Applications

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Automated genomic DNA purification in 96-well plate and 8-well strip format using the MACHEREY-NAGEL NucleoSpin® 8/96 Tissue kits on the ep*Motion*® 5075 from Eppendorf

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Abstract

In the current application note we demonstrate the integration of the MACHEREY-NAGEL NucleoSpin 8/96 Tissue kits into the ep*Motion* 5075 VAC automated pipetting system. The NucleoSpin 8/96 Tissue kits are based on a vacuum filtration based bind-wash-elute procedure. Protocols for the ep*Motion* 5075 VAC are available for medium throughput using the flexible 8-well strip based purification kit or for high throughput using the 96-well plate based kit. The use of NucleoSpin 8/96 Tissue kits on the ep*Motion* 5075 instrument allows the isolation of DNA from a wide range of sample materials. Application data for genomic DNA isolation from different mouse tissues, human cells or bacteria cells are presented.

Introduction

Typically, PCR based analytical methods in the field of transgenics, genotyping and SNP analysis require template DNA of high quality and reproducible yields. Purification of genomic DNA from different types of sample material is still challenging. First, the method should be sensitive, robust and easy to automate with little hands-on time. Second, besides a high degree of flexibility for the user, the procedure should avoid use of toxic solvents or precipitations which are very difficult to automate. Most of the drawbacks of conventional DNA isolation can be overcome by state-of-the-art silica membrane purification. Here, we describe the use of the MACHEREY-NAGEL NucleoSpin 8/96 Tissue kits for use on the ep*Motion* 5075 VAC automated pipetting system. Using the well proven bind-wash-elute procedure liquid-liquid extraction or DNA precipitation can be avoided. The NucleoSpin 8/96 Tissue kits provide excellent consistency, a high robustness even when using very diverse sample types such as tissues from various organs, mouse tail clippings, eukaryotic and bacterial cells, and uncompromised DNA yield and quality.

In addition, the kits can readily be automated on liquid handling systems. The procedure starts with an enzymatic sample digest at 56 °C. This heat incubation step can be either performed externally or on the instrument which is equipped with a heat incubator. All further steps are performed at room temperature. Following the lysis incubation the DNA is bound reversibly to the silica membrane of the NucleoSpin Tissue Binding Plate or strips. Following washing steps and an ethanol evaporation step the purified DNA is eluted in water or low salt elution buffer. The purified DNA is suitable for use in downstream applications like PCR, real-time PCR or restriction analysis. The kits are available in either 8-well strip format or 96-well plate format in order to meet the user requirements for sample throughput. The use of NucleoSpin 8/96 Tissue kits on the ep*Motion* 5075 automated pipetting system provides excellent results without the need for extensive programming, optimization and set-up time.

Materials and Methods

Eppendorf ep*Motion* 5075 VAC
Vac Frame 2
Vac Holder
Collection Plate Adapter for MN Tube Strips
Channeling Plate
Reservoir Rack with Reagent Reservoirs
MACHEREY-NAGEL NucleoSpin 96 Tissue kit
MACHEREY-NAGEL NucleoSpin 8 Tissue kit
tissue samples (e.g. mouse tail clippings, mouse ear punches, mouse organs), eukaryotic cells (HeLa S3 cells), bacterial cells

Product use limitations and safety information

Please read the MACHEREY-NAGEL NucleoSpin 8 Tissue or NucleoSpin 96 Tissue manual before performing the method for the first time.

Determination of yield and purity

Yield and purity were determined using a microplate reader (Biotek, Powerwave 200). DNA yield was calculated from A_{260} values. Purity was determined by calculating the $A_{260/280}$ ratio.

Agarose gel electrophoresis

Integrity of DNA and results of restriction analysis were analyzed by TAE agarose gel electrophoresis (1 % (w/v) agarose, ethidium bromide stain).

Restriction analysis

Approx. 1 µg DNA was incubated with *Eco*RI for 2 h at 37 °C according to manufacturers instructions (Invitrogen).

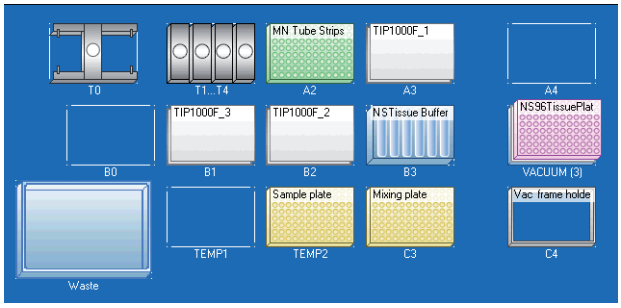

Figure 1: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for use with the NucleoSpin 96 Tissue kit.

