APPLICATION NOTE No. 365 I October 2015

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

[1]Florian Werner, [2]Renate Fröndt
[1]Macherey-Nagel, 52355 Düren, [2]Eppendorf AG, 22339 Hamburg

Abstract

The purification of genomic DNA from tissue samples with the NucleoMag 96 Tissue Kit is designed for manual or automated small-scale preparation of highly pure genomic DNA from tissue, cells or bacteria pellets. Here we show the implementation of the NucleoMag Tissue Kit from Macherey-Nagel on the ep*Motion* 5075t/m. The combination allows a walk away purification in less than 120 minutes for untreated cells and less than 100 minutes for prelysed tissue and bacteria pellets.

Introduction

The procedure of the NucleoMag 96 Tissue kit is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions.
Different samples, like tissue, cells or bacteria pellets are lysed with lysis buffer T 1 and Proteinase K. DNA is then bound to the magnetic beads. Contaminants are removed through a series of washing steps with different wash buffers. Finally, remaining salts are removed with an additional 80 % ethanol 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* 5075 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 Tissue Kit

Required Consumables:
epT.I.P.S.® Motion 1000 µL with filter
epT.I.P.S. Motion 300 µL with filter
Square-well Block as processing plate
Microtiterplate to collect the eluates

Samples
Tissue, cells, mouse tails or bacteria pellets

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 20 mg tissue, up to 10^6 cells or bacteria pellets from up to 1 mL overnight culture. For tissue purification 225 µL cleared lysate (external lysis step at 56°C for 1-3 hours or overnight, followed by centrifugation), for cell purification 200 µL suspension in T1 lysis buffer (without Proteinase K, which will automatically be added in the first epMotion step) is 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. After the lysis step magnetic beads and binding buffer are added. During mixing and incubation step the DNA is bound to the magnetic beads. Magnetic beads are separated with the NucleoMag SEP and the supernatant is removed. Unspecifically bound contaminats are removed through several washing steps with wash buffers MB3 and MB4. Remaining salts are removed with an additional 80 % ethanol wash step, which replaces the MB5 buffer. After the last washing step residual ethanol is removed in a drying step of 15 minutes at 70°C on the integrated TMX module of the epMotion. Finally, the eluate is be transferred to a dedicated elution plate. Both versions of the purification (for tissue or for cells and bacterias) are available as preprogrammed methods.
A purification process with 96 tissue 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	

