Applications

Note 175 | March 2010

Automated PCR clean-up in 96-well plate and 8-well strip format using the MACHEREY-NAGEL NucleoSpin® 8/96 Extract II kits on the ep*Motion*® 5075 from Eppendorf

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Abstract

In this application note we demonstrate the integration of the MACHEREY-NAGEL NucleoSpin 8/96 Extract II PCR clean-up 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 clean-up of PCR fragments requires minimal set-up time. Fragments from 65 bp up to 10 kbp can be purified. The purified PCR fragments were obtained with high recovery rates and were subjected to downstream applications like sequencing. For 96 samples a processing time of approximately 60 min was achieved.

Introduction

Purification of amplified PCR fragments is often necessary for several downstream applications like sequencing or microarray spotting. The NucleoSpin Extract II kits allow clean-up of PCR fragments using a silica membrane-based bind-wash-elute method. With the purification procedure undesired components (such as detergents, primers, or PCR additives) are removed efficiently [1].
The MACHEREY-NAGEL NucleoSpin Extract II kits allow for PCR clean-up in either medium throughput 8-well strip format or in the 96-well plate format for higher throughput. Both kits, NucleoSpin 8 Extract II or NucleoSpin 96 Extract II can be used fully automated on the ep*Motion* 5075 automated pipetting system. Amplified PCR products are mixed with a binding buffer and loaded onto the NucleoSpin Extract Binding strips or plate. The PCR fragments are bound reversibly to the silica membrane in a subsequent vacuum binding step. Following washing steps and an ethanol evaporation step the purified PCR fragments can be eluted in water or low salt elution buffer. The purified DNA is suitable for subsequent downstream applications, e.g., sequencing or microarray spotting.

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 8 Extract II kit only)
Channeling Plate
Reservoir Rack with Reagent Reservoirs
MACHEREY-NAGEL NucleoSpin 8 Extract II kit
MACHEREY-NAGEL NucleoSpin 96 Extract II kit
PCR fragments

Product use limitations and safety information
Please read the MACHEREY-NAGEL NucleoSpin 8 Extract II or NucleoSpin 96 Extract II manuals before performing the method for the first time.

PCR fragments
PCR products of different sizes (65 bp - 1484 bp) were amplified from a pGEM plasmid in a standard PCR reaction (2 ng template DNA, 0.4 µM forward primer, 0.4 µM reverse primer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 2.0 U *Taq* polymerase).

eppendorf

Determination of yield and purity:
Purified PCR fragments were dried and analyzed by agarose gel electrophoresis in comparison to not purified PCR reactions. DNA was quantified densitometrically using the Scanalytics ONE D Scan software.

Agarose gel electrophoresis:
PCR fragments were analyzed by TAE agarose gel electrophoresis (1 % (w/v) agarose, ethidium bromide stain).

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

Table 1: ep*Motion* 5075 VAC worktable details for NucleoSpin 8 Extract II protocol

Position	Labware	Comment
A2	MN Tube Strips (MN_TP_1200_48)	elution tubes* (***)
A3	epT.I.P.S Motion 1000 µL	
A4	PCR sample plate (EP_TT_PCR_150)	150 µL twin.tec PCR plate
B2	epT.I.P.S Motion 300 µL	
B3	Reagent Reservoirs Position 1: Buffer NT Position 2: Buffer NT3 Position 3: empty Position 4: Buffer NE	 100 mL reservoir 100 mL reservoir empty 30 mL reservoir
Vacuum	NucleoSpin Extract II Binding Strips (MN_FP_8_1400) n Column Holder A** Vacuum Frame 2 Reservoir 400 mL with channeling plate	DNA binding strips collar for vacuum manifold collects waste
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting tool
T2	TM 300-8	300 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 a part of the Starter Set A, see ordering information
***) 96 well MTP can be used optionally

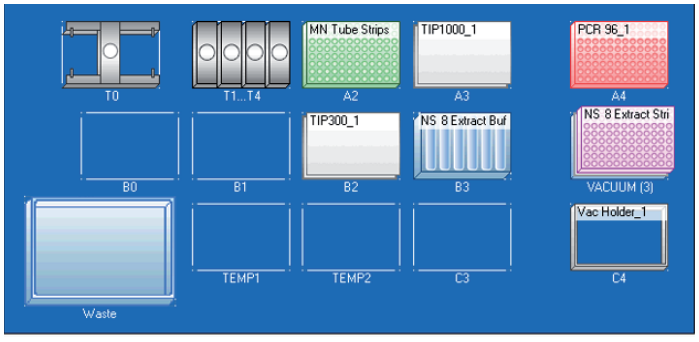


Fig. 1: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for use with the NucleoSpin 8 Extract II kit.