Table 1: ep*Motion* 5075 VAC worktable details for NucleoSpin 96 Tissue protocol

Position	Labware	Comment
A2	MN Tube Strips (MN_TP_1200)	elution tubes
A3	epT.I.P.S Motion 1000 µL	
B1	epT.I.P.S Motion 1000 µL	
B2	epT.I.P.S Motion 1000 µL	
B3	Reagent Reservoirs Position 1: empty Position 2: Buffer BQ1/EtOH Position 3: Buffer BW Position 4: Buffer B5 Position 5: Buffer B5 Position 6: Buffer BE Position 7: empty	 100 mL reservoir 100 mL reservoir 100 mL reservoir 100 mL reservoir 30 mL reservoir
Vacuum	NucleoSpin Tissue Binding Plate (MN_FP_96_1500 Vacuum Frame 2 Reservoir 400 ml with channeling plate	DNA binding plate (top) collar for vacuum manifold collects waste
C2	1.1 ml deep-well plate (MN_DWP_1100_RB_cd2)	sample plate
C3	2.1 ml deep-well plate (MN_DWP_2100)	for mixing sample with binding buffer
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting tool

Table 2: ep*Motion* 5075 VAC worktable details for NucleoSpin 8 Tissue protocol

Position	Labware	Comment
A2	MN Tube Strips (MN_TP_1200_48)	elution tubes* (***)
A3	epT.I.P.S Motion 1000 µL	
B1	epT.I.P.S Motion 1000 µL	
B2	epT.I.P.S Motion 1000 µL	
B3	Reagent Reservoirs Position 1: empty Position 2: Buffer BQ1-EtOH Position 3: Buffer BW Position 4: Buffer B5 Position 5: empty Position 6: Buffer BE Position 7: empty	 optional: Lysis buffer 100 mL reservoir 100 mL reservoir 100 mL reservoir 30 mL reservoir
Vacuum	NucleoSpin Tissue Binding Strips** (MN_FP_8_1400) Vacuum Frame 2 Reservoir 400 mL with channeling plate	DNA binding strips (top) collar for vacuum manifold collects waste
C2	1.1 mL deep-well plate (MN_DWP_1100_RB_cd2)	sample plate
C3	2.1 mL deep-well plate (MN_DWP_2100)	for mixing sample with binding buffer
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting tool

*) require Collection Plate Adapter for MN tube strips, see ordering information
**) 8-well strips are inserted into MACHEREY-NAGEL Column Holder A which is part of the Starter Set A, see ordering information
***) 96 well MTP can be used optionally

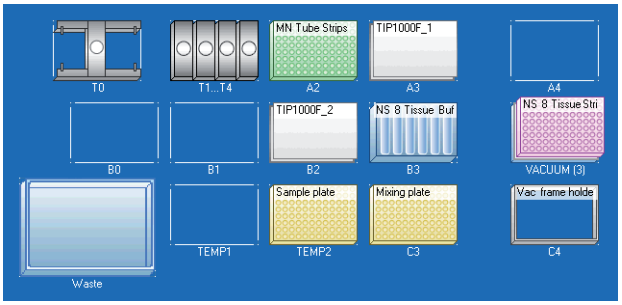

Figure 2: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for use with the NucleoSpin 8 Tissue kit.

