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Automated genomic DNA purification of marine organisms on the epMotion® 5075 VAC from Eppendorf

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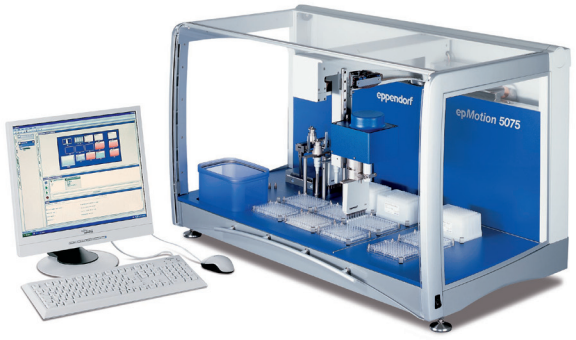
Abstract

In this application note, we describe the integration of the MACHEREY-NAGEL NucleoSpin® 8/96 Tissue kit into the epMotion® 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 epMotion 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. Application data for genomic DNA isolation from different marine organisms: Corals, red and yellow gorgonians, sponges, crustaceans and echinoderms are presented in this note. The extracted DNA is suitable for common downstream applications, such as PCR and sequencing.

Introduction

Typically, DNA isolation from marine invertebrates is difficult. Furthermore, yield and purity of the extracted nucleic acids are often of bad quality. Problems and difficulties in the extraction are caused by pigments, keratin and spicules present in marine invertebrates.
Studies on the genetic population of the invertebrates require specific genetic markers, which are only amplifiable if the isolated DNA is of high quality. In addition, genetic population analyses require large quantities of data sets therefore an automation of the DNA isolation is useful. In response to these requirements the MACHEREY-NAGEL kit NucleoSpin 8/96 Tissue was used in combination with the epMotion 5075 VAC automated pipetting system.
This set up provides a time-saving and robust procedure generating reproducible data of high quality. The MACHEREY-NAGEL NucleoSpin 8/96 Tissue procedure is applicable for fresh or frozen samples from the tested marine models.
The method starts with a mechanical lysis using stainless steel beads followed by an enzymatic sample digestion at 56°C over night. The heat incubation step can be either performed externally or on the instrument, which is equipped with a Thermomodule. All further steps are realized at room temperature. After a centrifugation, the recovered supernatant is bound reversibly to the silica membrane of the NucleoSpin Tissue Binding Plate or Strips. After the 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 such as PCR, real-time PCR or genotyping.
The kits are available in either 8-well strip format or 96- well plate format in order to meet the user requirement in sample throughput. The use of MACHEREY-NAGEL NucleoSpin 8/96 Tissue kits on the epMotion 5075 VAC automated pipetting system provides excellent results without the need for extensive programming, optimization, set-up time and is an overall user friendly procedure.



Materials and Methods

> Eppendorf ep*Motion*® 5075 VAC
> Vac frame 2
> Vac frame holder
> Collection Plate Adapter for MN Tube Strips
> Channeling Plate
> Reservoir Rack with Reagent Reservoirs
> MACHEREY-NAGEL NucleoSpin® 96 Tissue kit
> MACHEREY-NAGEL NucleoSpin 8Tissue kit
> Qiagen Tissue Lyser II
> Centrifuge
> Eppendorf Thermomixer comfort

Product use limitation and safety information
Please read the MACHEREY-NAGEL NucleoSpin 8/96 Tissue manual before performing the method for the first time.

Tissue samples
Each tissue is conserved at -20°C in absolute alcohol:
> Corals: *Corallium rubrum* (≈ 20 mg of tissue).
> Gorgonians: *Paramuricea clavata* (≈ 20 mg of tissue), *Eunicella cavolinii* (≈ 20 mg of tissue).
> Sponges: *Spongia sp* (≈ 45 mg of tissue).
> Crustacean: *Hemimysis margalefi* (≈ 3 mg of tissue).
> Echinoderms: *Ophioderma longicauda* (≈ 45 mg of tissue).

Sample preparation
Grinding step:
140 µl PBS are added to each sample with one 3 mm stainless steel bead. The grinding step is performed twice for 1 minute at 30 Hz.

