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Optimization of the PowerSoil®-htp 96 Well Soil DNA Isolation Kit on ep*Motion*® 5075 VAC

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Abstract

MO BIO Laboratories Inc. has developed the unique PowerSoil-htp 96 Well Soil DNA Isolation Kit for isolating genomic DNA from environmental samples while also removing PCR inhibitors. This kit was optimized on the ep*Motion* 5075 VAC automated pipetting system allowing greater pipetting accuracy while offering a more efficient workflow. DNA was extracted from six soil types from multiple sites in central and southwest Michigan using the PowerSoil-htp 96 Well Soil DNA Isolation Kit producing varying yields of DNA sufficient for subsequent testing and amplification of the DNA and showing little evidence of PCR inhibition from common inhibitors such as humic acids. Results demonstrated that DNA yields on the robot were similar to those obtained using individual silica spin filters.

Introduction

The PowerSoil DNA Isolation Kit from MO BIO Laboratories, Inc. is a popular method utilized for DNA isolation from a variety of samples types that contain PCR inhibitors. The PowerSoil DNA Isolation Kit is currently used by the Human Microbiome Project for analysis of microbial profiles in human samples including skin [1] and gut [2], and has been used successfully for the extraction of DNA from samples contaminated with oil [3, 4]. The PowerSoil method involves a patented system for inhibitor removal, called Inhibitor Removal Technology (IRT) that enables previously unamplifiable DNA to be successfully amplified in enzymatic applications.

One of the most difficult sample types to purify DNA from is soil. The major soil constituent affecting DNA purity is the level of humic substances (humic and fulvic acids) generated from decomposition of organic matter in the soil. Removal of humic substances is key to accurate quantification of DNA yields as well as success in complicated assays such as RFLP analysis and next generation sequencing.

An area of intense research is the composition and structure of microbial communities in agricultural lands. The changes in microbial diversity under various conditions of irrigation, plant life, and fertilizers are not well understood. Moreover, the significance of these conditions on communities with increasing depth in soil requires further study. For these reasons, high throughput analysis of multiple, replicate samples is required to capture spatial and temporal heterogeneity of complex microbial communities in soil.

Here, we report the successful use of the PowerSoil-htp 96 Well Soil DNA Isolation Kit with a cross section of agricultural soils optimized for automated extraction on the Eppendorf ep*Motion* 5075 VAC.

Materials and methods

1. Eppendorf ep*Motion* 5075 VAC
- Gripper
- Dispensing tool TM1000-8
- Vacuum with manifold
- Vac frame 1
- Thermomixer (TMX)
- Reservoir rack
- Eppendorf Consumables
  -9 x 100 mL reagent reservoirs
  -1,000 µL epTIPS

2. MO BIO Laboratories Inc. PowerSoil-htp 96 Well Soil DNA Isolation Kit (Catalog # 12955-4)

3. Retsch MM 400 96-Well Plate Shaker

4. 96-Well Plate Centrifuge

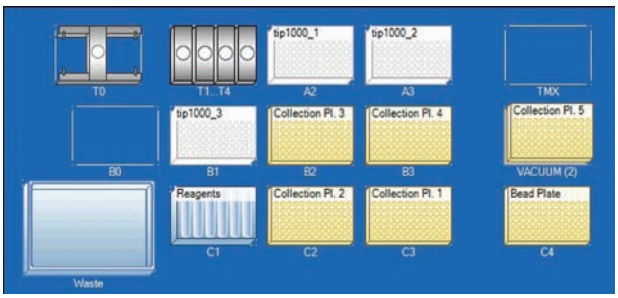


Figure 1: ep*Motion* 5075 worktable setup—Part 1.

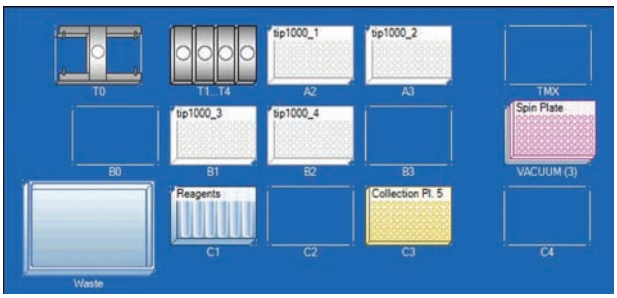


Figure 2: ep*Motion* 5075 worktable setup—Part 2.

