Applications

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

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Abstract

Purification of template DNA for sequencing is a time consuming step in genetic analysis. Automated DNA purification can help to overcome this bottleneck. In the current application note we demonstrate the integration of the MACHEREY-NAGEL NucleoSpin Robot-8/96 Plasmid kits into the ep*Motion* 5075 VAC automated pipetting system. Depending on the requirements in sample throughput DNA can be purified in 8-well strip format or in a 96-well plate format. The procedure for the purification of plasmid DNA requires minimal set-up time. The purified DNA was subjected to restriction analysis and sequencing. For 96 samples, a processing time of approximately 90 min was achieved.

Introduction

The use of silica membrane based DNA purification kits is a convenient way to prepare plasmid DNA samples for subsequent analysis, e.g. sequencing or restriction analysis. The MACHEREY-NAGEL NucleoSpin Plasmid kits allow for sample preparation in either medium throughput 8-well strip format or in the 96-well plate format for higher throughput. Both kits, NucleoSpin Robot-8 Plasmid or NucleoSpin Robot-96 Plasmid can be used fully automated on the ep*Motion* 5075 VAC automated pipetting system. The NucleoSpin procedure provides a modified alkaline lysis procedure based on the method described by Birnboim and Doly [1]. Bacteria are grown in culture tubes or 96-well plates. Following the removal of the culture medium the bacterial cells are lysed and chromosomal DNA and proteins are precipitated by addition of a combined neutralization and binding buffer.

The crude lysates are transferred to a filter plate and cleared by vacuum filtration. The cleared lysates are collected in the NucleoSpin Plasmid Binding Plate and the DNA is bound reversibly to the silica membrane in a subsequent vacuum binding step. Following washing steps and an ethanol evaporation step the purified DNA can be eluted in water or low salt elution buffer. The purified DNA is suitable for subsequent downstream applications, e.g. sequencing, restriction analysis or PCR reactions. The NucleoSpin Robot Plasmid kits are equipped with a powerful lysate clearing filter unit which allows processing of cultures grown in up to 5 mL LB medium or up to 2.5 mL enriched culture media (e.g. TB, 2YT). Furthermore, the design of the NucleoSpin Binding Plates prevents the bacterial lysate to drip out of the column outlets by gravity. This avoids contamination of the instrument with bacterial lysate.

Materials and Methods

Eppendorf ep*Motion* 5075 VAC
Vac Frame 2
Vac Holder
Reservoir 400 mL
Collection Plate Adapter for MN Tube Strips (for NucleoSpin Robot-8 Plasmid kit only)
Channeling Plate
Reservoir Rack with Reagent Reservoirs
Height Adapter 55 mm (2 pcs.)
MACHEREY-NAGEL NucleoSpin Robot-96 Plasmid kit
MACHEREY-NAGEL NucleoSpin Robot-8 Plasmid kit
E. coli cultures

Product use limitations and safety information

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

Bacteria cultures

PCR products of different size were cloned using pGEM T-Easy vector. After transformation into *E. coli* DH5α competent cells the cultures were grown overnight in LB or 2YT medium in culture flasks or in 96-well culture plate supplied with the kit. Aliquots of 1.5 mL were transferred into the culture plate supplied with the plasmid purification kits. Plates were centrifuged for 10 min at 1000 x g to harvest bacteria. Culture medium was discarded and plates were used immediately or after storage at -20 °C.

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

pGEM derived plasmid DNA was incubated with *Eco*RI for 2 h at 37 °C according to manufacturers instructions (Invitrogen).

DNA sequencing

Purified DNA samples were sequenced using ABI BigDye 3.1 chemistry. The sequence was determined using the ABI 3730 XL DNA Sequencer (MWG sequencing service).

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

Position	Labware	Comment
A2	Elution Plate (MN_MTP_320)	MTP for elution
A3	epT.I.P.S Motion 1000 µL	
A4	Bacteria culture plate (MN_DWP_2100)	
B0	Reservoir 400 mL with channeling plate	
B1	epT.I.P.S Motion 1000 µL	
B2	Reagent Reservoirs Position 1: Buffer A1 Position 2: Buffer A2 Position 3: Buffer A3 Position 4: Buffer AW Position 5: Buffer A4 Position 6: Buffer A4 Position 7: Buffer AE	 100 mL reservoir 100 mL reservoir 100 mL reservoir 100 mL reservoir 100 mL reservoir 100 mL reservoir 30 mL reservoir
B3	Height Adapter 55 mm	
Vacuum	NucleoSpin Plasmid Filter Plate (MN_FP_96_1500) Vacuum Frame 2 NucleoSpin Plasmid Binding Plate (MN_FP_96_1500)	lysate clearing plate (top) collar for vacuum manifold binding plate in manifold base
C3	Height Adapter 55 mm	
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 Plasmid protocol

