

Magnetic Bead Based Soil DNA Isolation using the ep*Motion*[®] and ClearMag[™] Technology

Vince Moroney, Heather Martinez Ph.D., Heather Callahan, Ph.D., Suzanne Kennedy Ph.D., MO BIO Laboratories, Inc. Carlsbad, CA, USA

Cheng Liu Ph.D., Jesse Cassidy Eppendorf North America, Hauppauge, NY, USA

Abstract

Many nucleic acid purification applications have been previously automated on the Eppendorf ep*Motion* 5075. The exception is purification of DNA from difficult samples high in PCR inhibitors, including soil, turbid water, biofilms, and human gut samples. While high-throughput purification of DNA can be achieved using

96-well silica plates, this method is time-consuming and not hands-free. This Application Note describes the successful automation of DNA isolation from these sample types with magnetic bead-based technology from MO BIO Laboratories.

Introduction

Typical DNA isolation from environmental samples releases compounds which inhibit PCR and other enzymatic reactions when present in the final DNA solution. In soil, these compounds include humic and fulvic acids, and in human gut or stool, these compounds include bile, bilirubin, and undigested food. Using MO BIO's[®] patented Inhibitor Removal Technology (IRT), the majority of inhibitors are removed prior to purification. However, with traditional silica coated magnetic beads, even trace amounts of inhibiting substances will bind and produce contaminated DNA unsuitable for analysis. To overcome this, MO BIO developed the PowerMag Soil DNA Isolation Kit with ClearMag magnetic bead technology. This technology enables purification of DNA without the typical surface binding to the beads, eliminating the adsorption of organic inhibitors and facilitating isolation of pure DNA. In addition, neither chaotropic salts nor ethanol are used in the binding and washing steps, removing a second source of contamination that can inhibit enzymatic reactions.

Eppendorf's ep*Motion* 5075 TMX Workstation presents as an effortless, hands-free companion for the purification of nucleic acids using magnetic bead based technologies. The PowerMag Soil DNA Isolation Kit has been optimized specifically for use on ep*Motion* Automated liquid handling workstation. The Eppendorf ep*Motion* 5075 TMX includes a heated thermomixer which is used for high efficiency binding of DNA to the beads, increasing DNA yield, and a gripper tool used to transport plates on the deck. The intuitive programming of the epBlue[™] software as well as six pipetting tools covering 1-1,000 µL range in both single and 8-channel format further simplifies the daily operation of this system.

Here, we demonstrate the isolation of high quality, inhibitor-free DNA using the PowerMag Soil DNA Isolation Kit on the ep*Motion* 5075 TMX and its successful use in downstream applications.

Materials and Methods

Organic garden soil (0.25 g) was measured into the PowerMag Bead Plate, followed by addition of PowerMag Bead Solution/RNase A and PowerMag Lysis Solution. Cell lysis was performed in a 96-well plate shaker. After centrifugation in a 96-well centrifuge to clear the lysates, the supernatants were transferred to a block containing the PowerMag IRT Solution, which removes the majority of the inhibiting substances. Following clearance of the inhibitors by centrifugation, the cleared lysates were transferred to a new block. The remaining purification steps were performed on the Eppendorf epMotion 5075 TMX. DNA was eluted in 100 μ L Elution Buffer at the end of the automation steps. Sample quality and yield were evaluated using a spectrophotometer and by running 10 μ L of each sample on a 1% TAE agarose gel. Sample purity was further examined by qPCR using primers for *Bacillus sp.*

Quadruplicates of undiluted elutions (1 μ L) and 1:10 dilutions were analyzed.

Table 1: epMotion 5075 TMX worktable details for the PowerMag Soil DNA Isolation Kit.

