorf DNA LoBind tubes. The required volume of buffers (binding and wash buffers, lysis and elution buffers, 80% ethanol) should be transferred respectively to 30 mL epMotion reservoirs. Proteinase K and resuspended NucleoMag B-Beads are positioned together in the reservoir rack tubes module within a reservoir rack on position B1 as described in Fig. 2. This automated protocol follows the procedure which is recommended by MACHEREY-NAGEL.

The protocol starts by adding Proteinase K and lysis buffer to the samples, followed by an incubation of 10 min at 25°C and simultaneous mixing at 1200 rpm. Afterwards, 300 µL of Binding Buffer MBL2 and 25 µL NucleoMag B-Beads are added to the samples followed by a 5 minutes mixing step. A subsequent 2 min magnetic separation allows the complete accumulation of the NucleoMag B-Beads. After the magnet bead separation, the supernatant is removed and discarded into the liquid waste tub. The genomic DNA attached to the magnetic beads is washed twice with 800 µL of the Wash Buffer MBL3. After second time removing the supernatant, a third washing step follows, using 80% ethanol. The NucleoMag B-Beads are air dried for 7 min at 55°C while mixing at 1200 rpm to remove traces of ethanol. The genomic DNA is eluted from the NucleoMag B-Beads by adding 105 µL of Elution Buffer MBL5, followed by mixing step at 1300 rpm and 55°C for 5 min. It is possible to adjust the volume of the Elution Buffer MBL5 according to the initial sample amount, to circumvent a strong dilution or concentration of the eluted genomic DNA. After a final magnetic separation step for 2 min, 100 µL supernatant containing the purified genomic DNA is transferred into fresh tubes in the Rack 24 on the C2 position.

Worktable Layout

| Position | Item |
|----------|---|
| TMX | PrepRack with DNA LoBind tubes 2.0 mL containing 200 µL blood sample. Be aware of the direction of tube placement, starting from position 1, 2, 3.... |
| A2 | 50 µL filter tips |
| B1 | Reservoir rack with 30 mL reservoirs and DNA LoBind tubes 2.0 mL containing reagents (Fig. 2) |
| B2 | 1000 µL filter tips |
| C2 | Rack 24 with fresh LoBind tubes 2.0 mL for eluted DNA |
| Waste | Tip waste and liquid waste tub |

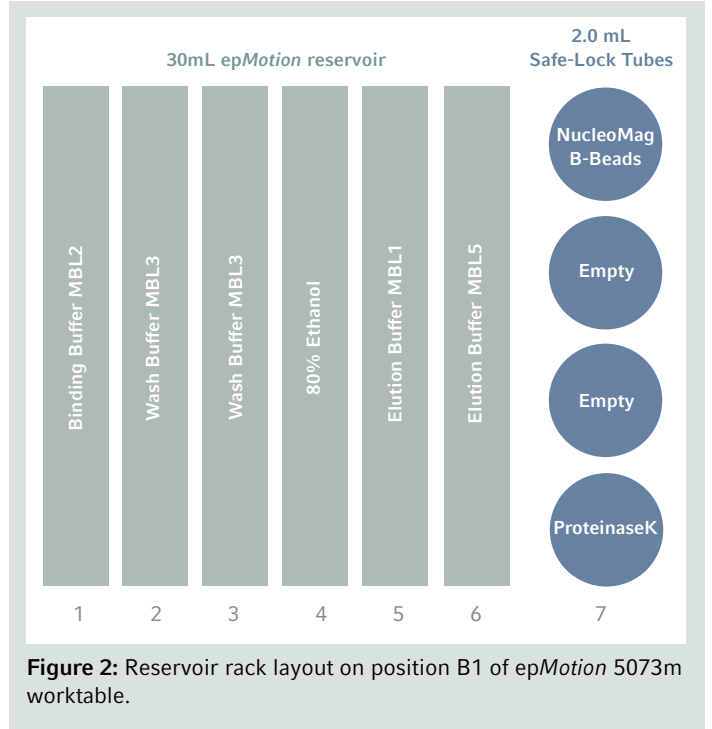


Figure 2: Reservoir rack layout on position B1 of epMotion 5073m worktable.