Position	Labware	Comment
A2	MN Tube Strips (MN_TP_1200_48)	elution tubes* (***)
A3	epT.I.P.S Motion 1000 µL	
A4	Bacteria culture plate (MN_DWP_2100)	
B0	Reservoir 400 ml with channeling plate	
B1	ep T.I.P.S Motion 1000 µL	
B2	Reagent Reservoirs Position 1: Buffer A1 Position 2: Buffer A2 Position 3: Buffer A3 Position 4: Buffer AW Position 5: Buffer A4 Position 6: empty Position 7: Buffer AE	 30 mL reservoir 30 mL reservoir 30 mL reservoir 100 mL reservoir 100 mL reservoir 30 mL reservoir
B3	Height Adapter 55 mm	
Vacuum	NucleoSpin Plasmid Filter Strips** (MN_FP_8_1400) Vacuum Frame 2 NucleoSpin Plasmid Binding Strips** (MN_FP_8_1400)	lysate clearing plate (top) collar for vacuum manifold binding plate in manifold base
C3	Height Adapter 55 mm	
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 wich is part of the Starter Set A, see ordering information
***) 96 well MTP can be used optionally

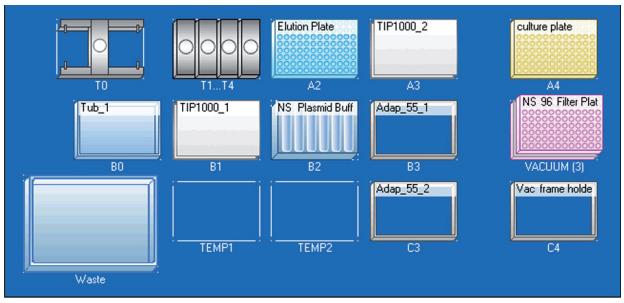

Figure 1: Screenshot from the ep*Motion* Editor showing the setup of the epMotion 5075 VAC worktable for use with the NucleoSpin Robot-96 Plasmid kit.

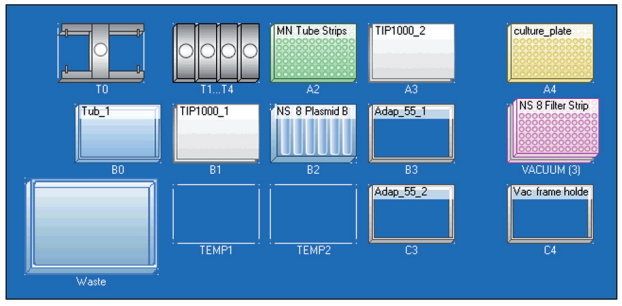

Figure 2: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for use with the NucleoSpin Robot-8 Plasmid kit.

Results

Yield and purity of plasmid DNA

Plasmid DNA yield depends on bacterial strain, copy number of plasmid, size of plasmid DNA construct, growing conditions and culture medium. Typically, from an *E. coli* strain with a high copy number plasmid (approx. 4 kbp) a total plasmid DNA yield of 4-6 µg/mL culture can be expected. In order to investigate the reproducibility of plasmid DNA purification cultures were grown in a flask and distributed for further processing in 96-well plates. Variation in DNA yield and DNA purity for a bacterial culture grown in a 96-well plate followed by purification using the NucleoSpin Robot-96 Plasmid kit is shown in figure 3. The results are summarized in table 3. Figure 4 and table 4 show the results of the purification of plasmids with different insert sizes.