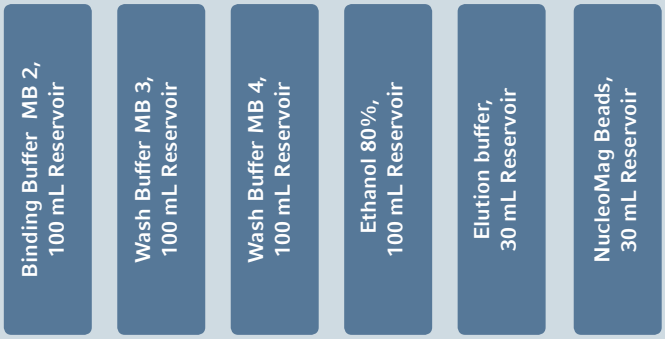


Figure 1: ReservoirRack Layout for cell purification

Figure 2: ReservoirRack Layout for bacteria pellets or tissue

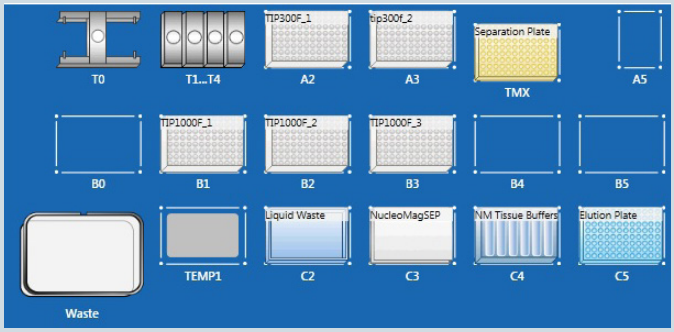


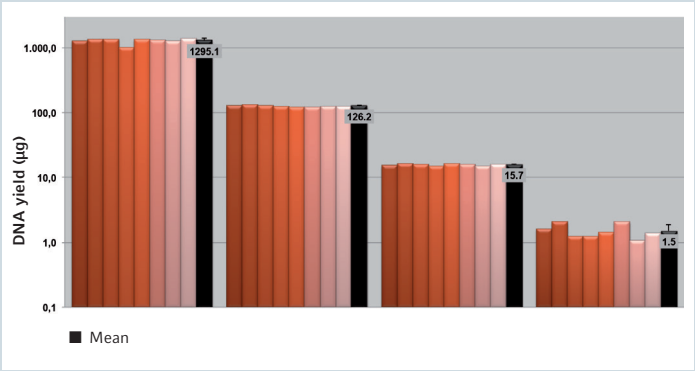
Figure 3: Worktable allocation for all purification versions

Results and Discussion

Purification results from tissue: Genomic DNA resulting from the aforementioned method was analyzed by gel electrophoresis of 10 µL eluate or PCR product via 1% TAE agarose gel; Yield and purity were determined by UV spectroscopy. Furthermore a qPCR with SensiFast® Probe Lo-Rox Kit (Bioline®) on an Applied Biosystems® 7500 was used to check for the absence of PCR inhibitors.

Purification of cells

HeLa cells in the range of 10^6 down to 10^3 were tested.



	10^6 cells	10^5 cells	10^4 cells	10^3 cells
DNA yield (ng)	1263.8	128.4	15.2	1.6
	1353.0	131.2	16.4	2.1
	1362.7	129.7	15.9	1.2
	1024.2	126.0	14.9	1.2
	1352.8	121.2	16.4	1.4
	1315.0	122.1	15.8	2.1
	1281.4	126.3	15.2	1.1
	1408.2	124.9	16.0	1.4
MEAN	**1295.1**	**126.2**	**15.7**	**1.5**
STDEV	**118.9**	**3.5**	**0.6**	**0.4**

Figures 4 and 5: Results from cultured eukaryotic cells. DNA yield and purity determination with Synergy™ HT Multi-detection microplate reader (BioTek®)

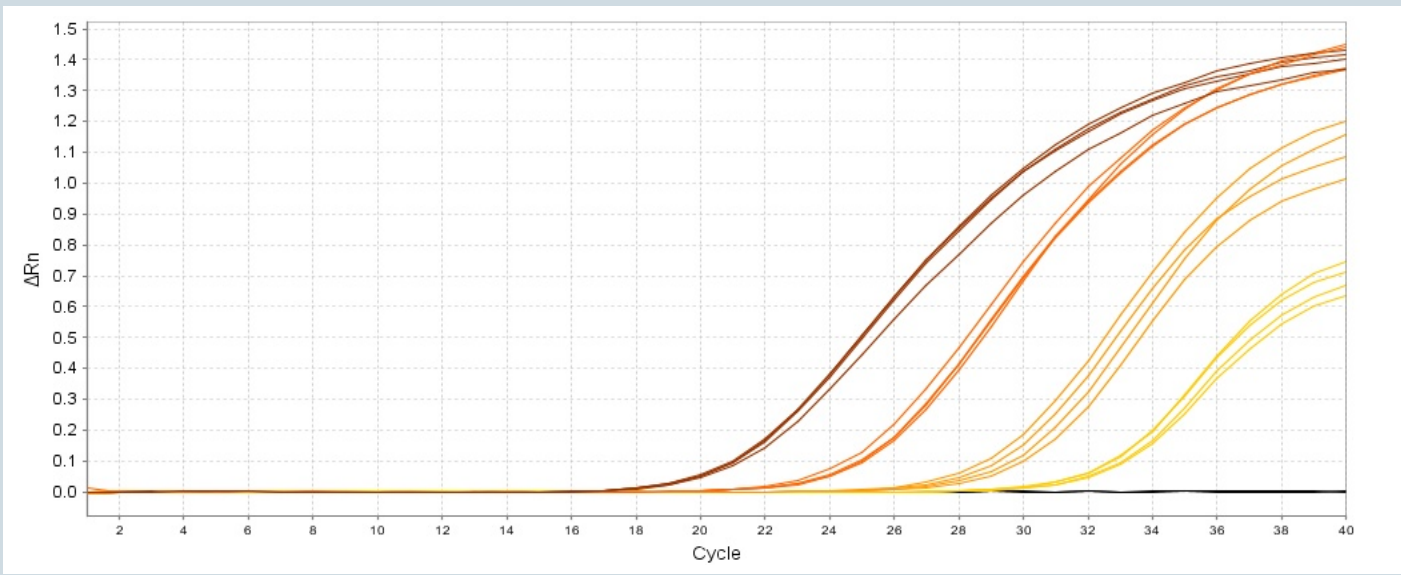
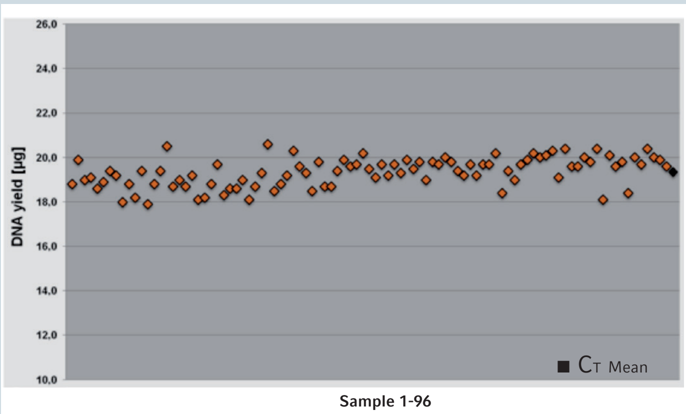


