

Automated purification of high quality genomic DNA

from various tissues using the Eppendorf MagSep Tissue gDNA Kit on the Eppendorf epMotion® M5073

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Abstract

The Eppendorf MagSep Tissue gDNA Kit has been specifically developed to be used with the Eppendorf epMotion M5073 automated pipetting system. It provides a flexible, easy to use solution for magnetic bead-based, automated purification from 1-24 samples of high quality, ready to use genomic DNA from a broad variety of sample sources, such as tissue, mouse tails, cultured cells and bacteria. In this study different mouse tissues were subjected to automated

DNA purification using the Eppendorf MagSepTissue gDNA Kit. Genomic DNA purified in this manner showed yields and qualities that were comparable to manual methods and even superior to silica column-based technologies. No cross contamination was detectable and the genomic DNA was directly compatible with downstream real-time PCR amplification. Typical yields ranged from 13.5 µg (10 mg mouse tail material) to 33 µg (10 mg mouse liver).

Introduction

With the introduction of the Eppendorf epMotion M5073 automated pipetting system with integrated Thermomixer (TMX) and Magnetic Finger Module a powerful tool for the automation of magnetic bead-based applications became available (Figure 1). The Eppendorf MagSep Tissue gDNA Kit is a suitable addition that enables the user to easily perform hands free, walk away automated genomic DNA purification. This combination of instrument and kit delivers ready to use, high quality, high yield genomic DNA that is directly compatible with downstream applications. The purification process benefits from the combination of the well-known Eppendorf TMX module and a magnetic separator, allowing the entire process being performed without any labware transport steps. In addition the reagents of the Eppendorf MagSep Tissue gDNA Kit are delivered in a tray that can directly be placed into a ReagentRack on the worktable of the Eppendorf epMotion, rendering error prone buffer decanting obsolete.



Figure 1: Eppendorf epMotion M5073

After use the individual bottles can be simply recapped and stored in the tray for future use, adding extra convenience. A Prep-Assistant software (Figure 2) guides the user through the entire setup process, with features such as selecting the type of vessel for the eluates or the adjustment of the elution volume to get the optimum yield or the maximum concentration of DNA. Further novelties of the system are the option to re-use tips (Eppendorf SafeRack tips are recommended to avoid cross contamination), decreasing the amount of tips required per run and the integration of a removable liquid waste tub into the waste position, freeing up deck space and facilitating the separate disposal of liquid and solid waste. Supplied with the kit are Eppendorf DNA LoBind Tubes to guarantee the highest possible recovery of nucleic acids. The method offers great flexibility since the purification process is performed in single 2 mL Eppendorf tubes thus allowing variable sample numbers from 1 – 24.

Materials and Methods

Materials

Eppendorf epMotion M5073 delivered with

- Dispensing Tool TS 1000
- Dispensing Tool TS 50
- Liquid waste tub
- Waste tub
- ReagentRack
- PrepRack
- Rack 24 Eppendorf Safe-Lock

Eppendorf consumables

- epT.I.P.S. Motion Filter SafeRacks 1000 µL
- epT.I.P.S. Motion Filter SafeRacks 50 µL
- Eppendorf MagSep Tissue gDNA Kit
- 2 mL DNA LoBind Tubes (included in kit)

Methods

If not stated otherwise, 10 mg of mouse tissue were used as starting material. For the processing of tissue samples, 25 µL Proteinase K solution and 200 µL lysis buffer were added manually to 10 mg tissue cuts and the lysis was performed overnight in a Thermomixer comfort at 56 °C with shaking. After complete lysis, the DNA-containing supernatant was cleared by centrifugation at 5600 x g in an Eppendorf Centrifuge 5424 and 225 µL tissue lysate were subsequently transferred into a fresh 2 mL Eppendorf DNA LoBind Tube (provided with the kit) for subsequent automated processing by the epMotion M5073. For 24 tissue samples the total

processing time was approximately 2 hours excluding overnight lysis. The automated purification of gDNA from tissue samples was compared to a manual purification using the same reagents and conditions plus two widely used competitor kits based on silica column technology, following the manufacturer's instructions. The isolation performance of the individual purification methods was assessed by UV measurements of 3 µL (Eppendorf µCuvette G1.0 + Eppendorf BioSpectrometer kinetic) and gel electrophoresis of the final eluates.