Lysis Buffer:
Prepare the Proteinase K solution as described in the MACHEREY-NAGEL NucleoSpin 8/96 Tissue user manual. Store it at -20°C for long time storage.
For each series of extraction, prepare a mixture of T1 Buffer and Proteinase K in the following proportions: 180 µl Buffer T1 + 25 µl Proteinase K per sample.

Agarose gel electrophoresis:
Integrity of DNA and PCR results were analyzed by TBE agarose gel electrophoresies (1 % (w/v) agarose, stained with ethidium bromide).

PCR analysis
For each sample, amplification was performed with a nuclear or mitochondrial marker, specific for each species. The PCR reaction conditions cannot be described in this Application Note because the data are not published yet. PCR was performed with an Eppendorf Mastercycler® gradient pro S instrument with the Promega GoTaq® Flexi DNA polymerase kit and specifics primers for each species.

Determination of yield and purity
Yield and purity of DNA were determined using an Eppendorf Biophotometer® Plus with a Hellma® Tray Cell. DNA yield was calculated from A260 values. Purity was determined by calculating the A260/A280 ratio. 4 µL of DNA was analyzed. The correction at 340nm is applied.

Cross contamination Assay
To ensure that the automated pipetting process is reliable and accurate, a negative control was included in each series of extraction to verify the absence of-cross-contamination between the samples.

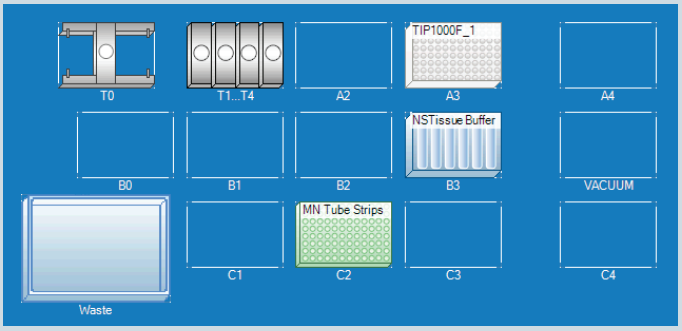


Figure 1: Screenshot from the epMotion® Editor showing the setup of the epMotion® 5075 VAC worktable for use with the MACHEREY-NAGEL NucleoSpin® 96 Tissue kit for **lysis step**.

Table 1: epMotion 5075 VAC worktable details for the MACHEREY-NAGEL NucleoSpin 96 Tissue kit for **lysis step**.

Position	Labware	Comments
T0	Gripper	
T1…T4 A3	TM 1000-8 Dispensing Tool ep T.I.P.S. Motion 1000 µL, filter	8-channel pipetting tool 1000 µL pipette tips
B3	Reagent Reservoirs Position 1 : PBS Position 2 : Buffer T1 - Proteinase k Position 3 : empty Position 4 : empty Position 5 : empty Position 6 : empty Position 7 : empty	 100 mL reservoir 100 mL reservoir
C2	MN Tube Strips	Samples Plate

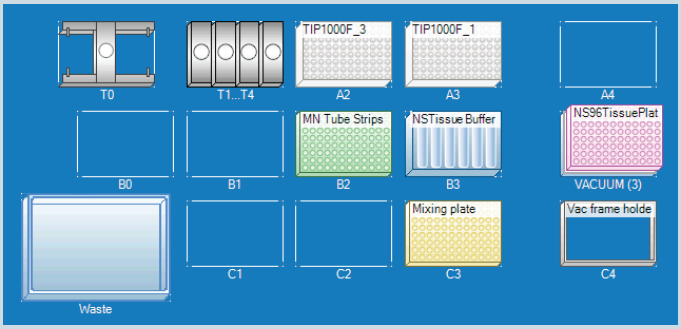


Figure 2: Screenshot from the epMotion® 5075 VAC worktable showing the setup of using the MACHEREY-NAGEL NucleoSpin® 96 Tissue kit for **washing** and **elution steps.**

Table 2: epMotion 5075 VAC worktable details for the MACHEREY-NAGEL NucleoSpin 96 Tissue kit for **washing and elution steps.**