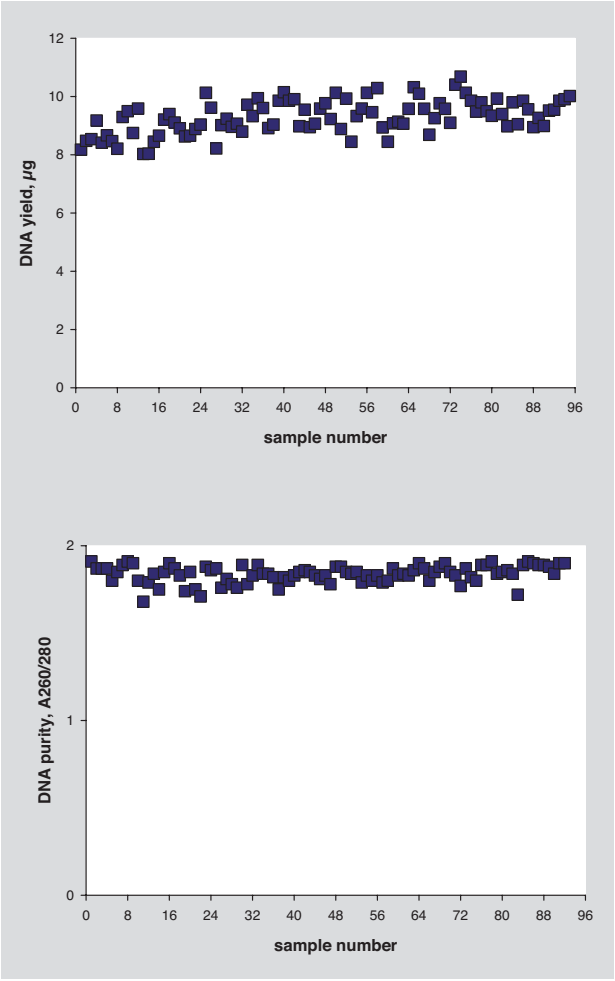

Figure 3: Reproducibility of DNA purification using NucleoSpin 96 Tissue kit.
Genomic DNA was isolated from mouse tail samples (master lysate) using NucleoSpin 96 Tissue kit on the ep*Motion* 5075 VAC automated pipetting system. DNA yield and purity were determined spectrophotometrically.

Results

Reproducibility of yield and purity of genomic DNA isolated from mouse tail clippings using NucleoSpin 96 Tissue kit

In order to demonstrate the reproducibility of the purification method genomic DNA was isolated from a master lysate of mouse tail clippings representing identical sample material. Each lysate aliquot corresponds to a 4 mm mouse tail sample. The mouse tail samples were lysed with proteinase K overnight at 56 °C. Heat incubation for lysis was performed in a external incubator. Following lysis the lysate was centrifuged in order to precipitate remaining debris and hairs. The cleared lysate was transferred into the wells of a deep-well plate and placed on the instrument for further processing. DNA yield and purity are shown in figure 3. The results are summarized in table 3. Highly reproducible results for yield and purity were obtained. With a CV of 6.2 % an average yield of 9.3 µg genomic DNA was obtained from the mouse tail clippings.

Table 3: Yield and purity of DNA isolated from mouse tail clippings

	DNA yield (µg)	DNA purity ($A_{260/280}$)
average yield / purity	9.30	1.84
standard deviation	0.58	0.05
min. yield / purity	8.03	1.68
max. yield / purity	10.69	1.91

Quality of DNA and structural integrity

In order to demonstrate quality and structural integrity of the isolated DNA the purified samples were analyzed by agarose gel electrophoresis. From the recovered 150 µL of purified DNA 20 µL were loaded on the agarose gel. The DNA migrates as high molecular weight band of approx. 20-30 kbp. The appearance of the tight DNA band and the absence of low molecular weight smear demonstrate the excellent quality of DNA and well to well consistency. The results are shown in figure 4.

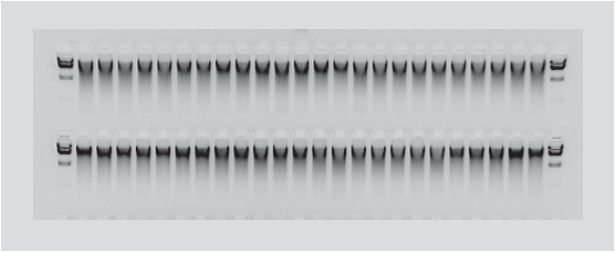

Figure 4: Agarose gel analysis of purified DNA isolated from mouse tail clippings.
Aliquots of 20 µL from randomly selected purified genomic DNA samples were analyzed on an 1 % agarose gel. High molecular weight DNA with a good consistency of yield was obtained.

Isolation of genomic DNA from different mouse organ tissues using NucleoSpin 8 Tissue kit

In addition to the 96-well plate based NucleoSpin 96 Tissue kits for high sample throughput the NucleoSpin 8 Tissue kit offers high flexibility using the NucleoSpin 8 Tissue binding strips. This kit is specially designed for medium throughput and allows for the processing of flexible sample numbers in multiples of 8 samples. The use of individual 8-well strips avoids the sealing of unused wells of a 96-well plate when processing less than 96 samples. Reusing partially used 96-well filterplates introduces a risk of contamination or sample carry-over and thus should be avoided. As an example for the use of the NucleoSpin 8 Tissue kit DNA purification from different mouse organs is shown in figure 5. The results for yield and purity are summarized in figure 6. The mouse organs were incubated for 6 h at 56 °C in the supplied lysis buffer including proteinase K using an external incubator.

Figure 5: DNA isolation from mouse organs.
DNA was isolated from 20 mg tissue samples as described before. Aliquots of 20 µL from the purified samples were analyzed by agarose electrophoresis. High molecular weight DNA with consistent yield was obtained.