To assess whether cross contamination due to the re-use of tips occurred, tissue lysates of 5 mg mouse tail material were processed in a checkerboard pattern with PBS buffer and the resulting eluates were subjected to real-time PCR.

Results and Discussion

Tip Usage

A purification run with 24 tissue samples with re-use tips function for the wash steps (default setting) requires 76 1000 µL tips, whereas a single use would result in a total consumption of 124 tips.

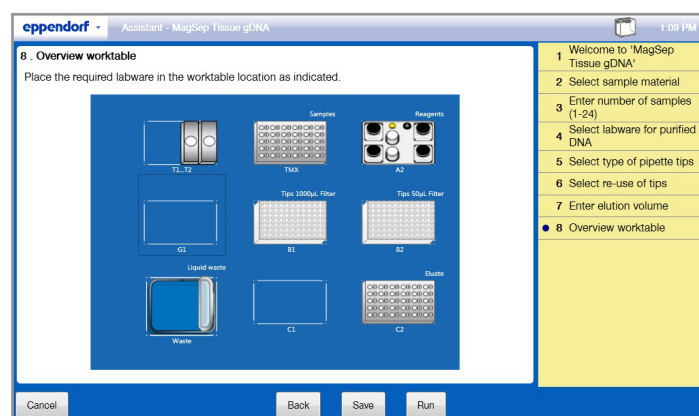


Figure 2: Screenshot from the epMotion Prep-Assistant showing the setup of the epMotion M5073 worktable for the use with the Eppendorf MagSep Tissue gDNA Kit.

Yield and Purity of gDNA from tissue

Depending on the type of tissue, typical DNA yields from 10 mg sample material were in the range of 13.5 – 33 µg for the automated method (Figure 3). DNA purity was good as indicated by A260/280 ratios between 1.67 – 1.89. A detailed overview of the different tissue types is given in Table 1.

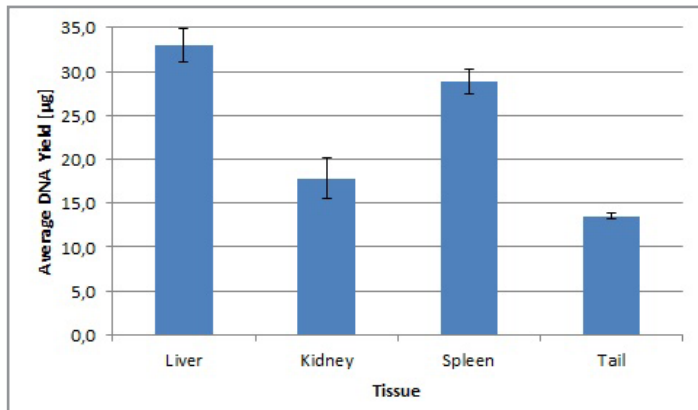


Figure 3: DNA yields from various tissue types

Genomic DNA was purified from 10 mg mouse tissue samples using the Eppendorf MagSep Tissue gDNA Kit on the epMotion M5073. Each bar represents the average DNA yield with standard deviations from six replicates.

Table 1: Yield and Purity of DNA

Tissue type	Mean DNA Yield (n=6)		Mean DNA Purity (n=6)	
	[µg]	%CV	(A260/280)	%CV
Mouse liver	33.0	5.83	1.67	3.31
Mouse kidney	17.8	12.82	1.72	2.59
Mouse spleen	28.8	4.75	1.89	0.72
Mouse tail	13.5	2.85	1.84	1.00

Reproducibility

In a separate experiment, DNA isolated from 24 mouse tail clippings (10 mg each, combined in a master lysate) using the Eppendorf epMotion M5073 showed yields from 16.6 – 17.8 µg, with an average yield of 17.3 µg and a CV of 2.09 %, giving evidence for a high consistency of the automated method (Figure 4a). The obtained genomic DNA was of high molecular weight as indicated by a distinct band and the absence of low molecular weight smear in gel electrophoresis (Figure 4b).

