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APPLICATION NOTE No. 460

Automated Microbial DNA Purification Using the MagAttract® PowerSoil® Pro Kit on ep*Motion*® 5075t or 5075vt

Authors

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Abstract

This application note describes the configuration and methods for automated microbial DNA purification from different types of soil and stool samples using QIAGEN® MagAttract PowerSoil Pro DNA Kit. The setup for the ep*Motion* 5075t and 5075vt platform allows for the purification of up to 96 samples at once. Samples were manually lysed and treated by a one-step inhibitor removal procedure to eliminate contaminants, followed by an automated purification protocol to extract and clean up microbial DNA. Automated DNA purification by ep*Motion* helps to reduce the workload needed for this routine procedure and ensures reproducibility.
Additionally, the results demonstrate that the new MagAttract PowerSoil Pro DNA Kit improves the process and efficiency of soil DNA extraction over its predecessor MagAttract PowerSoil DNA Kit. When using the new kit, the automated DNA extraction processes enables higher yields of pure, high-quality DNA from challenging soil samples, which is suitable for use in Next-Generation Sequencing (e.g. 16S rRNA sequencing).

Introduction

Metagenomics has opened new doors in the analysis of complex microbial communities without having to rely on culture methods. Unculturable bacteria constitute a significant part of the gut microbiome [1], soil and other environmental samples, and were previously difficult to characterize with standard techniques. With increasing understanding of the microbial world, it becomes evident that the microbiome plays a key role in both shaping human health and our ecosystems [1, 2]. However, some challenges remain when it comes to the reproducibility of experiments (mostly 16S rRNA gene sequencing) aiming to represent the total microbial diversity. Setting up the optimal DNA isolation procedure is crucial for minimizing biases [3]. Some organisms are more sensitive to lysis then others, so an efficient and uniform lysis - preferably by a combination of mechanical and chemical methods - increases the reliability of the data obtained and gives a more precise picture on the microbial community really present in the sample. Moreover, stool and soil samples contain inhibitory substances which impair downstream processing of the isolated DNA [4]. And finally, manual handling of a multitude of samples can also introduce errors in the experimental results due to the repetitive and tedious nucleic acid isolation procedures. Here we introduce a microbial DNA purification protocol automated on the ep*Motion* 5075t or 5075vt using the MagAttract PowerSoil Pro DNA Kit with improved sample lysis and inhibitor removal technology. The resulting purified and inhibitor-free DNA can be used in sensitive downstream applications such as PCR, qPCR and NGS. Automation of the protocol ensures great reproducibi-lity of your experiments and frees up time for the evaluation and interpretation of your results!

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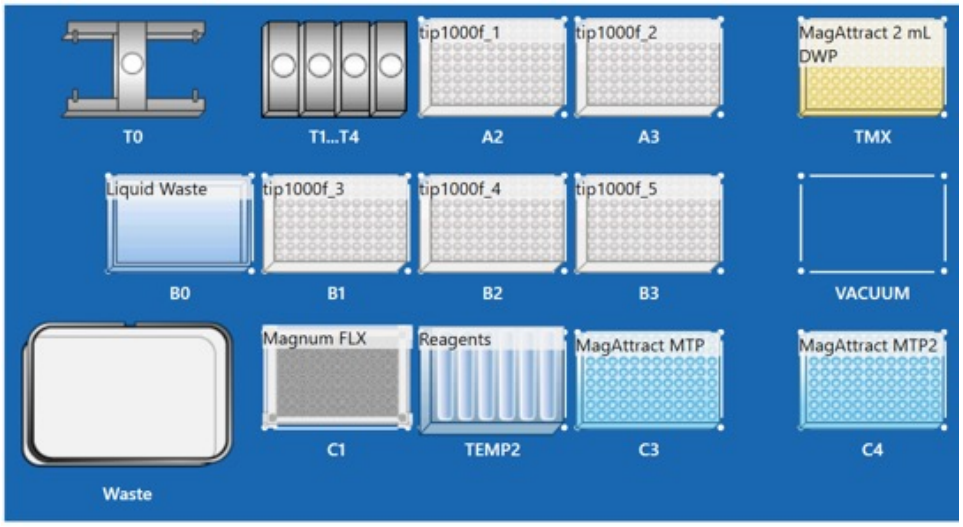
Materials and Methods

In this application note, all DNA isolation steps have been demonstrated using the ep*Motion* 5075vt model, however, each step can be carried out on the ep*Motion* 5075t in the same way. A representative image of the ep*Motion* worktable for the isolation of 96 samples with the MagAttract PowerSoil Pro DNA Kit is shown in Figure 1 [5].