Table 2: ep*Motion* 5075 VAC worktable details for NucleoSpin 96 Extract II protocol

Position	Labware	Comment
A2	Elution Plate (MN_MTP_320)	MTP for elution
A3	epT.I.P.S Motion 1000 µL	
A4	PCR sample plate (EP_TT_PCR_150)	150 µL twin.tec PCR plate
B2	epT.I.P.S Motion 300 µL	
B3	Reagent Reservoirs Position 1: Buffer NT Position 2: Buffer NT3 Position 3: Buffer NT3 Position 4: Buffer NE	 100 mL reservoir 100 mL reservoir 100 mL reservoir 30 mL reservoir
Vacuum	NucleoSpin Extract II Binding Plate (MN_FP_96_1500) Vacuum Frame 2 Reservoir 400 mL with channeling plate	DNA binding plate collar for vacuum manifold collects waste
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting tool
T2	TM 300-8	300 µL 8-channel pipetting tool

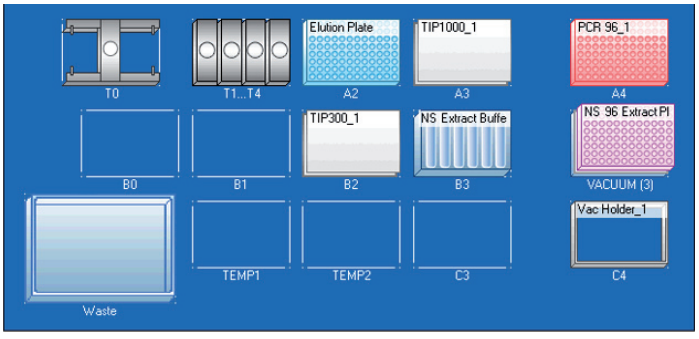


Fig. 2: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for use with the NucleoSpin 96 Extract II

Result

Recovery of PCR fragments
Total yield of PCR fragments after purification usually depends on the PCR reaction itself, size of the amplified PCR fragment, PCR reaction buffer and PCR reaction additives (e.g. betaine, DMSO, detergents). The NucleoSpin Extract II kits show an excellent performance with a broad range of PCR reaction buffer systems. The kits can be used for purification of PCR fragments down to 65 bp. Figure 3 shows a typical result for the purification of PCR fragments of different sizes. The results for the recovery of the individual PCR fragments are summarized in figure 4.

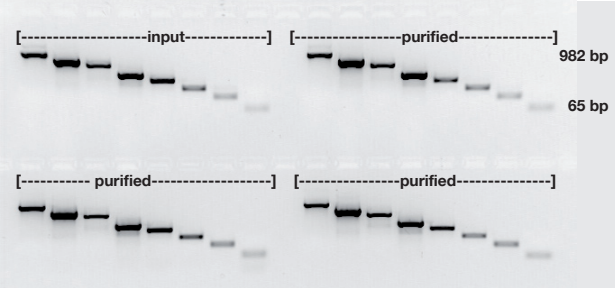


Fig. 3: : Purification of PCR fragments of different sizes. PCR fragments of 65 bp, 164 bp, 252 bp, 359 bp, 459 bp, 645 bp, 782 bp, and 982 bp were purified using the NucleoSpin 96 Extract II kit. Samples were analyzed before purification and following purification on a 1 % TAE agarose gel. The figure shows the purification of samples with the indicated sizes in triplicate. High recovery rates were obtained even with small fragments.