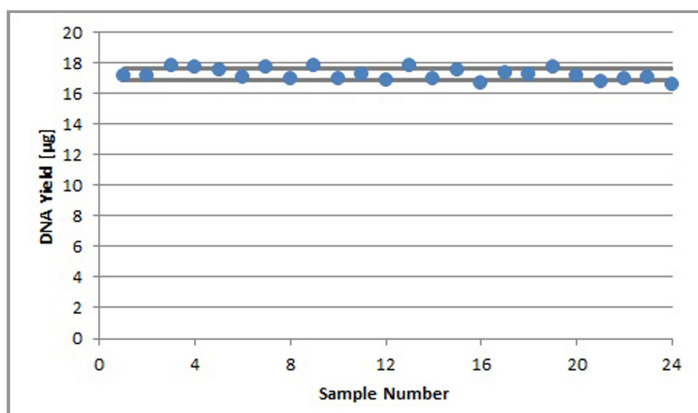


Figure 4a: Reproducibility of DNA purification

Genomic DNA was isolated from 24 mouse tail samples, 10 mg each. The average yield was 17.3 µg gDNA with a CV value of 2.09 %, giving evidence for a high level of consistency over 24 replicates. Each symbol represents the DNA yield of one sample; grey bars denote the average yield +/- 1 SD.

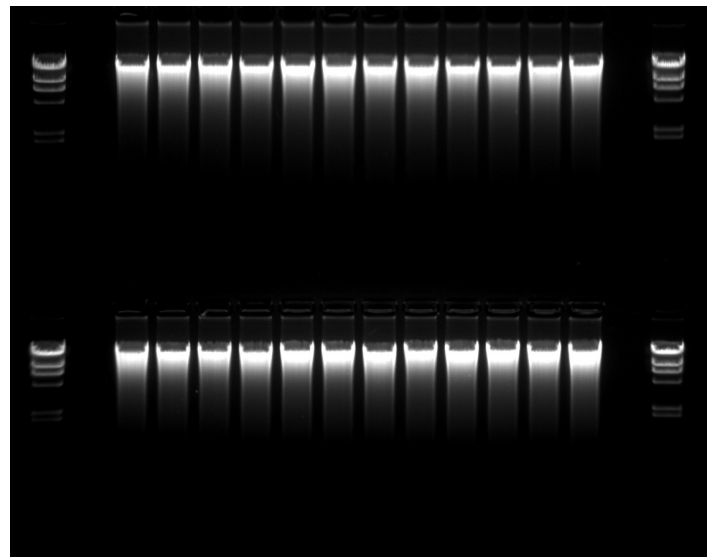


Figure 4b: High quality genomic DNA

On a 1 % agarose gel 7.5 µL out of 75 µL mouse tail gDNA eluates were separated electrophoretically. The obtained DNA displayed a high molecular weight as indicated by the absence of low molecular weight smear. DNA size standard: Lambda HindIII (Fermentas).

Performance comparison

The isolation performance of the automated Eppendorf MagSep Tissue gDNA Kit was compared to two widely used silica column based kits and the manual method using the same reagents. Figure 5 clearly shows that the magnetic bead-based methods (both automated and manual) yielded up to twice as much DNA with purities comparable to the silica column methods, with higher reproducibility of the automated method.

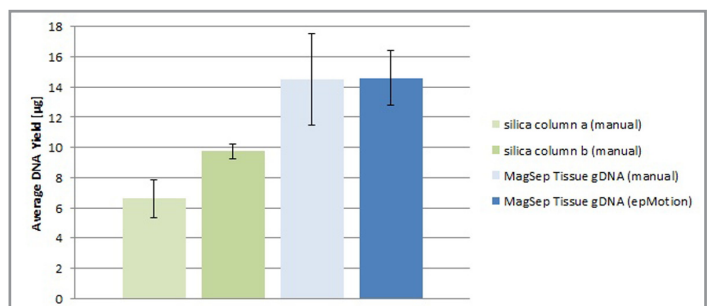


Figure 5: DNA yields achieved with different methods

Genomic DNA was isolated with different methods from 10 mg mouse tail samples. For each method 6 samples were processed in parallel. Other supplier's methods a and b were based on silica column technology. DNA yields obtained by the magnetic bead based manual and automated methods were considerably higher than the yields from silica column based methods.

Cross contamination analysis

DNA samples obtained from tissue samples gave a positive real-time PCR signal with a CP value around 23, whereas signals with CP values >35 were obtained from eluates that originated from PBS pre filled positions, indicating the absence of cross contamination (Figure 6). This finding was also verified by melt curve analysis and by the absence of PCR products as shown by gel electrophoresis (Figure 7).