Position	Item
A2	1,000 µL filter tips
A3	1,000 µL filter tips
TMX	MagAttrackt 2 mL DWP
B0	400 mL reservoir
B1	1,000 µL filter tips
B2	1,000 µL filter tips
B3	1,000 µL filter tips
C1	Magnum FLX Magnet Adapter
C2	ReservoirRack + 100 mL reservoir (5x)
C3	MagAttract MTP
C4	MagAttract MTP2
Parking positions	1,000 µL filter tips (4x)

Sample number	Tip consumption (1000 µL with filter)	ep*Motion*	Nr. of user interventions
24	224	2h 30	0
48	416	3h 00	1
94	800	4h 14	3

Figure 1: Worktable layout for the MagAttract PowerSoil Pro DNA Kit – For processing of up to 96 samples with tip consumption and runtime information. The consumables labelled with MagAttract are part of the isolation kit, the other consumables and accessories are listed in detail in the ordering information. (DWP: Deepwell Plate, MTP: Microtiter Plate)

1 Comparison of DNA yield and purity after isolation from various soil and stool samples using either the MagAttract PowerSoil Pro DNA Kit or the legacy MagAttract PowerSoil DNA Kit on the ep*Motion* 5075vt

DNA was isolated from soil (250 mg) and stool (100 mg). Samples were added to a 96 well bead beating plate (PowerBead Pro Plate). Cell lysis occurred by a combination of mechanical and chemical methods (Figure 2). In the following step humic acids were removed using Inhibitor Removal Technology®. At this point 450 µL lysate per well was transferred to a clean plate and automatically processed on the ep*Motion* 5075vt. Total genomic DNA was captured using specialized magnetic beads. During the following purification steps the beads were pulled using a Magnum FLX ring magnet for 96 well plates allowing for gentle and complete automated removal of the supernatant. Beads were washed three times (MW1 buffer for wash step 1 and 80% ethanol for wash step 2 and 3) before the DNA was eluted using a 10 mM Tris (pH 8) buffer. Samples isolated by the legacy MagAttract PowerSoil Pro DNA Kit (entire lysate) were processed as previously described [6]. The DNA concentration, as well as 260/280 nm and 260/230 nm ratios to address DNA purity, were determined using the QIAxpert® System.

2 Assessment of inhibitor removal in DNA samples isolated by using either the MagAttract PowerSoil Pro DNA Kit or the MagAttract PowerSoil DNA Kit on the ep*Motion* 5075vt

The co-isolation of inhibitors was assessed via Inhibition qPCR. The internal control (IC) from the QuantiFast Pathogen + IC Kit was spiked with 4 µL isolated DNA. After IC amplification via qPCR, the Ct values were compared to the Ct values of water added to the IC (representing no inhibition) by calculating:

$$\Delta Ct = Ct_{\text{PCR spiked with isolated DNA}} - Ct_{\text{PCR spiked with water}}$$

3 Assessment of consistency and cross-contamination during the isolation process using the MagAttract PowerSoil Pro DNA Kit on the ep*Motion* 5075vt

In order to test the reproducibility of the automated DNA isolation steps the results for yield and quality measures were compared across different wells of a 96 well plate. Furthermore, to exclude cross-contaminations between wells a 96 well plate was filled with sample lysate and water in a checkerboard pattern. Following the isolation steps, all wells were analyzed in a 16S qPCR reaction to estimate copy numbers in the eluate.