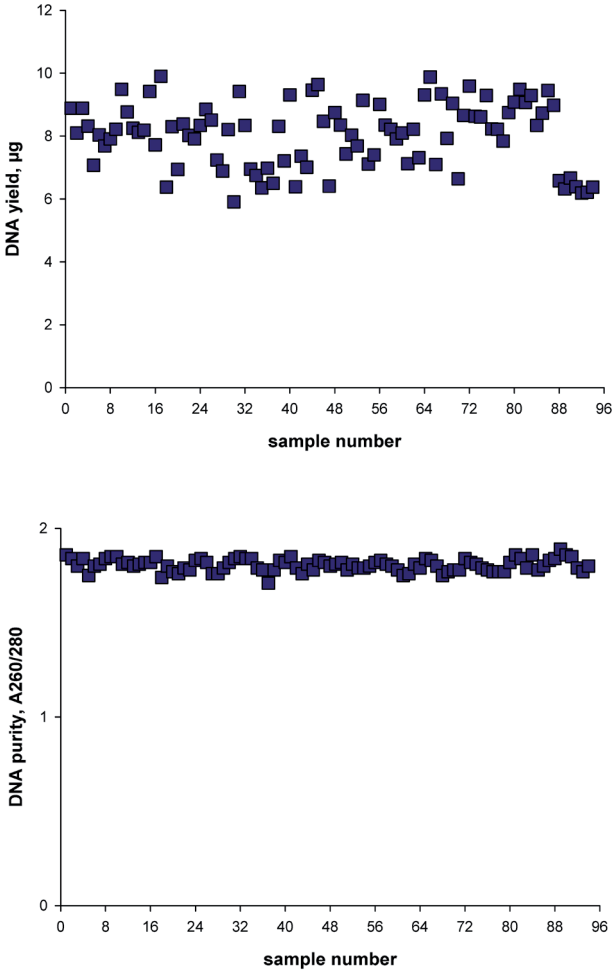

Figure 3: Reproducibility of DNA purification using NucleoSpin Robot-96 Plasmid kit.
Plasmid DNA was isolated from 1.5 mL aliquots of an *E. coli* DH5α culture grown in 2YT medium for 14 h. DNA yield and purity were determined spectrophotometrically.

Table 3: Yield and purity of DNA

	DNA yield (µg)	DNA purity ($A_{260/280}$)
average yield / purity	8.04	1.81
standard deviation	1.03	0.03
min. yield / purity	5.21	1.71
max. yield / purity	9.90	1.89

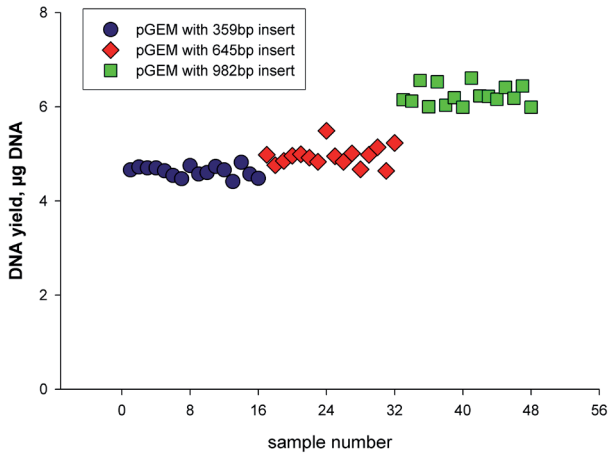

Fig. 4: DNA yield from plasmids with different insert sizes using NucleoSpin Robot-8 Plasmid kit.
Plasmid DNA was isolated from 1.5 mL aliquots of an *E. coli* DH5α culture grown in LB medium for 14 h. Insert lengths of the pGEM plasmids are as indicated. DNA yield and purity were determined spectrophometrically.

Table 4: Yield of DNA

	DNA yield, µg		
	pGEM 359	pGEM 645	pGEM 982
average yield	4.63	4.95	6.23
standard deviation	0.11	0.21	0.21
min. yield	4.41	4.64	5.99
max. yield	4.82	5.49	6.61

Quality of DNA and structural integrity

In order to demonstrate quality and structural integrity of the isolated DNA the purified plasmids were 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 5.

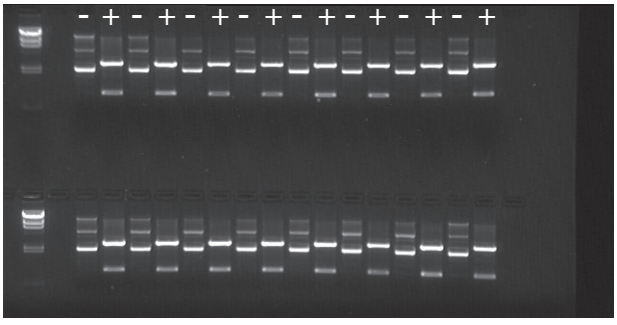

Fig. 5: Agarose gel analysis of extracted plasmids.
An aliquot of 10 µL from randomly selected purified plasmid DNA samples were incubated for 2 h at 37 °C with *Eco*RI restriction enzyme (+). In all samples treated with *Eco*RI enzyme the cloned insert was cut out of the plasmid. A distinct band of the expected size was obtained. Another aliquot was incubated with the restriction enzyme buffer only at 37 °C for 2 h (-). The samples incubated only with enzyme reaction buffer show structural integrity of >90 % of the ccc monomer plasmid form. Distinct bands in these samples indicate the absence of DNase activity.