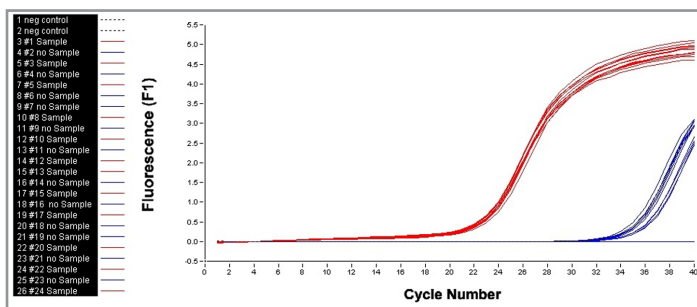


Figure 6: No cross contamination detectable

5 mg tissue samples were processed in a checkerboard pattern along with PBS, re-using the tips. The final eluates were diluted 1:10 and used in a real-time PCR to amplify a 212 bp fragment. Specific amplification was only detectable for positions with tissue lysates (red); from PBS filled positions no specific amplification was obtained (blue). Real-time PCR was performed on a Roche LightCycler system.

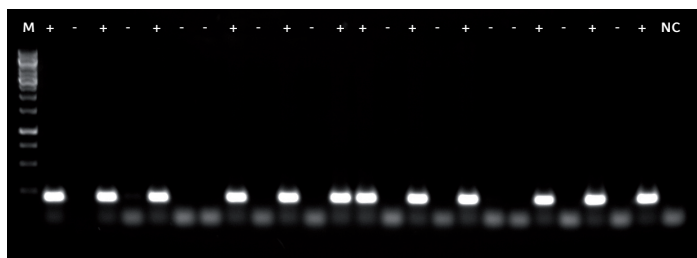


Figure 7: No cross contamination detectable

Real-time PCR products were loaded on a 1 % agarose gel and separated electrophoretically. Only eluates originating from tissue samples showed expected amplification of a 212 bp product, PBS filled positions did not show any amplification.

M: DNA size standard, 1 kb Ladder (Fermentas), + denotes tissue sample, - denotes PBS and NC denotes the PCR negative control.

Conclusion

The Eppendorf *epMotion* M5073 system in combination with the Eppendorf MagSep Tissue gDNA Kit allows the user to conveniently and efficiently purify genomic DNA from tissue and cell samples with minimum hands on time and a maximum level of automation.

A broad range of sample material can be processed and reproducibly yields large amounts of high quality, ready to use genomic DNA. For a 24 samples run overall consumption of 1000 µL tips is reduced from 124 to 76 by the implementation of the Eppendorf SafeRack that allows re-using tips during a run, directly decreasing the cost per sample. The safety and reliability of this feature was proven by the fact that no cross contamination was detectable by real-time PCR.

Due to the flexibility and usability of the system – 1 to 24 samples can be processed; reagents are supplied ready to use in a tray and recappable, assistant guided run setup - the *epMotion* M5073 and the Eppendorf MagSep Tissue gDNA Kit form an attractive automation bundle for low to medium throughput DNA purification requirements from a broad variety of sample types.

Ordering information

Description	International Order no.	North America Order no.
epMotion® M5073 for automated nucleic acid preparation of 1–24 samples with Eppendorf MagSep Kits, 100–240 V/50–60 Hz, with Control-Panel, Software epBlue and Prep-Assistants, TS 50, TS 1000, PrepRack, ReagentRack, Rack 24 Eppendorf Safe-Lock Tubes	5073 000.205	5073 000.205
Centrifuge 5424	5424 000.410	022620401
Eppendorf µCuvette™ G1.0	6138 000.018	6138000018
Eppendorf BioSpectrometer® kinetic	6136 000.002	952000006
Thermomixer® comfort	5355 000.011	6136000010
Thermoblock, 24 x 2.0 mL	5362 000.019	022670107
MagSep Tissue gDNA Kit	0030 450.000	022670549
2 mL DNA LoBind® Tubes	0030 108.078	0030 450.000
epT.I.P.S.® Motion Filter SafeRacks 1000 µL	0030 014.650	022431048
epT.I.P.S.® Motion Filter SafeRacks 50 µL	0030 014.618	0030 014.650

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