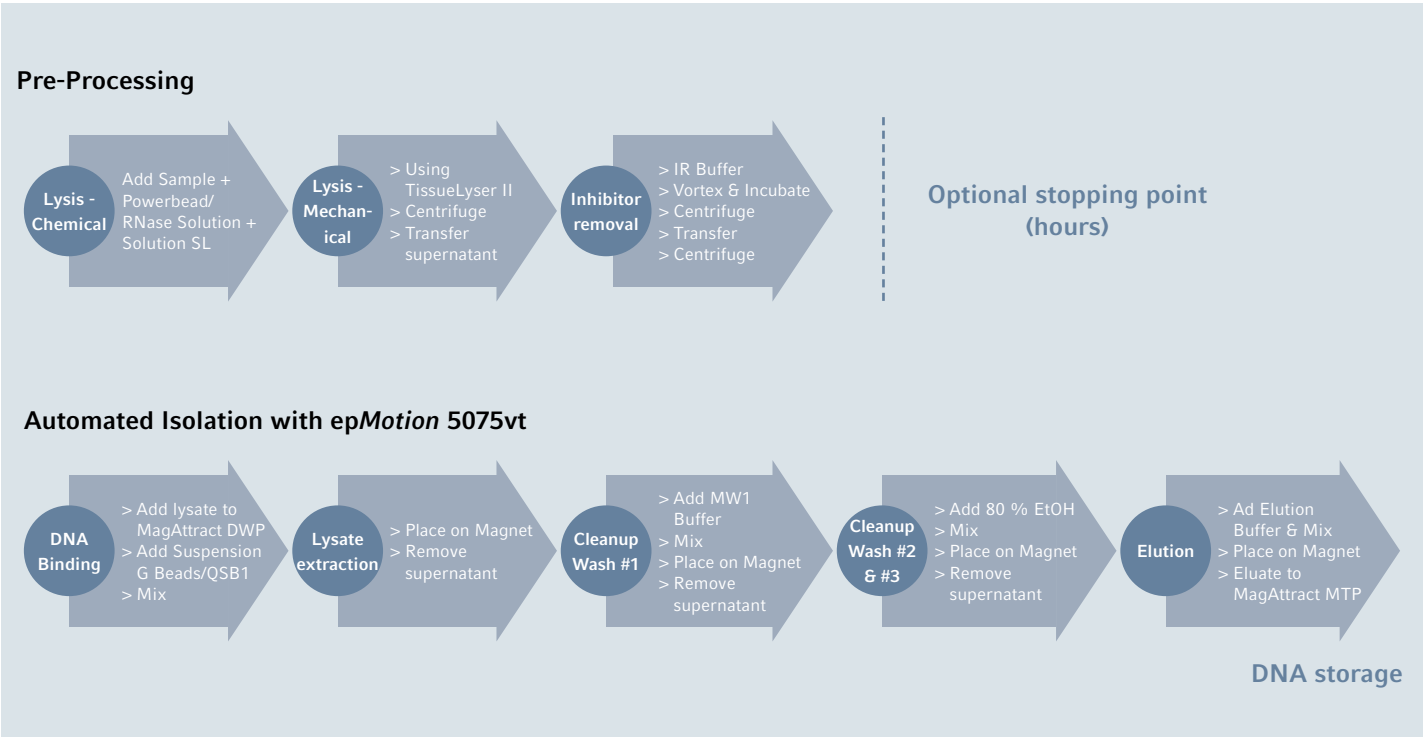


Figure 2: Overview of the DNA isolation process including pre-processing steps and details of the automated workflow on the ep*Motion* 5075.

Result and Discussion

1 Comparison of DNA yield and purity after isolation from various soil and stool samples

With the MagAttract PowerSoil Pro DNA Kit (Figure 3.A, red) higher DNA yields were obtained for all sample types compared to the legacy MagAttract PowerSoil DNA Kit (Figure 3.A, dark blue). The 260/280 ratios of the samples isolated via the new kit (Figure 3.B) were around 1.8 (indicating uncontaminated DNA). The deviating 260/280 ratios when using the legacy kit indicate protein and residual RNA carry-over. The 260/230 ratios for all samples were higher, thereby having a higher purity, with the MagAttract PowerSoil Pro DNA Kit (Figure 3.C).