Position	Labware
T0	Gripper
T1	TM 1000-8
A2	epTIPS 1,000 µL *Motion*
A3	epTIPS 1,000 µL *Motion*
TMX	Thermomixer
B1	epTIPS 1,000 µL
B2	1 mL collection plate
B3	1 mL collection plate
Vacuum	2 mL collection plate
C1	Reagents Reservoir (100 mL Reservoirs)
	Position 1: PowerSoil-htp bead solution
	Position 2: PowerSoil-htp solution C1
	Position 3: PowerSoil-htp solution C2
	Position 4: PowerSoil-htp solution C3
	Position 5: PowerSoil-htp solution C4
	Position 6: PowerSoil-htp solution C4
C2	1 mL collection plate
C3	1 mL collection plate
C4	Bead plate

Table 1: ep*Motion* 5075 Worktable details for the PowerSoil-htp 96 Well Soil DNA Isolation Kit—Part 1

Position	Labware
T0	Gripper
T1	TM 1000-8
A2	epTIPS 1,000 µL *Motion*
A3	epTIPS 1,000 µL *Motion*
TMX	Thermomixer
B1	epTIPS 1,000 µL *Motion*
B2	epTIPS 1,000µL *Motion*
B3	empty
Vacuum	Spin Plate (on frame)
	Vacuum frame1
	400 mL Reservoir
C1	Reagents Reservoir Module (100 mL Reservoirs)
	Position 1: PowerSoil-htp solution C5-D
	Position 2: PowerSoil-htp solution C5-D
	Position 3: PowerSoil-htp solution C6
C2	empty
C3	2 mL collection plate
C4	empty

Table 2: ep*Motion* 5075 Worktable details for the PowerSoil-htp 96 Well Soil DNA Isolation Kit—Part 2

Six soil types (Table 5) were weighed out in duplicate 0.25 g portions and added to the bead plate included in the PowerSoil-htp 96 Well Soil DNA Isolation Kit from MO BIO Laboratories.

The plate was then added to the worktable and the automated method started. Several points along the process required user intervention for centrifugation. The automated method itself was divided into two parts to accommodate the numbers of reagents and plates used in the protocol. The DNA was eluted in 100 µL of PowerSoil-htp C6 buffer.

Step	Command	Description
1	Number of samples	Input sample number
2	Reagent transfer	Add 750 µL of bead solution
3	Reagent transfer	Add 60 µL of solution C1
4	User intervention	Transfer to plate shaker speed 25 for 10 mins. Centrifuge 10 mins. at 2,500 x *g*.
5	Sample transfer	Transfer 500 µL supermatant to 1 mL collection plate 1
6	Reagent transfer	Add 250 µL solution C2
7	Transport	Move 1 mL collection plate 1 to TMX
8	Thermomixer	Shake at 1,200 rpm for 30 seconds. Incubate at 4 °C for 10 mins.
9	Transport	Move 1 mL collection plate 1 to C3
10	User intervention	Centrifuge 10 mins. at 2,500 x *g*
11	Sample transfer	Transfer 650 µL supernatant to 1 mL collection plate 2
12	User intervention	Centrifuge 10 mins. at 2,500 x *g*
13	Sample transfer	Transfer 600 µL supernatant to 1 mL collection plate 3
14	Reagent transfer	Add 200 µL solution C3
15	Transport	Move 1 mL collection plate 3 to TMX
16	Thermomixer	Shake at 1,200 rpm for 30 seconds. Incubate at 4 °C for 10 mins.
17	Transport	Move 1 mL collection plate 3 to B2
18	User intervention	Centrifuge 10 minutes at 2,500 x *g*
19	Sample transfer	Transfer 700 µL supernatant to 1 mL collection plate 4
20	User intervention	Centrifuge 10 minutes at 2,500 x *g*
21	Sample transfer	Transfer 650 µL supernatant to 2 mL collection plate 5
22	Reagent transfer	Add 650 µL supernatant C4 and mix
23	Reagent transfer	Add second 650 µL solution C4
24	End protocol	