Position	Labware	Comments
T0	Gripper	
T1…T4	TM 1000-8 Dispensing Tool	8-channel pipetting tool
A2	ep T.I.P.S. Motion 1000 µL, filter	1000 µL pipette tips
A3	ep T.I.P.S. Motion 1000 µL, filter	1000 µL pipette tips
B2	MN Tube Strips	elution tubes*
B3	Reagent Reservoirs	
	Position 1 : empty	
	Position 2 : Buffer BQ1	100 mL reservoir
	Position 3 : Abs Ethanol	100 mL reservoir
	Position 4 : Buffer BW	100 mL reservoir
	Position 5 : Buffer B5	100 mL reservoir
	Position 6 : Buffer B5	100 mL reservoir
	Position 7 : Buffer BE**	100 mL reservoir
Vacuum	NS Tissue Binding Strips ***	DNA binding plate
	Vacuum Frame 2	Collar for vacuum manifold
	Reservoir 400 mL with Channeling Plate	Collects waste
C3	MN Square-well Block	Lysis supernatant collected after centrifugation
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2

* Require Collection Plate Adapter for MN tube strips, see ordering information
** Precaution: warm the buffer to 70°C before use.
*** 8-well strips are inserted into MACHEREY-NAGEL Column Holder A which is part of a Starter Set A, see ordering information

Processing

User Intervention – sample addition:
For each sample, a certain quantity of tissue (as described in Tissue sample) was placed into the provided sample lysis tube containing the stainless steel bead. The sample tubes were then deposited on C2 position (Figure 1) on the ep*Motion* 5075 VAC.

Automation:
The automated procedure started with the addition of 140 µL of PBS (Figure 1 - B3 - position 1) to each sample.

User Intervention – grinding step:
Lysis tubes were removed from the ep*Motion*® and sealed with the provided caps.
Grinding was performed twice for 1 minute at 30Hz on the Tissue Lyser II. After grinding, the samples were shortly centrifuged at 4°C for 1 minute to remove any debris. The sealing caps were removed and the plate was returned to position C2.

Automation:
The automated procedure continued with the addition of 200 µL lysis buffer (Buffer T1 + Proteinase K, Figure 1- B3 -position 2) to each sample lysis tube.

User intervention – sample lysis:
Lysis tubes were removed from the ep*Motion*® and sealed with the provided caps. The samples were homogenized by inverting the plate. In a second short centrifugation step at room temperature for 1 minute cell debris was removed. The plate was placed on an Eppendorf Thermomixer comfort at 56 °C, 600 rpm overnight.
After the incubation step, samples were centrifuged at top speed for 10 minutes. The supernatant (240 µL was transferred into the MN Square-well Block, taking care to avoid any potential sample cross contamination. Samples were placed in C3 position (Figure 2).

Automation:
The automated protocol continued by first adding 240 µL BQ1 Buffer (Figure 2 - B3 - position 2), and afterwards 200 µL absolute ethanol (Figure 2 - B3 - position 3). Afterwards, the mixtures were homogenized by pipetting and transferred into the NS Tissue Binding Binding Plate (Vacuum).
Genomic DNA was bound by a subsequent vacuum binding step at 400 millibar for 2 minutes. The following three washing steps were performed with 600 µL BW Buffer for the first washing step (Figure 2 - B3 - position 4), and 600 µL Buffer B5 for the second and third washing step (Figure 2 - B3 - position 5 and 6). Each washing step was performed at 400 millibar for 2 minutes, followed by an ethanol evaporation step at 400 millibar for 10 min, drying the silica membrane.

User intervention - elution:
Insert the 70 °C pre-warmed Elution Buffer BE Buffer at B3 position (Figure 2).

Automation:
The final automated elution was performed in two steps by adding 100 µl Buffer BE each. For both elution steps vacuum was applied at 400 millibar for two minutes to receive a final 200 µl elution fraction.

Results

Table 3: DNA Yield and purity.