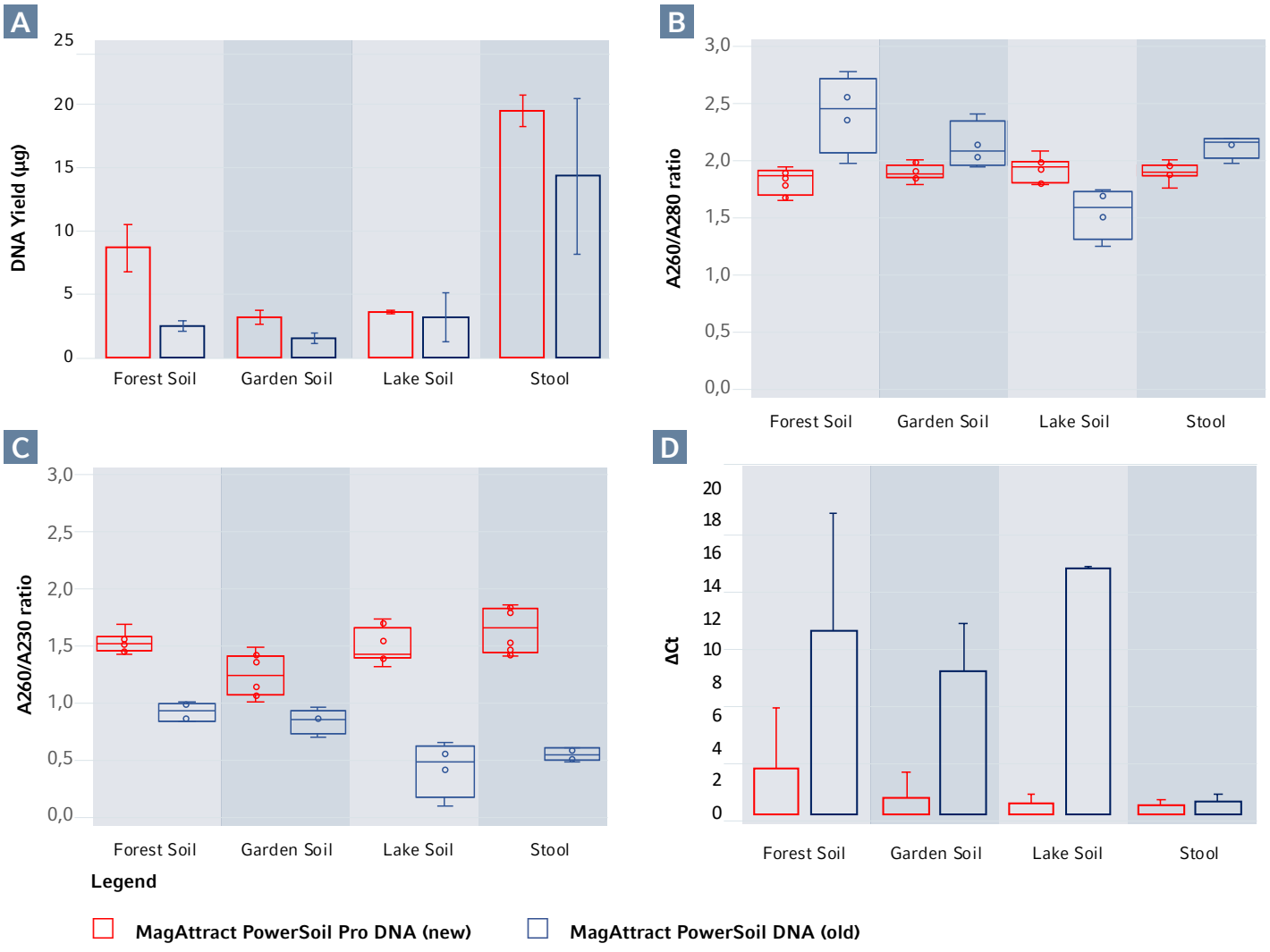


Figure 3: DNA yield (A), A260/A280 ratio (B), and A260/A230 ratio (C) were determined following isolation from 3 different soil samples and a stool sample using MagAttract PowerSoil Pro DNA Kit (n=4-8) or the MagAttract PowerSoil DNA Kit (n=4). (D) Efficiency of inhibitor removal quantified by qPCR (n=4). Higher ΔCt values indicate increased amount of inhibitors in the amplified DNA sample.

2 Assessment of inhibitor removal in isolated microbial DNA samples

DNA isolated with MagAttract PowerSoil Pro DNA Kit showed little to no PCR inhibition, whereas the legacy MagAttract PowerSoil DNA Kit co-isolated inhibitors from all processed soil samples (Figure 3.D).

3 Assessment of consistency and cross-contamination during the isolation process

When comparing DNA yield and quality measures across full isolated 96 well plates (column wise), the values deviate only slightly around the full plate average, thereby proving the robustness and great reproducibility of the isolation method (Figure 4.A). The 16S qPCR performed on samples and negative controls (arranged in checkerboard pattern on the plate) is a very sensitive method for the detection of microbial presence (Figure 4.B). After calculating the input copy numbers, our results show that, during the automated DNA isolation on the ep*Motion*, problems with cross-contamination can be excluded.