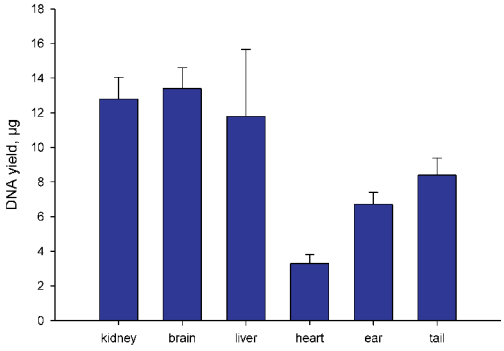

Figure 6: DNA isolation with NucleoSpin 8 Tissue from different mouse tissue samples.
DNA was isolated from approx. 20 mg of the indicated tissues. Each bar represents the average of eight extractions. Error bars indicate the standard deviation for each set of extractions.

DNA yield as shown in figure 6 represents the amount of genomic DNA in different organs. Different yields are also represented by the DNA intensities in figure 5. Within one sample (e.g., mouse kidney tissue) DNA yields are consistent with a small variation as indicated by the error bars in figure 6.

DNA quality and suitability for downstream applications

In order to demonstrate quality (e.g., absence of DNase activity) and suitability for downstream applications isolated DNA was analyzed by agarose gel electrophoresis. Samples were analyzed with and without treatment with restriction enzyme. Furthermore, samples were mixed with restriction enzyme incubation buffer and incubated without restriction enzyme for 2 h at 37 °C to demonstrate the absence of DNase activity. The results are shown in figure 7.

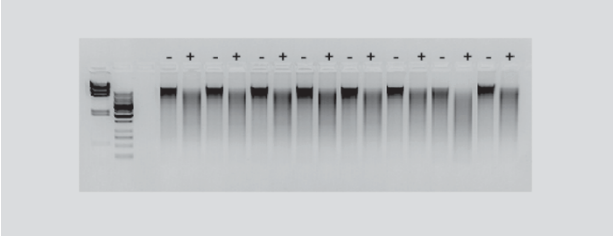

Figure 7: Restriction digest of isolated genomic DNA.
An aliquot of 10 µL from randomly selected purified DNA samples isolated from mouse tail clippings were incubated for 2 h at 37 °C with *Eco*RI restriction enzyme (+). In all samples treated with *Eco*RI enzyme DNA was restricted. Another aliquot was incubated with the restriction enzyme buffer only at 37 °C for 2 h (-). The samples incubated with enzyme reaction buffer only show a distinct high molecular weight band. Distinct bands in these samples indicate the absence of DNase activity demonstrating the high quality of DNA.

In order to demonstrate the suitability of the purified DNA for real-time PCR analysis, DNA isolated from mouse tail samples was used as template for PCR in a Roche Lightcycler instrument. The purified DNA was diluted 1:10 and used in 40 cycle reactions. A primer set amplifying a 212 bp fragment of *Mus musculus* cytoplasmatic aconitase exon (aco l) gene was used.

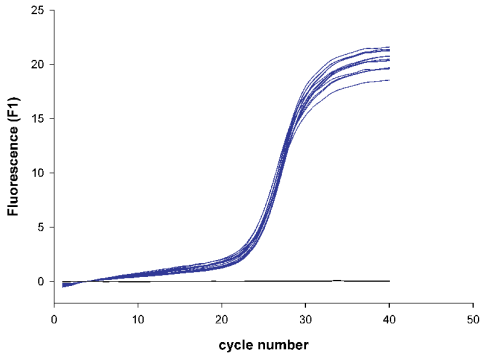

Figure 8: Real time PCR of DNA isolated from mouse tails.
DNA isolated from mouse tails was used in a SYBR Green PCR assay. PCR amplification plots show reproducible amplification and no evidence for PCR inhibitors. The average crossing point for all sixteen samples was 23.79 with a CV of 2.43 % demonstrating the high reproducibility of DNA yield and amplification. The specificity of the amplified PCR product was verified by melting curve analysis and analysis of the amplified PCR products by agarose electrophoresis (data not shown).

DNA isolation from cells

With a slightly modified procedure the NucleoSpin 8/96 Tissue kits can be also used for DNA isolation from cultured eukaryotic or bacterial cells. DNA isolation from eukaryotic cells was exemplified with HeLa cells. In contrast to the applications described before the lysis with proteinase K supplemented buffer and all purification steps were performed at room temperature on the instrument. After lysis RNase A was added to digest RNA. The results of the extraction of gDNA from HeLa cells are shown in the figure 9.

