eppendorf

SHORT PROTOCOL No. 45

Automated gDNA Purification from various mouse tissue types using the NucleoMag® Tissue kit on ep*Motion*® 5073m

Introduction

This protocol describes the automated process on the liquid handling system ep*Motion* 5073m using the NucleoMag Tissue Kit from MACHEREY-NAGEL.

We show the configuration and pre-programmed method for automated genomic DNA purification from different types of mouse tissue for up to 24 samples.

Configuration and method procedure

Method name: NucleoMag_Tissue_5073m.export
This protocol is programmed to process up to 24 samples in parallel on the epMotion 5073m. The NucleoMag Tissue kit is based on reversible adsorption of nucleic acids to magnetic beads under appropriate buffer conditions. The ep*Motion* 5073m is by default equipped with an 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 ep*Motion* 5075m as well. Four different types of mouse tissue were analyzed: Lung, heart, kidney and tail. The lysis step was performed manually according to the manufacturer´s recommendations. A total volume of 20 mg tissue was lyzed overnight by adding 200 µL Lysis Buffer T1 and 25 µL proteinase K. After spinning down the samples for 5 min at 6000 x g the cleared lysates (225 µL) were transferred into 2.0 mL tubes. To maximize the efficiency of DNA recovery, it is recommended to use the Eppendorf DNA LoBind tubes. These tubes are placed in the PrepRack on the TMX position on the deck of the epMotion 5073m (Fig. 1).
The required volume of buffers (Binding and Wash Buffers, 80% ethanol and Elution Buffer) should be transferred to 30 mL ep*Motion* reservoirs. NucleoMag B-Beads need to be provided in one 2.0 mL Eppendorf Safe-Lock Tube within a reservoir rack on position B1 as described in Fig. 2.

The automated protocol starts by adding 24 µL of NucleoMag B-Beads to the samples, followed by mixing at 1200 rpm for 30 sec. 360 µL of Binding Buffer MB2 are added to the lyzed samples followed by a 5 minutes mixing at 900 rpm. A subsequent 2 min magnetic separation allows the complete accumulation of the NucleoMag B-Beads. After magnetic separation, the supernatant is removed and discarded into the liquid waste tub. The genomic DNA attached to the magnetic beads is washed twice: First with 600 µL of the Wash Buffer MB3 followed by a removal of the supernatant. Then 600 µL of the Wash Buffer MB4 is added, followed by a removal of the supernatant. A third washing step is subsequently performed with 900 µL of 80% ethanol and the supernatant is removed afterwards. To remove all traces of ethanol, the NucleoMag B-Beads are air dried for 7 min at 55°C while the magnetic separator remains switched on. In a second phase the drying continues with the magnetic separator switched off for 7 more minutes at 55°C and mixing at 1200 rpm. The genomic DNA is eluted from the NucleoMag B-Beads by adding 105 µL of Elution Buffer MB6 and intermittent mixing at 1300 rpm and 55°C for 5 min. It is possible to adjust the volume of the Elution Buffer MB6 according to the initial sample amount, to circumvent a strong dilution or concentration of the eluted genomic DNA. Then, the magnetic separation is switched on again for 2 min before 100 µL of the supernatant containing the purified genomic DNA is transferred into fresh tubes in the Rack 24 on the worktable.

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

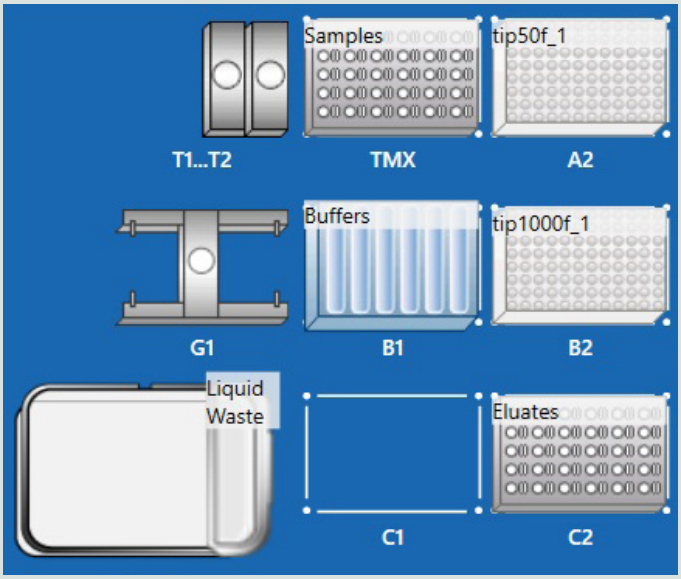

Figure 1: ep*Motion* M5073m worktable layout.