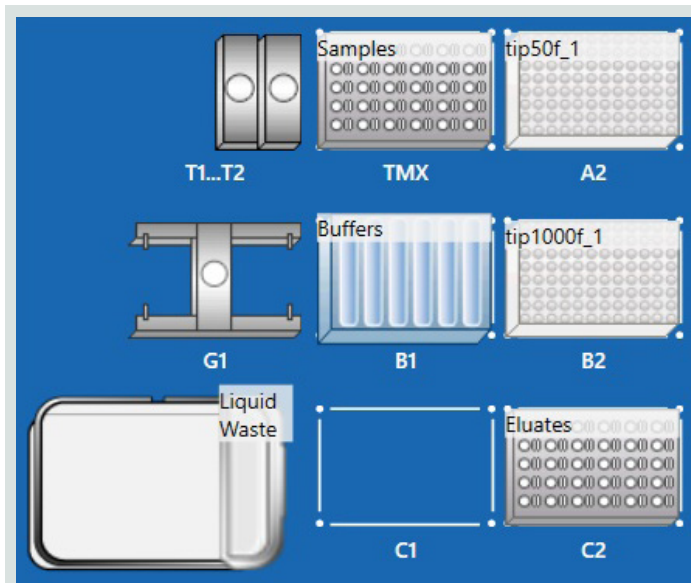
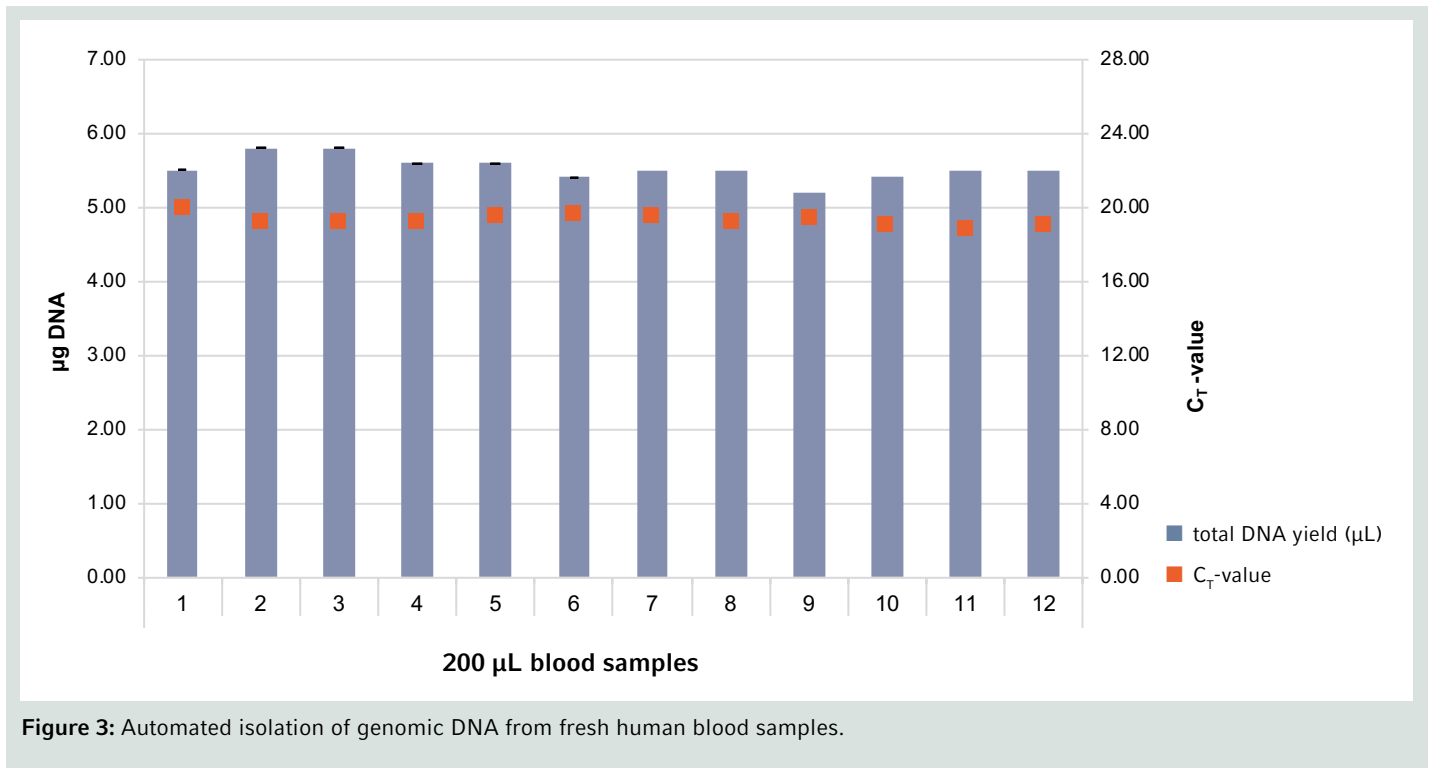


Figure 1: epMotion M5073m worktable layout.

Results

In order to prove the efficiency of the automated protocol on the *epMotion 5073m* described in this publication, one human blood pool was split into 12 x 200 μL fractions.

The blood samples were processed by using the following method: NucleoMag_Blood200_5073m.export.



DNA was isolated from fresh 200 μL human blood samples ($n = 12$) using the NucleoMag Blood 200 μL kit on an *epMotion 5073m* worktable. The elution of genomic DNA was carried out in 105 μL Elution Buffer MBL5. The DNA concentration of all 12 samples was determined by

UV-spectroscopy (Fig. 3, dark blue bars). A subsequent qPCR (orange squares) was performed with a Taqman[®] Probe for a 250 bp β -Actin amplicon using the Sensifast[™] Probe Lo-ROX kit from Biorline on an Applied Biosystems[®] 7500 Real-Time PCR System (Fig. 3).

