

Magnetic Bead Based Soil DNA Isolation using the Thermo Scientific KingFisher Flex and KingFisher Duo and ClearMag™ Technology

Vince Moroney, Heather Martinez, Suzanne Kennedy, MO BIO Laboratories, Carlsbad, CA, USA
Marika Suomalainen, Thermo Fisher Scientific, Vantaa, Finland

Key Words

DNA purification, sample preparation, automation, magnetic bead, soil, clay, sand.

Goal

Certain sample materials offer more challenges in DNA purification than the others. Soil samples, for instance, contain lot of PCR inhibitors, which are difficult to remove during sample preparation. The PowerMag™ Soil DNA Isolation Kit was used together with the Thermo Scientific KingFisher Flex and KingFisher Duo magnetic particle processors for DNA purification from several different soil samples. The aim was to optimize an efficient sample preparation system ensuring high yield and quality DNA without inhibitors.

Introduction

Many nucleic acid purification applications have been successfully automated on robotic platforms. The exception is purification of DNA from 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 not hands-free or walk-away. Magnetic bead based technologies are currently the preferred method for hands-free, automated DNA isolation.

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®, 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 the difficulties in isolation of inhibitor-free DNA from soil and environmental samples using magnetic beads, MO BIO developed the PowerMag Soil DNA Isolation Kit with ClearMag™ magnetic bead technology. ClearMag 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.

Magnetic particle processors such as KingFisher® Flex and KingFisher® Duo enable hands-free purification of nucleic acids using magnetic bead based technology. With



the KingFisher Flex it is possible to purify 96 samples per run, and with the KingFisher Duo, 12 samples per run. The PowerMag Soil DNA Isolation Kit (27000-4-KF) has been optimized specifically for use on KingFisher Flex and KingFisher Duo magnetic particle processors.

Here, we demonstrate the isolation of high quality, inhibitor-free DNA using the PowerMag Soil DNA Isolation Kit on the KingFisher Flex and KingFisher Duo and its successful use in downstream applications.

Methods

Soil samples (0.25 g) were 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 robotic deck of either the KingFisher Flex or KingFisher Duo. Sample quality and yield were evaluated using a Thermo Scientific NanoDrop 2000 Spectrophotometer and by running 10 µl of each sample on a 1% TAE agarose gel. Sample purity was further examined by qPCR using primers for *Bacillus*. Quadruplicates of undiluted elutions (1 µl) and 1:10 dilutions were analyzed.

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 a panel of six soil samples using the PowerMag Soil DNA Isolation Kit on the KingFisher Duo. Gel electrophoresis analysis demonstrated high quality and high molecular weight DNA in all samples (Figure 1). DNA yield and purity were examined using a NanoDrop® 2000 spectrophotometer. The yield of each soil type varied depending on its biomass. Yields ranged from 2.3 – 14.7 µg, and A_{260}/A_{280} ratios ranged from 1.77 – 1.90 (Table 1).

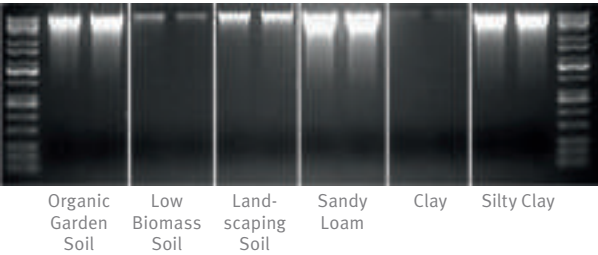


Figure 1. DNA isolated from a panel of six soil samples (0.25 g) using the PowerMag Soil DNA Isolation Kit on the KingFisher Duo. High quality, high molecular weight DNA was observed in each soil sample with yields varying based on microbial load. No differences in yield or quality were observed between the replicate samples.

Table 1. Consistent yields of pure DNA isolated from a panel of six soil samples using the PowerMag™ Soil DNA Isolation Kit on the KingFisher Duo.

Sample	Average Yield (µg)	Average A_{260}/A_{280}
Organic Garden Soil	7.8	1.87
Low Biomass Soil	2.3	1.77
Landscaping Soil	3.9	1.78
Sandy Loam	14.7	1.90
Clay	1.8	1.92
Silty Clay	8.7	1.90

We next isolated DNA from a panel of two soil samples using either the KingFisher Flex or KingFisher Duo. We observed minimal differences in yield between the two instruments, and the differences are most likely due to a lack of homogeneity in the soil samples (Table 2).

Table 2. Average of two samples per instrument and soil type. High yields of pure DNA were isolated using both KingFisher Flex and KingFisher Duo instruments.