Figure 6: 4 µL of randomly selected eluates from all extracted cell numbers underwent a quantitative PCR with a Taqman® probe for beta-Actin and 250bp amplicon size using the SensiFast Probe Lo-Rox Kit (Bioline) on an Applied Biosystems 7500 instrument

Mousetails

DNA isolation from 20 mg mousetails



20 mg mousetail												
DNA yield (µg)	18.8	18.0	18.7	18.3	18.5	18.7	19.1	19.0	19.2	19.9	19.6	18.4
	19.9	18.8	19.0	18.6	18.8	18.7	19.7	19.8	19.7	20.2	20.0	20.0
	19.0	18.2	18.7	18.6	19.2	19.4	19.2	19.7	19.7	20.0	19.8	19.7
	19.1	19.4	19.2	19.0	20.3	19.9	19.7	20.0	20.2	20.1	20.4	20.4
	18.6	17.9	18.1	18.1	19.6	19.6	19.3	19.8	18.4	20.3	18.1	20.0
	18.9	18.8	18.2	18.7	19.3	19.7	19.9	19.4	19.4	19.1	20.1	19.9
	19.4	19.4	18.8	19.3	18.5	20.2	19.5	19.2	19.0	20.4	19.6	19.6
	19.2	20.5	19.7	20.6	19.8	19.5	19.8	19.7	19.7	19.6	19.8	Blank
MEAN	19.4											
STDEV	0.7											

Figures 7 and 8: DNA yield from 95 individual prelysed mousetail samples determined with Synergy HT Multi-detection microplate reader, Biotek.

20 mg mousetail												
Purity (260/280 nm)	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
	1.8	1.8	1.8	1.8	1.8	1.7	1.8	1.8	1.8	1.8	1.7	1.8
	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
	1.8	1.7	1.8	1.8	1.8	1.8	1.8	1.7	1.8	1.8	1.8	1.8
	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	Blank
MEAN	1.8											
STDEV	0.0											

Figure 9: Purity of 95 mousetail samples. A 260 / 280 ratio of 1.8 indicates DNA of optimal purity. The absorption was determined with Synergy HT Multi-detection microplate reader, Biotek.