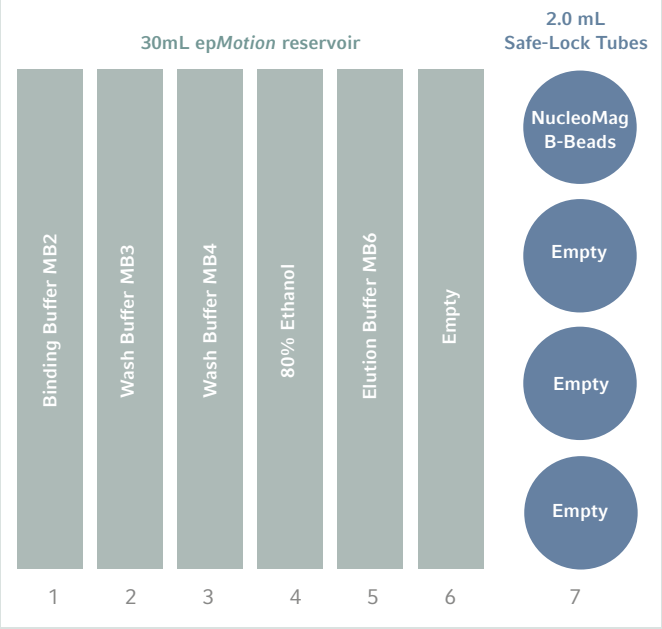

Figure 2: Reservoir rack layout on position B1 of ep*Motion* 5073m worktable.

Results

Here we demonstrated the usage of the NucleoMag Tissue kit from MACHEREY-NAGEL with the application method "NucleoMag_Tissue_5073m.export" on the ep*Motion* 5073m. Four different types of mouse tissue samples (lung, heart, kidney and tail) have been extracted in 4 replicates respectively, by using the NucleoMag Tissue kit from MACHEREY-NAGEL and application method NucleoMag_Tissue_5073m.export. The elution of the genomic DNA was performed in 105 µL of Elution Buffer MB6. The DNA concentration of all 16 samples was determined by UV-spectroscopy and analyzed for each tissue type separately (Fig. 3, dark blue bars). The extraction efficiency was further proven by a qPCR assay (oranges squares) using a Taqman® Probe for a GAPDH amplicon using the Sensifast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System (Fig. 3, orange squares). The total purity was determined by UV-spectroscopy (resulting into an average A260/A280 value between 1.70 and 1.96 depending on the tissue type (Fig. 4).

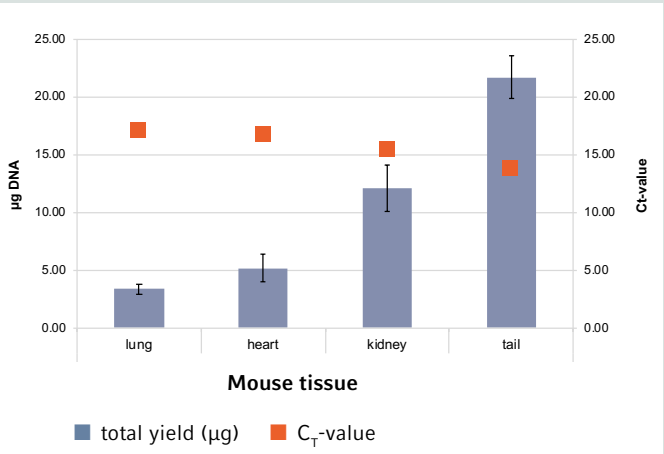

Figure 3: Total DNA concentration (dark blue bars) and CT value (oranges squares) of the eluted mouse tissue samples.
The standard deviation bars are the deviation among 4 samples of the same tissue.

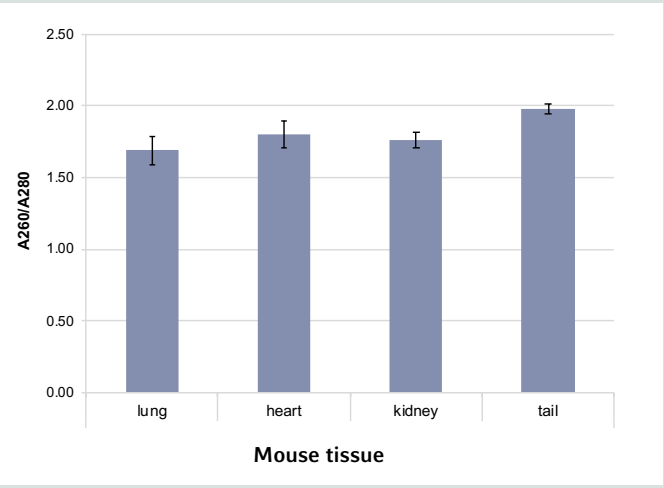

Figure 4: Total purity of the eluted DNA tissue sample.
The ration of A260/A280 value for different tissue type.

Ordering information

Description	Order no. International
ep*Motion*® 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
ep*Motion*® 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
Eppendorf AG · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com