Sample	Instrument	Average Yield (µg)	Average A_{260}/A_{280}
Sand	Duo	2.2	1.8
	Flex	1.9	1.9
Sandy and Stony Soil	Duo	2.4	1.8
	Flex	2.4	1.9

ClearMag magnetic beads and Inhibitor Removal Technology (IRT) have been optimized to remove humic substances and other PCR inhibitors from soil, stool and environmental samples. We next assessed the purity of DNA isolated from organic garden soil, low biomass soil and landscaping soil via qPCR using primers for *Bacillus* on 1µl of each elution. Undiluted samples and 1:10 dilutions were examined. All samples amplified and Cq values fell within the standard curve (Figure 2). 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.

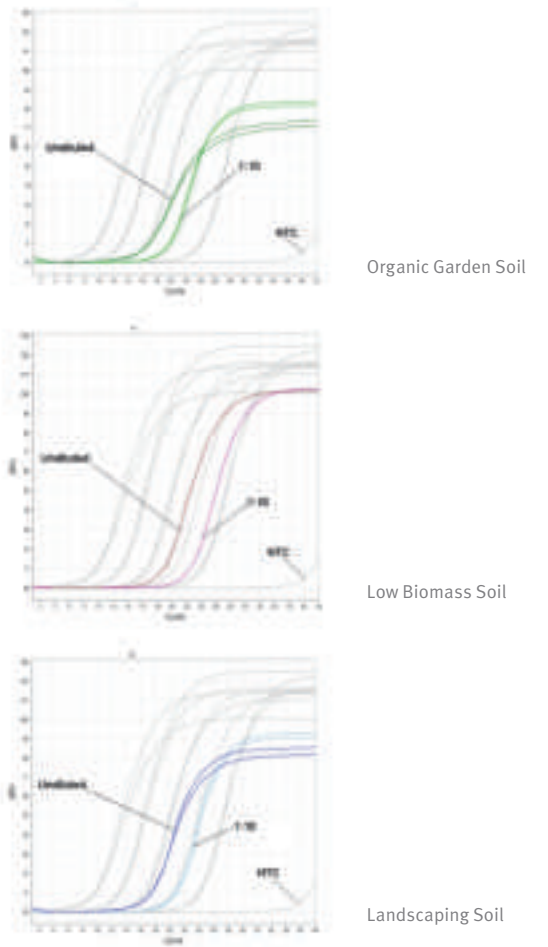


Figure 2. qPCR was performed on 1 µl of DNA from three of the soil types described in Figure 1 that are known to be high in PCR inhibitors, using primers for *Bacillus*. Undiluted DNA and samples diluted 1:10 fell within the standard curve (grey lines), and the difference between the diluted and undiluted samples was approximately 3 cycles, indicating the DNA was free of inhibitors.

To further demonstrate the importance of IRT to successful PCR amplification, we performed the PowerMag Soil DNA Isolation Kit protocol as described above, but omitted the IRT step in one set of samples. As expected, samples isolated without IRT or with water in place of IRT failed to amplify, while samples isolated with IRT amplified successfully and the C_q value fell within the standard curve (Figure 3.)

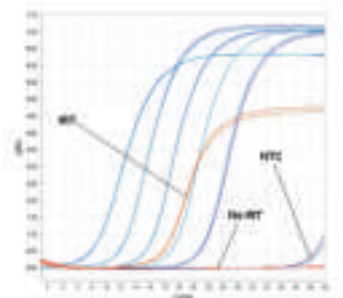


Figure 3. Inhibitor Removal Technology (IRT) is essential for removal of PCR inhibitors. DNA was isolated from organic garden soil samples using the PowerMag Soil DNA Isolation Kit with or without the IRT step, and purified using the KingFisher Duo. qPCR was performed as described in Figure 2. Samples processed without IRT failed to amplify, while samples with IRT were successfully amplified and fell within the standard curve (blue lines).

Conclusion

Using the PowerMag Soil DNA Isolation Kit on a KingFisher Flex or KingFisher Duo is a successful automated method for magnetic bead based DNA purification from soil. A combination of Inhibitor Removal Technology and the inhibitor-resistant ClearMag magnetic bead technology enables DNA purification from the most difficult sample types on the KingFisher Flex and KingFisher Duo magnetic particle processors. Here, we have shown that consistent yields of high quality DNA can be obtained from a variety of soil samples using this method. Moreover, DNA isolated from soil containing high levels of humic acids was shown to be free of PCR inhibitors and was successfully used for qPCR. In conclusion, the PowerMag Soil DNA Isolation Kit together with the KingFisher Flex and KingFisher Duo enables rapid, hands-free isolation of high quality, inhibitor-free DNA from soil, stool and environmental samples.

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North America:

USA/Canada +1 603 595 0505

USA toll free 800 345 0206

Europe:

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Belgium +32 53 73 42 41

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France +33 2 2803 2000

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Russia/CIS +7 (495) 739 76 41

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UK/Ireland +44 870 609 9203

Asia:

Australia +613 9757 4474

China +86 21 6865 4588 or +86 10 8419 3588

China toll free 800-810-5118, 400-650-5118

India +91 22 6716 2200

Japan +81-3-5826-1616

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