Other tissues

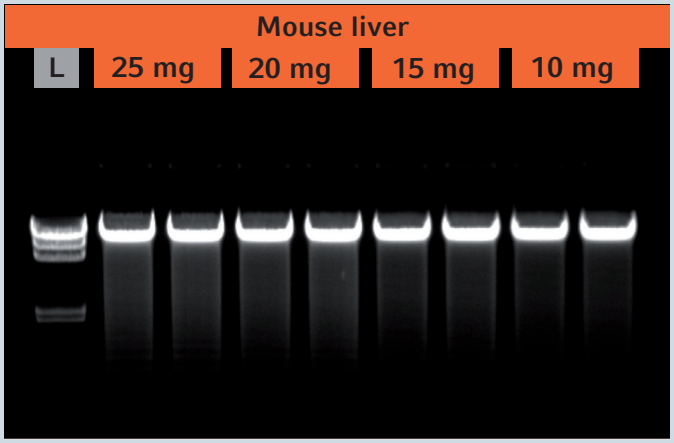


Figure 10: Purified gDNA from 4 different input amounts of mouse liver samples. 10 µL eluate respectively per lane of an agarose gel (1 %)

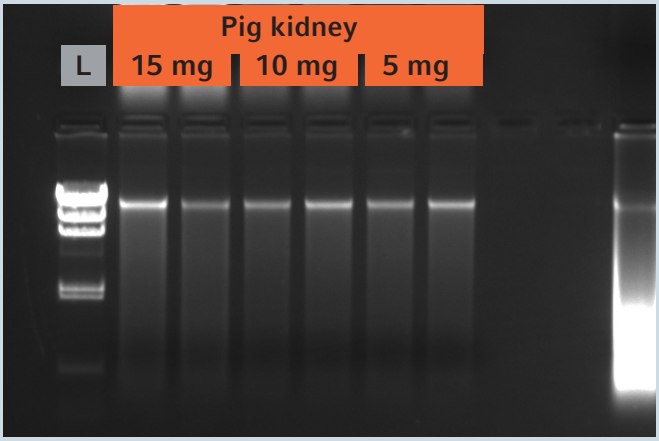


Figure 13: Results from pig kidney.

	Mouse liver				Mouse lung			
	25 mg	20 mg	15 mg	10 mg	25 mg	20 mg	15 mg	10 mg
DNA yield (µg)	29.2	25.9	20.5	16.8	14.1	12.3	9.5	7.1
	27.0	25.8	20.7	16.9	14.8	12.1	10.4	7.7
	28.1	25.7	20.9	16.9	14.6	12.9	10.0	7.9
	29.6	25.3	20.7	16.9	14.3	12.6	10.1	7.6
	29.4	24.3	20.0	16.5	15.1	12.0	10.0	7.8
	29.2	22.4	21.7	16.6	15.7	12.0	11.0	8.1
	28.9	24.9	19.3	17.8	14.8	11.3	10.9	7.0
	29.1	24.2	19.4	16.2	14.0	11.6	9.9	7.2
MEAN	**28.8**	**24.8**	**20.4**	**16.8**	**14.7**	**12.1**	**10.2**	**7.6**
STDEV	**0.9**	**1.2**	**0.8**	**0.5**	**0.6**	**0.5**	**0.5**	**0.4**

Figure 11: DNA yield from several amounts of mouse liver and mouse lung, respectively

	Mouse liver				Mouse lung			
	25 mg	20 mg	15 mg	10 mg	25 mg	20 mg	15 mg	10 mg
Purity (260/280 nm)	1.9	1.9	1.9	1.9	1.9	2.0	1.9	1.9
	1.8	1.8	1.7	1.9	1.9	2.0	2.0	1.9
	1.9	1.9	1.9	1.9	1.9	2.0	1.9	1.9
	1.9	1.9	1.9	1.9	2.0	2.0	1.9	2.0
	1.9	1.9	1.9	1.9	2.0	1.9	2.0	2.0
	1.8	1.9	1.9	1.9	2.0	2.0	1.9	1.9
	1.9	1.9	1.9	1.9	2.0	2.0	1.9	1.9
	1.8	1.9	1.9	1.9	2.0	2.0	1.9	1.9
MEAN	**1.8**	**1.9**	**1.8**	**1.9**	**2.0**	**2.0**	**1.9**	**1.9**
STDEV	**0.1**	**0.1**	**0.1**	**0.0**	**0.1**	**0.1**	**0.1**	**0.1**

Figure 12: The 260 / 280 ratio of at least 1.8 indicated highly pure DNA extracted from different starting amounts of mouse liver and mouse lung, respectively.

Cross contamination

The cross contamination determination was done with mousetail samples in a checker board patter. No amplification for empty wells proves the absence of cross contamination.