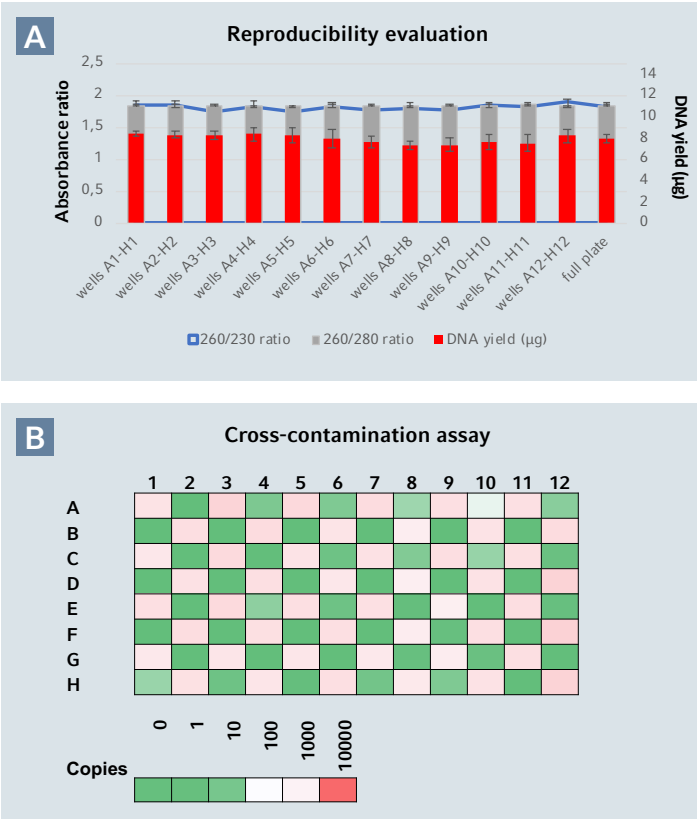


Figure 4: (A) DNA yield, A260/A280 and A260/A230 ratios of stool samples isolated on one 96 well plate with the MagAttract PowerSoil Pro DNA kit. The values are depicted column wise, together with the average values for the full plate.
(B) Copy number distribution on a 96 well plate – estimated following 16S qPCR - indicates no cross-contamination between neigbouring wells (Every second well starting from A1 contains sample lysate; every second well starting from A2 contains negative control).

Conclusion

A new protocol for automated sample processing was developed and validated on the ep*Motion* 5075vt to isolate microbial gDNA from various soil and stool samples. The automated protocol was proven to be highly reproduceable, resulting in a stable amount of purified gDNA of high quality. A better recovery yield and higher purity of microbial gDNA was obtained with the MagAttract PowerSoil Pro DNA compared to the legacy kit for all the samples tested. Overall, the gDNA extracted with the MagAttract PowerSoil Pro DNA kit has little to no co-inhibitor content which makes it favorable for downstream applications such as qPCR and sequencing.

Literature

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[5] QIAGEN GmbH, MagAttract PowerSoil Pro DNA Kit Handbook 11/2020, for hands-free isolation of DNA from soil and stool using automated processing and liquid handling systems. HB-2834-001 11/2020
[6] QIAGEN GmbH, MagAttract PowerSoil DNA EP Kit Handbook 03/2018, for hands-free isolation of DNA from soil using automated processing and liquidhandling systems, 1104583 HB-2278-001 03/2018

Ordering information

Ordering information

Description	Order no. international	Order no. North America
DNA purification kit and additional equipment for sample processing and analysis		
QIAGEN® MagAttract® PowerSoil Pro Kit	47109	47109
QIAGEN® QuantiFast Pathogen RT-PCR +IC Kit	211454	211454
QIAGEN® TissueLyser II	85300	85300
QIAGEN® Adapter plates	11990	11990
QIAGEN® QIAxpert System	9002340	9002340
Eppendorf 5910 centrifuge with S-4xUniversial rotor	5943 000.316	5943 000.343
Equipment and consumables for DNA purification on ep*Motion*® 5075t		
epMotion® 5075vt with Eppendorf ThermoMixer®, Vacuum module, epBlue software	5075 000.304	5075 006.035
OR epMotion® 5075t with Eppendorf ThermoMixer®, epBlue software	5075 000.302	5075 006.022
Waste box 45 mm UV cover	5075 751.976	5075 751.976
TM 1000-8 eight-channel dispensing tool	5280 000.258	960001061
Gripper	5282 000.018	960002270
Reservoir Rack 7	5075 754.002	960002148
Eppendorf Magnum FLX® Magnet Adapter	5075 751.836	5075 751.836
epMotion® reservoir 100 mL for use with Reservoir Rack 7	0030 126.513	960051017
epMotion® reservoir 400 mL	5075 751.364	960002229
epT.I.P.S.® Motion 1,000 µL, Filter	0030 014.499	0030 014.499
Waste bags	5075 751.763	5075 751.763
Optional ep*Motion* Accessoires		
Work Surface Adapter	5070 752.001	5070 752.001
Waste box 100 mm UV cover	5075 751.992	5075 751.992
Enhanced Feature Set 1	5075 000.964	5075 000.964

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