Sample	Initial Sample Weight (mg)	n	Average purity A_{260}/A_{280}	Average concentration (ng/µl)	Average yield (µg)
P. clavata	20	8	1.83	55.77	11.15
E. cavolinii	20	8	1.80	33.84	6.77
C. rubrum	20	8	1.95	34.51	6,90
Sponge	45	14	1.95	114.16	22.83
H. margalefi	3	8	1.53	8.29	1.66
O. longicauda	45	8	1.91	74.6	14.92

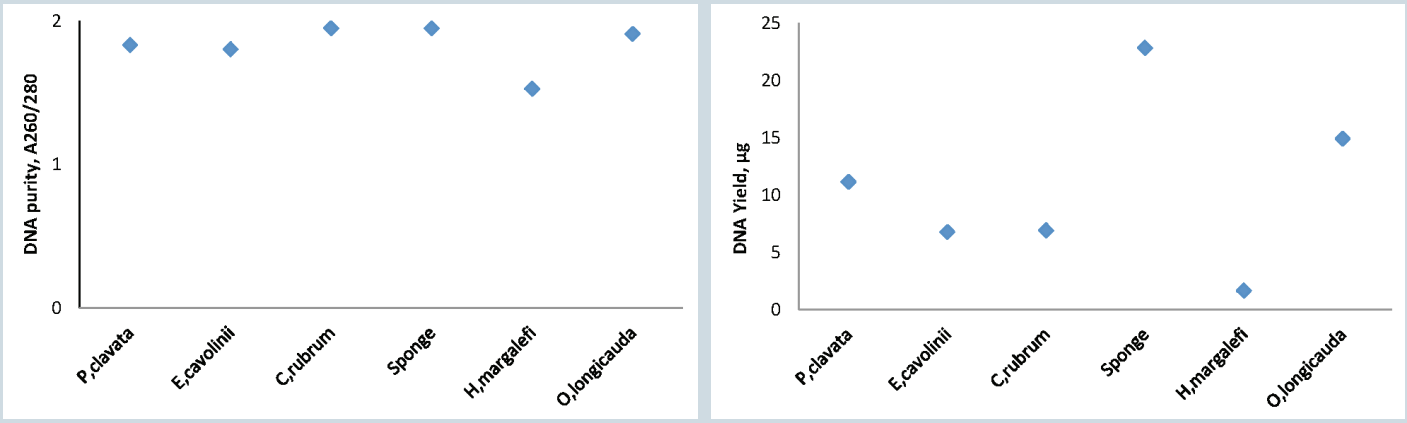


Figure 3: DNA yield and purity.

DNA yield and purity

As shown in Table 3 and Figure 3, DNA from various marine invertebrate samples can easily be purified with the MACHEREY-NAGEL NucleoSpin® 8/96 Tissue kit and the automated epMotion® 5075 VAC system. The method delivers consistently high purity DNA with an average A260/280 ratio of 1.83, indicating low protein contamination. The average yield across the sample types was 10.71 µg.

DNA Quality

In order to demonstrate the quality of the isolated DNA, the purified DNA samples have been analyzed by PCR using several species specific markers. The data cannot be shown, because the results are of confidential nature.

Cross contamination

Both the spectrophotometric assays and the PCR analysis did not detect DNA in the negative controls indicating an extraction without cross contaminant.

Conclusion

The integration of the MACHEREY-NAGEL NucleoSpin® 96 Tissue kit into the epMotion® 5075 VAC platform provides a reliable, convenient and flexible system for the automated purification of high quality DNA from invertebrate marine models. The 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. The purified genomic DNA is of excellent quality and suitable for downstream applications such as PCR or DNA sequencing. Combining the NucleoSpin® technology and the epMotion 5075 VAC automated pipetting system forms an attractive and versatile system saving time to increase the throughput for reproducible purification.

References

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

Macherey-Nagel
NucleoSpin 8 Tissue kit user manual
NucleoSpin 96 Tissue kit user manual

Ordering Information Eppendorf

Description	Order no. International	Order no. North America
ep*Motion*® 5075 VAC 100 - 240 V (vacuum chamber included)	5075 000.016	960020014
ep*Motion*® 5075 VAC PC version (vacuum chamber included)	5075 000.768	960020222
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 126.505	960051009

Ordering Information MACHEREY-NAGEL

Description	Order no.
NucleoSpin® 8 Tissue (12 x 8 preps)	740740
NucleoSpin® 8 Tissue (60 x 8 preps)	740740.5
NucleoSpin® 96 Tissue (2 x 96 preps)	740741.2
NucleoSpin® 96 Tissue (4 x 96 preps)	740741.4
NucleoSpin® 96 Tissue (24 x 96 preps)	740741.24
Starter Set A (Vacuum adapter set for NucleoSpin 8 Tissuel kit only) 1 set	740682

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