eppendorf

APPLICATION NOTE No. 419

Purification of High-Quality DNA from FFPE Samples

Abstract

DNA purification of formalin-fixed, paraffin embedded samples is an important standard procedure used in hospitals worldwide. The analysis of these samples can lead to insights in the fields of oncology, hematology or immunology and is needed for drug development, diagnostic purposes or experimental research. However DNA purification is not trivial and contaminants present in the FFPE-sample can hinder the process. Furthermore, manual processing of the sample and DNA purification can have multiple diffculties resulting in low DNA yield, or low DNA quality. We show an automated method, using mouse FFPE samples and the NucleoMag® DNA FFPE kit together with ep*Motion*® 5075t for optimal DNA quality and yield. Additionally, we compared manual and automated processing showing the reliability of the automated solution.

Introduction

The collection and storage of formalin-fixed, paraffin-embedded (FFPE) clinical samples is a standard procedure in hospitals worldwide and represent an invaluable resource for retrospective analyses. To breakdown and identify molecular correlations between, e.g., carcinogenesis, patient treatment, and disease outcomes, the purification of substantial amounts of high quality DNA is essential. However, there are several issues in using FFPE material for subsequent downstream molecular applications. The fixation process leads to cross-linked nucleic acids resulting in severe fragmentations. Moreover, FFPE extraction protocols often involve tedious manual steps and hazardous chemicals such as xylene.
MACHEREY-NAGEL® now provides the automation ready, magnetic bead based NucleoMag DNA FFPE extraction kit to speed up the purification process. MACHEREY-NAGEL developed an odorless Paraffin Dissolver (patented) allowing the effective lysis in a convenient two phase system, followed by a decrosslinking step to ensure optimal performance in downstream applications. The extracted DNA can be directly used as a template for PCR, NGS, blotting, or various other enzymatic reactions.
MACHEREY-NAGEL is continuously expanding on its collaborations with automation partners in order to offer more support to high throughput customers. We now present the first automation of the NucleoMag DNA FFPE kit on the ep*Motion* 5075t system including the time consuming deparaffinization and sample lysis procedure. The method eliminates a maximum of hands on time, by automating the deparaffinization, sample lysis, DNA decrosslinking and the DNA isolation. Our optimized protocol requires only one defined manual intervention, allowing the full processing of 24 samples, including deparaffinization and lysis within approximately 5 h 50 min.

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Table 1: Product at a glance

NucleoMag DNA FFPE	
Technology	Magnetic beads
Sample material	≤ 5 mg tissue, < 15 mg paraffin
Preparation time	Approx. 5 h 50 min including sample preparation and extraction for 24 samples. (Note: Preparation time can be shortened.)
Typical yield	Strongly depending on sample type, quality, and amount
Elution volume	> 25 µL

Table 2: Product at a glance

ep*Motion* 5075t	
Pipetting type	Air-cushion
Volume range	0.2 µL – 1 mL
SLAS/ANSI deck positions	14.5
Dimensions (W × D × H)	107 × 61 × 67 cm / 43 × 24 × 27 in Weight w/o accessories 87 kg / 191.8 lb

Materials and methods

The paraffin is removed from the tissue with the Paraffin Dissolver followed by sample lysis in a convenient two phase system with an effective lysis buffer. The released DNA in the lower aqueous phase is still highly crosslinked. Therefore, heat incubation in a special Decrosslinking Buffer is carried out to separate the DNA (see the NucleoMag DNA FFPE kit protocol for more detailed information). The lower aqueous phase is transferred manually to a fresh 1.5 mL reaction tube in order to ensure a reliable DNA extraction procedure.

Subsequent DNA isolation is performed on the automation platform ep*Motion* 5075t. The isolation principle is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions during the binding step. Contaminants, such as salts or lipids, are then removed by three washing steps, while nucleic acids are reversible bound to the paramagnetic beads. Pure DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

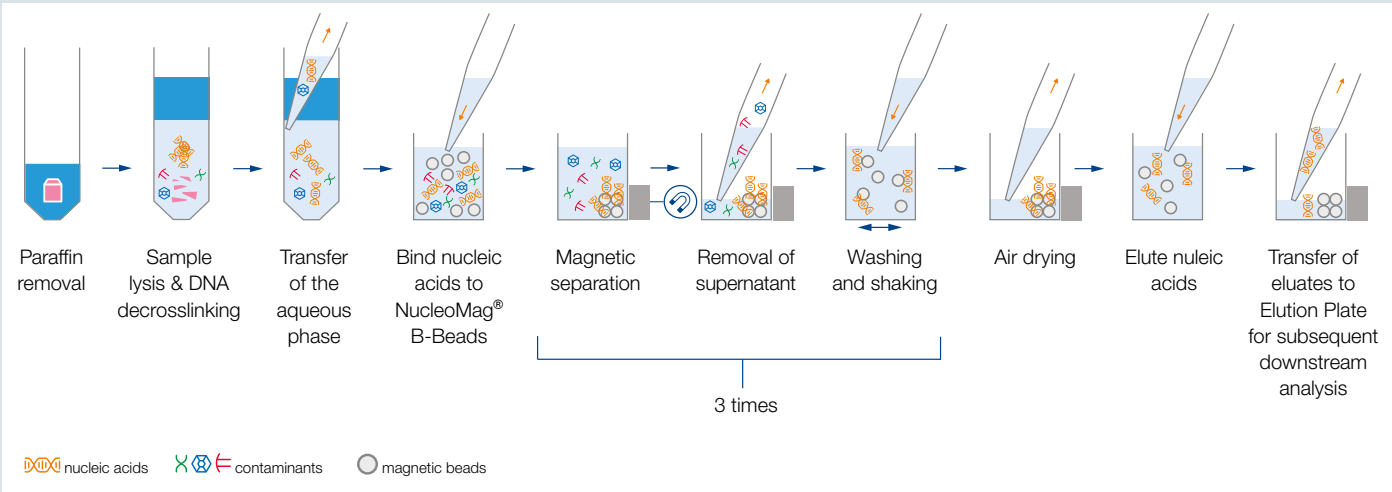

Figure 1: Schematic description of all steps during DNA purification of FFPE samples using a liquid handling workstation.