Position	Labware	Comment
A2	epT.I.P.S.® Motion 1,000 μ L, filtered	
A3	epT.I.P.S. Motion 1,000 μ L, filtered	
TMX	MO BIO® 2 mL Deep Well Plate (MoBio_DWP)	Sample lysate plate
B0	Reservoir 400 mL	Waste collector
B1	MO BIO MTP 2 (MoBio_MTP)	Elute collection plate
B2	epT.I.P.S. Motion 1,000 μ L, filtered	
B3	epT.I.P.S. Motion 1,000 μ L, filtered	
B4	MO BIO PowerMag Magnetic Separator (MOBIO_POWERMAG_Magnet)	
C2	Reservoir 400 mL	Wash buffer
C3	Beads/Bind & Elution Reservoir Rack (MoBio_PowerMag)	
	Position 1: Beads/Bind	100 mL tub
	Position 3: Elution	30 mL tub
C4	MO BIO MTP 1 (MoBio_MTP)	
T0	Gripper	
T1	TM1000-8	

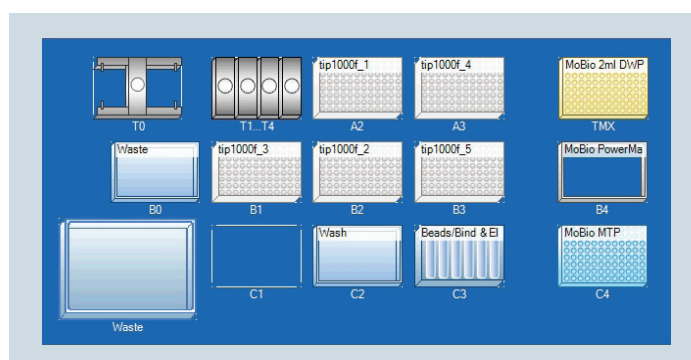


Figure 1: Screenshot of the epBlue software showing the setup of the epMotion 5075 TMX worktable for use with MO BIO PowerMag Soil DNA Isolation Kit.

Results

When performing high-throughput purification, it is essential to achieve consistent, reproducible results with respect to DNA quality and yield. Here, we examined DNA isolated from eight replicates of organic garden soil using the PowerMag Soil DNA Isolation Kit on the epMotion 5075 TMX. Gel electrophoresis analysis demonstrated high quality, high molecular weight DNA in all samples (Figure 2).

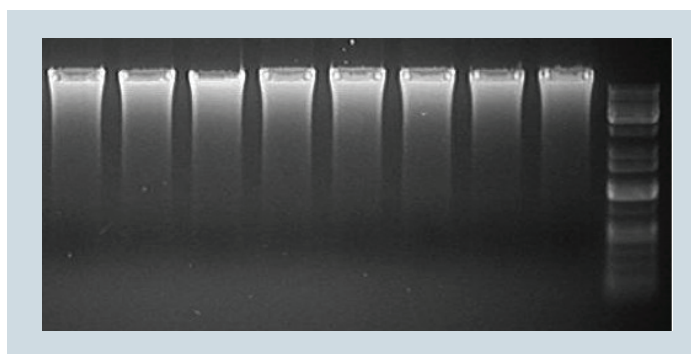


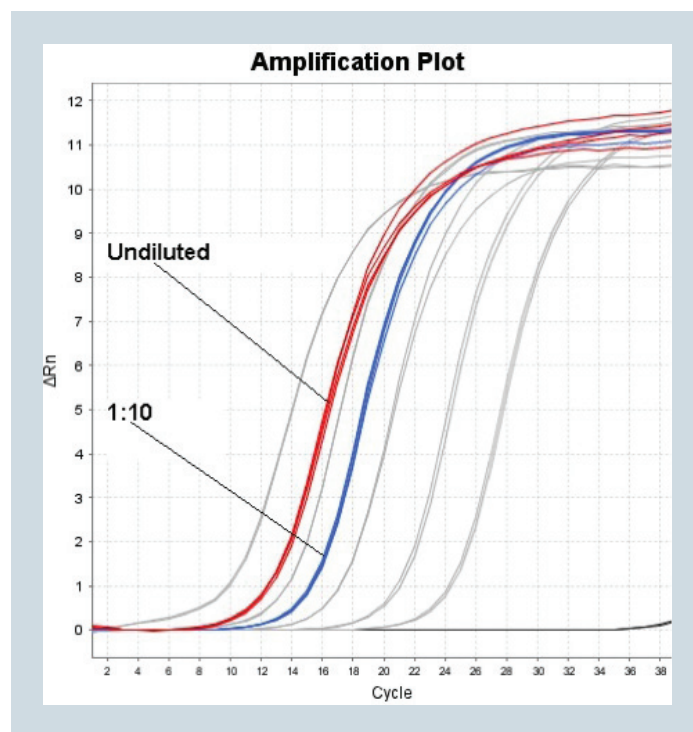
Figure 2: High quality DNA isolated from 0.25 g of organic garden soil using the PowerMag Soil DNA Isolation Kit on the epMotion 5075 TMX. High quality, high molecular weight (40 kb) DNA was observed in eight replicate samples examined on a 1% agarose gel. No differences in yield or quality were observed between the replicate samples.