DNA yield [µg] of 20 mg mousetail

	1	2	3	4	5	6	7	8	9	10	11	12
A	20.2	0.0	20.8	0.0	20.2	0.0	20.3	0.0	21.7	0.0	22	0.0
B	0.0	20.7	0.0	20.3	0.0	21.1	0.0	20.8	0.0	21.9	0.0	21.5
C	19.1	0.0	19.6	0.0	20.7	0.0	21.5	0.0	21.7	0.0	21.1	0.0
D	0.0	20	0.0	20.6	0.0	20.8	0.0	21.7	0.0	22.2	0.0	21.9
E	18.3	0.0	19.6	0.0	20.3	0.0	21.8	0.0	21.7	0.0	22.1	0.0
F	0.0	20.3	0.0	20.3	0.0	20.8	0.0	20.9	0.0	22.4	0.0	21.7
G	20.2	0.0	20.1	0.0	20.5	0.0	21	0.0	21.7	0.0	21.8	0.0
H	0.0	20.8	0.0	20.8	0.0	21.3	0.0	20.9	0.0	22.7	0.0	20.2

Figure 14: No PCR amplification was observed with eluates from negative controls.

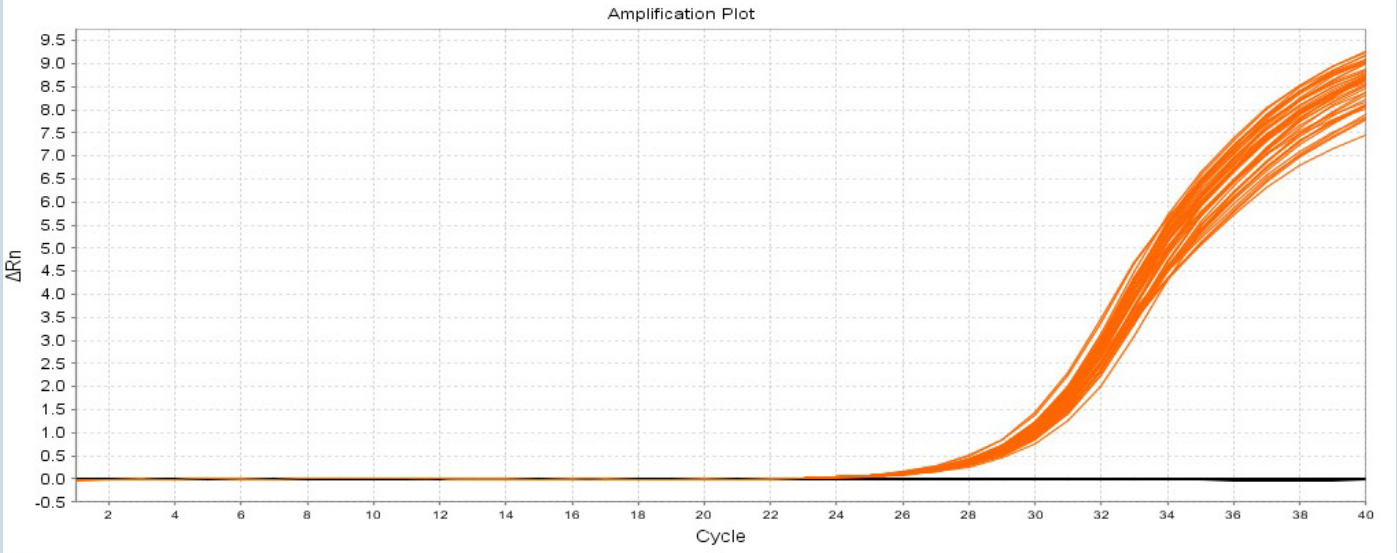


Figure 15: Amplification curves of the cross-contamination experiment

Conclusion

The purified DNA is suitable for a full range of downstream methods. The results from the electrophoresis analysis, qPCR as well as photometric measurements show the performance of the described procedure. The run time for the automated extraction of 96 samples is between 100 and 120 minutes depending on the sample material. The use of epT.I.P.S. in SafeRacks along with the re-use tips function, has a direct impact on cost.

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 Tissue	REF 744300
NucleoMag® SEP	REF 744900
Square well block	REF 740481

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · Barkhausenweg 1 · 22331 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com