Application data

Automated isolation of DNA from various mouse FFPE samples
DNA was isolated from various mouse FFPE samples (n = 4; section size muscle: 1 mm^2; liver: 12 mm^2; esophagus: 3 mm^2; lung: 5 mm^2; kidney: 8 mm^2; brain: 4,5 mm^2) using the NucleoMag DNA FFPE kit on an ep*Motion* 5075t system. The total yield was determined by UV spectrometry (Figure 2, dark blue bars). A subsequent qPCR analysis was performed with a Taqman® Probe for a GADPH amplicon using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System (orange squares) using 2 µL undiluted eluat. The results demonstrate a reliable qPCR-performance for all tested mouse FFPE samples.

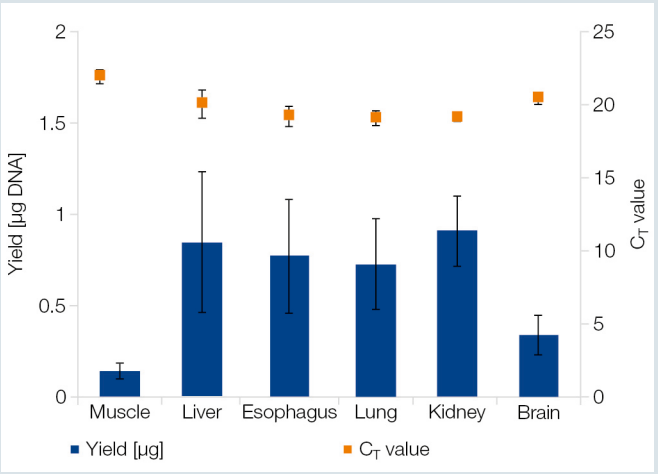

Figure 2: Automated isolation of DNA from various mouse FFPE samples

Comparison of automated and manual processing
DNA was isolated from mouse FFPE samples (n = 4; 10 mg each) using the NucleoMag DNA FFPE kit in an automated manner on the ep*Motion* 5075t system (dark blue bars) or manually (grey bars, both figure 3). A subsequent qPCR analysis was performed with a Taqman Probe for a GADPH amplicon using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems 7500 Real-Time PCR System. 2 µL undiluted eluat were used for qPCR analysis. The results demonstrate a reliable performance of the established, automated method.

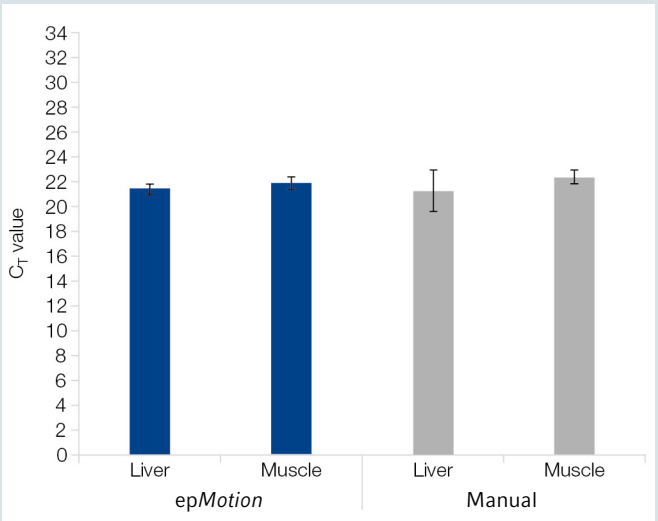

Figure 3: Comparison of automated and manual processing

Automate your FFPE sample handling

MACHEREY-NAGEL delivers a ready to go solution for your automated FFPE sample handling. We adapted the NucleoMag DNA FFPE procedure on the ep*Motion* 5075t system to facilitate your DNA extraction workflow.

> Automated sample preparation and sample extraction with only one manual user intervention
> Reliable performance using NucleoMag DNA FFPE on the ep*Motion* 5075t system
> Excellent recovery and easy handling due to paraffin removal buffer

Ordering information

Description	Pack of	Order no. International
ep*Motion*® 5075t, basic device incl. Eppendorf ThermoMixer®, epBlue™ software, mouse, waste box, 100 – 240 V ± 10% / 50 – 60 Hz ± 5%, 0.2 µL – 1 mL		5075000969
NucleoMag®, Kit based on magnetic bead technology for the isola-tion of genomic DNA from formalin-fixed paraffin-embedded samples	1 x 96	744320.1
	4 x 96	744320.4
NucleoMag® SEP*, Static magnetic separator	1	744900
Square-well Block*, 96-well deep-well block with 2.5 mL square-wells, u-bottom for magnetic separation	4	740481
	24	740481.24
Elution plate U-bottom*	24	740486.24

*available at www.mn-net.com/

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