DNA sequencing

Sequencing the purified plasmid DNA is the most frequent downstream application for miniprep plasmid DNA. In order to demonstrate the quality of the purified DNA for sequencing some purified DNA samples were sequenced using the ABI BigDye chemistry. DNA sequence was determined on ABI 3730 XL capillary sequencer. High reading length combined with high quality scores were obtained. A typical electropherogram is shown in figure 6.

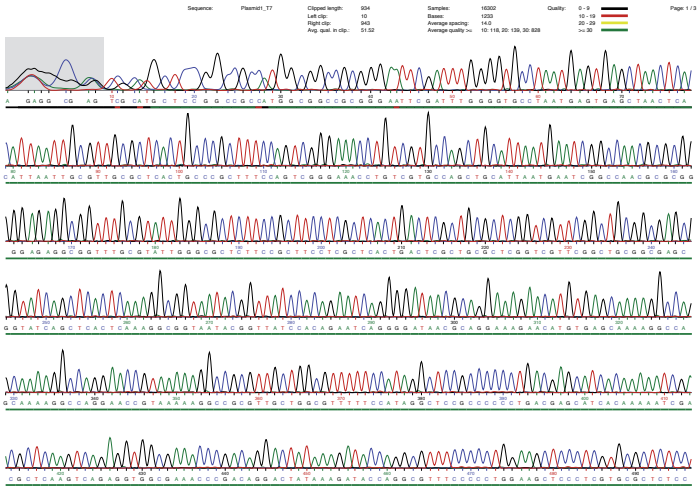

Fig. 6: DNA sequencing of purified plasmid DNA.
Typical sequence of a plasmid DNA purified using NucleoSpin Robot-96 Plasmid kit on the ep*Motion* 5075 VAC. Typically reading lengths of approx. 800 nt with high quality were obtained.

Conclusion

The integration of the MACHEREY-NAGEL NucleoSpin Robot-8 and Robot-96 Plasmid kits into the ep*Motion* 5075 VAC resulted in a flexible system for automated purification of high quality plasmid DNA. The system can be used either for low to medium throughput using the 8-well strip based NucleoSpin Robot-8 Plasmid kit or, for higher throughput, using the 96-well based NucleoSpin Robot-96 Plasmid 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. From typical bacterial cultures of 1.5 mL, average yields of about 6.0 µg with an average $A_{260/280}$ ratio of 1.80 were obtained. The extracted plasmid DNA is of excellent quality and suitable for downstream applications such as restriction analysis or DNA sequencing. Using the described protocols for the NucleoSpin Robot Plasmid kits on the ep*Motion* 5075 VAC automated pipetting system will provide excellent results without the need for extensive method programming or optimization and requires minimum set-up time.

References

[1] Birnboim, H.C. & Doly, J. (1979) Nucleic Acids Res. 7, 1513-1523
Eppendorf Operating Manual ep*Motion* 5075

MACHEREY-NAGEL
NucleoSpin Robot-8 Plasmid kit user manual
NucleoSpin Robot-96 Plasmid kit user manual

Eppendorf Ordering Information

Product	Order no. international	Order no. North America
ep*Motion*® 5075 VAC 230 V (vacuum chamber included)	5075 000.164	n/a
ep*Motion*® 5075 VAC 120 V (vacuum chamber included)	n/a	960020014
Collection Plate Adapter MN	5075 785.064	960002571
Channeling Plate	5075 794.004	960002540
Height Adapter 55 mm	5075 752.000	960002113
Vac Frame 2	5075 785.005	960002261
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® Robot-8 Plasmid kit	
12 x 8 preps	740 730.1
48 x 8 preps	740 730
NucleoSpin® Robot-96 Plasmid 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 Robot-8 Plasmid only)	
1 set	740 682

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