Table 3: ep*Motion* program description for part 1 of the PowerSoil-htp 96 Well DNA isolation protocol

Step	Command	Description
1	Number of samples	Input sample number
2	Sample transfer	Transfer 650 µL from 2 mL collection plate 5 to Spin Plate
3	Vacuum	900 mBar vacuum for 3 mins.
4	Sample transfer	Transfer 650 µL from 2mL collection plate 5 to Spin Plate
5	Vacuum	900 mBar vacuum for 3 mins.
6	Sample transfer	Transfer 650 µL from 2 mL collection plate 5 to Spin Plate
7	Vacuum	900 mBar vacuum for 4 mins.
8	Reagent transfer	Add 500 µL C5-D to Spin Plate
9	Vacuum	900 mBar vacuum for 4 mins.
10	Reagent transfer	Add 500 µL C5-D to Spin Plate
11	Vacuum	900 mBar vacuum for 5 mins.
12	User intervention	Centrifuge for 10 minutes at 2,500 x *g*
13	Reagent transfer	Add 100 µL solution C6 to Spin Plate
14	User lintervention	Centrifuge for 5 minutes at 2500 x *g*
15	End protocol	

Table 4: ep*Motion* Program description for Part 2 of the PowerSoil-htp-96 Well DNA isolation protocol.

Soil Site	Soil Type	Average DNA Yield (ng/µL)
KBS LTER T1R1	Standard row crop agriculture historically tilled	6.95 ± 0.2
KBS LTER T8R1	Managed grassland never tilled	20.3 ± 5.4
KBS LTER DFR1	Deciduous forest	18.0 ± 4.6
GLBRC Extensive Site PR06	Prairie	11.6 ± 1.1
GLBRC Extensive Site SW10	Switchgrass	17.6 ± 1.9
GLBRC Extensive Site C26	Corn	9.10 ± 1.6

Table 5: DNA yield by soil type as measured by gel quantification

Results and Discussion

Soil samples were collected from a range of sites in central and southwest Michigan. The sites included three from the Kellogg Biological Station Long Term Ecological Research sites (additional information about the soils can be found at http://lter.msu.edu/about/site_description/soils.php) and three from the Great Lakes Bioenergy Research Center extensive sites. DNA yields varied by soil source (Table 5). The results were comparable to what can be expected from MO BIO Laboratories individual extraction PowerSoil kit following the included protocol.

After the DNA was extracted from soils, the 16S rRNA genes were PCR amplified as they are commonly used to assess microbial diversity [5]. Primers 8F and 1492R were tested [6] and showed little evidence of PCR inhibition from common inhibitors such as humic acids (Figure 3).

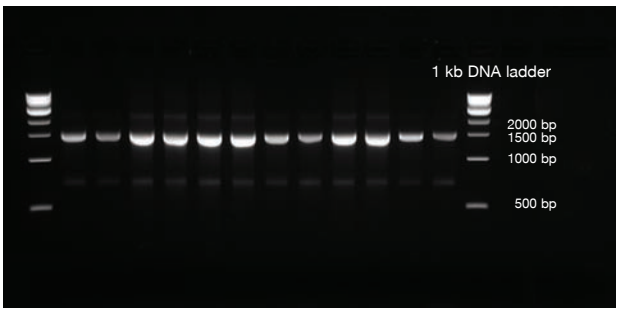


Figure 3: 16S rDNA PCR fragments were separated on a 1.2 % agarose gel and visualized by ethidium bromide staining. Each soil type was run in duplicate in the following order: T1R1, T8R1, DFR1, PR06, SW10, and C26.

Conclusion

The PowerSoil-htp 96 Well Soil DNA Isolation Kit enables high-throughput extraction of DNA from soils and can be automated for use on the Eppendorf ep*Motion* 5075. DNA yields using automation and the PowerSoil-htp kit were typical of expected results from the manual PowerSoil kit.

References

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Ordering information

Product	Order no. International	Order no. North America
ep*Motion*® 5075 VAC	5075 000.768	960020222
Dispensing Tool TM1000_8	5280 000.258	960001061
Reservoir Rack	5075 754.002	960002148
Reservoirs 100 mL	0030 126.513	960051017
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