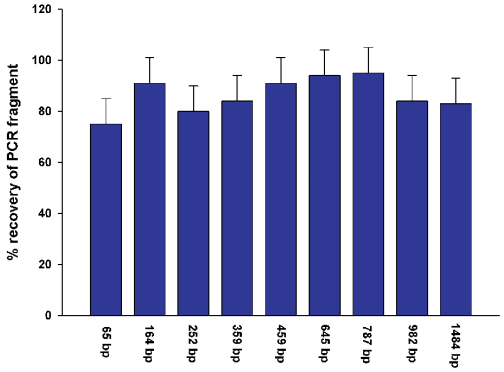
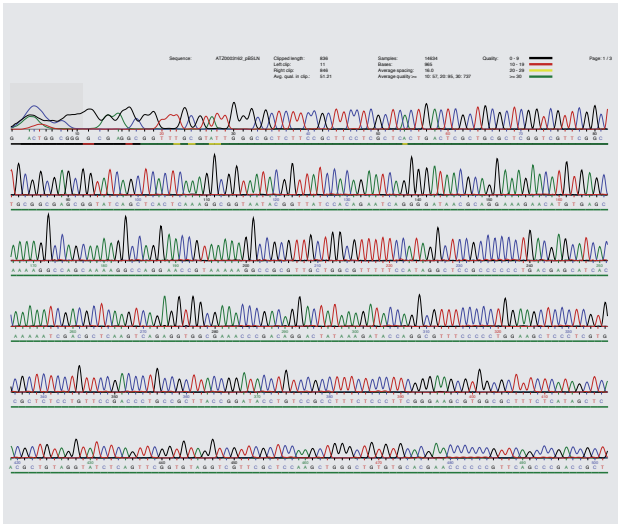


Fig. 4: Recovery rates of PCR fragments. PCR fragments with the indicated sizes were purified with the NucleoSpin 96 Extract II kit. Recovery rate was determined densitometrically. Each value represents the average of six individual purifications with the input set to 100 %. High recovery rates of 75 % to 95 % were achieved.

Sequencing of purified PCR fragments

Primer removal is a critical step for good quality sequencing results of PCR fragments. Incomplete primer removal causes double signals at the beginning of the sequence and may lead to sequencing failure. Using the NucleoSpin 96 Extract II kit allows for purification of small size PCR fragments together with complete removal of PCR primers or primer-dimers. Figure 5 shows the result of a sequencing reaction of a 982 bp PCR fragments. High quality sequencing data together with long reading lengths were obtained.



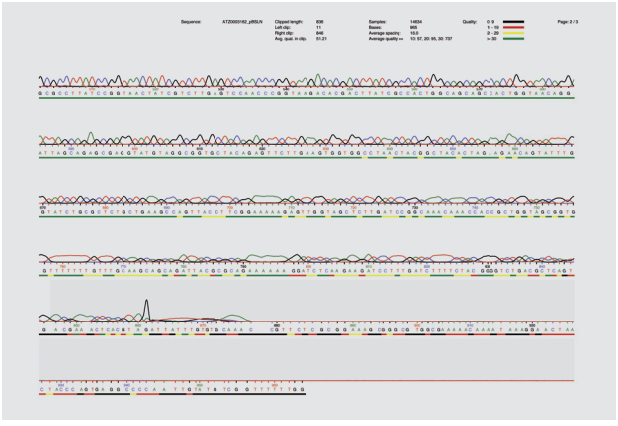


Fig. 5: Automated sequencing of a 982 bp PCR fragment purified with NucleoSpin 96 Extract II kit

Conclusion

The integration of the MACHEREY-NAGEL NucleoSpin 8 Extract II and 96 Extract II kits into the ep*Motion* 5075 VAC resulted in a flexible system for automated purification of PCR fragments. The system can be used either for low to medium throughput using the 8-well strip based NucleoSpin 8 Extract II kit or for higher throughput using the 96-well based NucleoSpin 96 Extract II 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. PCR fragments of different sizes from 65 bp to 1482 bp were purified with recovery rates of 75 % to 95 %. The purified PCR products are of excellent quality and suitable for downstream applications such as DNA sequencing. Combining the NucleoSpin technology and the ep*Motion* 5075 VAC automated pipetting system forms an attractive and versatile system for the automated clean-up of PCR fragments.

References

Eppendorf
Operating Manual for ep*Motion* 5075
[1] Birnboim, H.C. & Doly, J. (1979) *Nucleic Acids Res.* 7, 1513-1523
Macherey-Nagel
NucleoSpin 8 Extract II kit user manual
NucleoSpin 96 Extract II kit user manual

Ordering Information Eppendorf

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
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

Ordering Information MACHEREY-NAGEL

Product	Order no.
NucleoSpin® 8 Extract II kit 12 x 8 preps	740 668
NucleoSpin® 8 Extract II kit 60 x 8 preps	740 668.5
NucleoSpin® 96 Extract II kit 1 x 96 preps	740 658.1
NucleoSpin® 96 Extract II kit 2 x 96 preps	740 658.2
NucleoSpin® 96 Extract II kit 4 x 96 preps	740 658.4
NucleoSpin® 96 Extract II kit 24 x 96 preps	740 658.24
Starter Set A (for NucleoSpin 8 Extract II kit only) 1 set	740 682

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