DNA yield and purity were examined using a NanoDrop® 2000 spectrophotometer, revealing consistent yields ranging from 4.25–5.27 μ g, with $A_{260/280}$ ratios ranging from 1.87–2.01 (Table 2).

Table 2: Consistent yields of pure DNA isolated from organic garden soil using the PowerMag Soil DNA Isolation Kit on the epMotion 5075 TMX.

Sample	Concentration (ng/ μ L)	A _{260/280}	Yield (μ g)
1	46.84	1.90	4.68
2	52.65	1.90	5.27
3	49.84	1.95	4.98
4	50.28	1.87	5.03
5	48.81	2.01	4.88
6	47.96	1.95	4.80
7	42.45	1.99	4.25
8	47.41	1.93	4.74

ClearMag magnetic beads and Inhibitor Removal Technology have been optimized to remove humic substances and other PCR inhibitors. As organic garden soil is known to contain high levels of humic substances, we next assessed the purity of the DNA via qPCR using primers for *Bacillus sp.* on 1 μ L of each elution. Undiluted samples and 1:10 dilutions were examined. All samples amplified and Cq values fell within the standard curve (Figure 3). Nearly identical Cq values were observed in each series for both the undiluted and 1:10 diluted quadruplicate samples, and the difference between the diluted and undiluted samples was approximately 3 cycles, indicating the DNA was free of inhibitors.



Sample	Cq Values	
	Undiluted	1:10 Dilution
1	13.73	16.53
2	13.79	16.33
3	13.92	16.51
4	13.78	16.42

Figure 3: Successful amplification of DNA isolated using the PowerMag Soil DNA Isolation Kit. qPCR was performed using primers for *Bacillus sp.* on 1 μ L of DNA isolated from organic garden soil. Undiluted DNA and samples diluted 1:10 fell within the standard curve (grey lines), and the difference between the diluted and undiluted samples was approximately 3 cycles, indicating the DNA was free of inhibitors.

Summary

The PowerMag Soil DNA Isolation Kit is the first successful method of magnetic bead based automated DNA purification for soil. A combination of Inhibitor Removal Technology and the inhibitor-resistant ClearMag magnetic bead technology enables DNA from the most difficult sample types to be purified on the ep*Motion* 5075 TMX Automated Pipetting System. Here, we have shown that consistent yields of high quality DNA can be obtained from soil samples using this method. Moreover, DNA isolated from organic garden soil, a sample known to contain high

levels of humic acids, was shown to be free of PCR inhibitors and was successfully used for qPCR. Although a limited number of samples were tested, this study demonstrated that the Eppendorf ep*Motion* 5075 TMX is fully compatible to the ClearMag technology. Equipped with 12 deck spaces for additional tips and other consumables as well as 8-channel pipettes for increased throughput, this method can be easily scaled up to process up to 96 samples in a single run, allowing better use of your valuable time while high-quality results can still be ensured.

Ordering information

Description	Order no. international	Order no. North America
Eppendorf		
epMotion® 5075 TMX PC complete with gripper		960020555
Dispensing tool TM 1000-8, 40-1,000 µL	5280 000.258	960001061
Reservoir Rack	5075 754.002	960002148
Reservoirs 30 mL	0030 126.505	960051009
Reservoirs 100 mL	0030 126.513	960051017
400 mL reservoir, set of 10	5075 751364	5075751364
epT.I.P.S.® Motion 40–1,000 µL, filtered	0030 114.499	0030014499
MO BIO®		
PowerMag™ Soil DNA Isolation Kit		27200-4-EP
PowerMag™ Magnetic Separator		27400

Your local distributor: www.eppendorf.com/contact
 Eppendorf AG · 22331 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

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