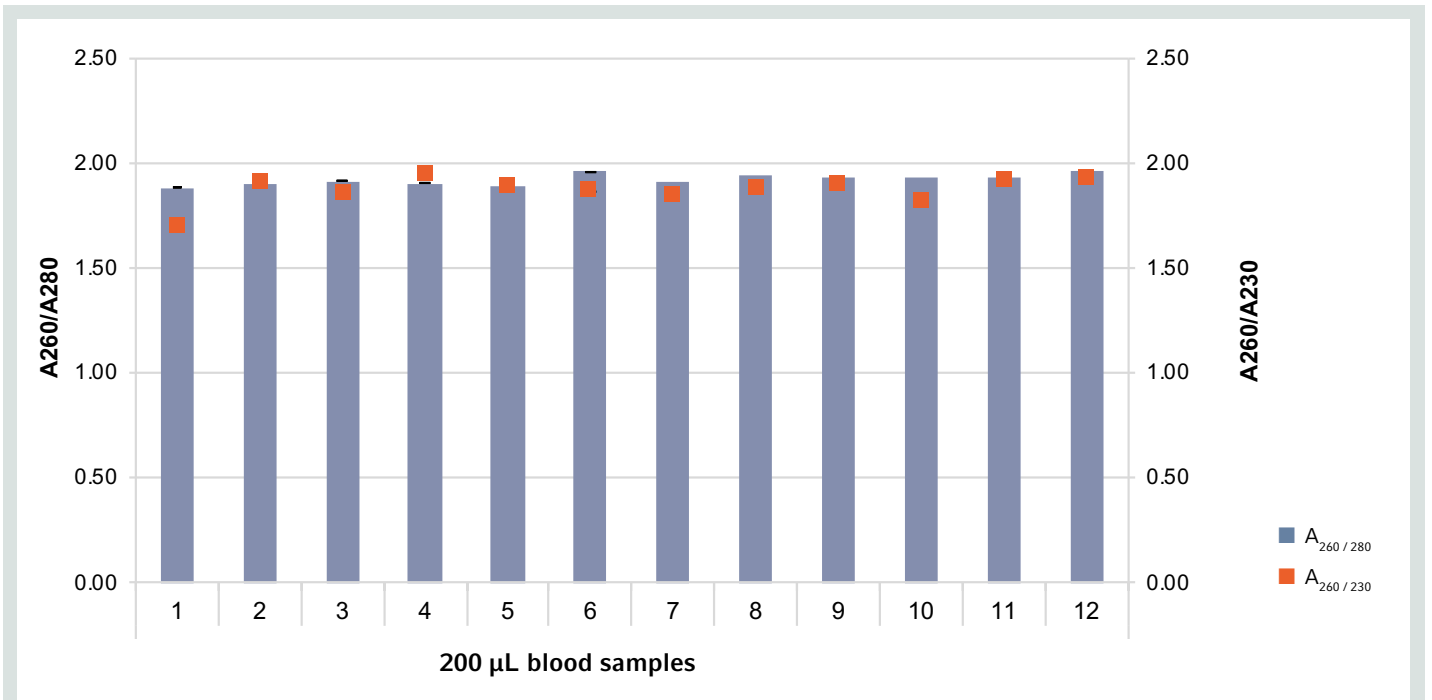


Figure 4: Purity of genomic DNA isolated from fresh human blood samples

DNA was isolated from fresh 200 µL human blood samples (n = 12) using the NucleoMag Blood 200 µL kit on a epMotion 5073m worktable. The total purity was determined by UV-spectroscopy. The ration of A260/280 value is showed in dark blue bars and ration of A260/A230 value in oranges squares.

DNA quality analysis resulted into an average A260/A280 value of 1.92 +/- 0.02 and into an average A260/A230 value of 1.86 +/- 0.06.

Ordering information

| Description | Order no. International |
|--|----------------------------|
| epMotion® M5073 | 5073 000.205 |
| TS 50 single channel dispensing tool | 5280 000.010 |
| TS 1000 single channel dispensing tool | 5280 000.053 |
| PrepRack for 24 Eppendorf Safe-Lock Tubes 2 mL | 5073 751.006 |
| Reservoir Rack | 5075 754.002 |
| Reservoir rack module TC for 4x Safe-Lock tubes 0.5/1.5/2.0 mL | 5075 799.081 |
| Reservoir 30 mL | 0030 126.505 |
| Eppendorf Safe-Lock LoBind Tubes, 2.0 mL | 0030 120.094 |
| Eppendorf Rack for 24 x Safe Lock 2,0 mL tubes | 5075 751.275 |
| epT.I.P.S.® Motion 50 µL Filter | 0030 014.413 |
| epT.I.P.S.® Motion SafeRack 1000 µL Filter | 0030 014.650 |
| epMotion® Tub for liquid waste 400 mL | 5075 210.401 |
| NucleoMag® Blood 200 µL 1 x 96 / 4 x 96 | 744501.1 /4 |

Your local distributor: www.eppendorf.com/contact

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