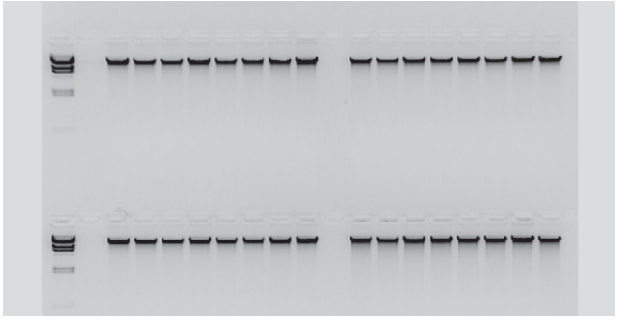

Figure 9: DNA isolation from HeLa cells
HeLa cells were grown in culture bottles, harvested after trypsin treatment and pelleted into the wells of the lysis block. Each well represents approx. 5 x 10^5 cells. gDNA was isolated according to the NucleoSpin 96 Tissue protocol using lysis with proteinase K followed by RNase A treatment. Average yield of DNA was 2.9 µg. The high molecular weight band and absence of smear indicate the excellent quality of the DNA.

DNA isolation from bacteria cells

DNA isolation from gram-negative bacteria may be performed according to the standard protocol of the NucleoSpin 8/96 Tissue kit protocol. For Gram-positive bacteria the lysis procedure has to be modified. For these difficult to lyse bacteria the use of a modified lysis buffer, including lysozyme or lysostaphin, is recommended. For the isolation of genomic DNA from *Bacillus subtilis*, *Pseudomonas fluorescence* and *Escherichia coli* the standard lysis procedure was used.

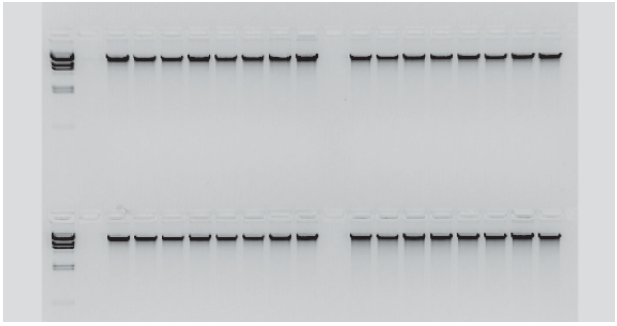

Figure 10: DNA isolation from bacterial cells
DNA was isolated from 1 mL of an overnight culture of bacteria. For lysis, a buffer supplemented with lysozyme was used. Reproducible yields of high molecular weight DNA were obtained.

Conclusion

The combination of the MACHEREY-NAGEL NucleoSpin 8 Tissue and 96 Tissue kits and the ep*Motion* 5075 VAC resulted in a flexible system for automated purification of high quality genomic DNA from a broad range of various sample materials. The compact ep*Motion* 5075 VAC automated pipetting system can be used either for low to medium throughput using the 8-well strip based NucleoSpin 8 Tissue kit or for higher throughput using the 96-well based NucleoSpin 96 Tissue kit. Both kits can be used with the same hardware allowing the user to switch between the two methods according to the requirements in sample throughput. DNA purification is achieved using the NucleoSpin Technology based on a vacuum driven bind- wash-elute procedure. The use of an optimized silica membrane in the NucleoSpin 8/96 Tissue kits allow the purification of DNA from various sample materials without a risk of clogged columns. The purified DNA is of excellent quality and suitable for downstream applications such as restriction analysis or PCR based analysis. In summary, the NucleoSpin technology and the ep*Motion* 5075 VAC automated pipetting system form an attractive and versatile system for the automated isolation of genomic DNA from different sample materials.

Eppendorf Ordering Information

Product	Order no. International	Order no. North America
ep*Motion*® 5075 VAC (vacuum chamber included)	5075 000.164	960020014
Vac Frame 2	5075 785.005	960002261
Collection Plate Adapter	5075 785.064	960002571
Channeling Plate	5075 794.004	960002540
Dispensing tool TM 1000-8	5280 000.258	960001061
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

Macherey-Nagel Ordering Information

Product	Order no.
NucleoSpin® 8 Tissue kit	
12 x 8 preps	740730.1
48 x 8 preps	740 730
NucleoSpin® 96 Tissue kit	
2 x 96 preps	740 708.2
4 x 96 preps	740 708.4
24 x 96 preps	740 708.24
Starter Set A (for NucleoSpin 8 Tissue only)	
1 set	740 682

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