Validation of MO BIO's PowerMag™ Soil DNA Isolation Kit on the Automated Liquid Handling VERSA 1100 Workstation

Sikander Gill PhD[1], Rajwant Gill PhD[1], Matthew Lemay PhD[2] and Dong Liang PhD[1]

Aurora Biomed Inc., Vancouver, BC, Canada[1]

Department of Botany, University of British Columbia, Vancouver, BC, Canada[2]

I. Summary

The soils being very difficult sample types due to the presence of inhibitors of downstream applications, need special kit chemistry and process for efficient removal of such inhibitors. The automation of MO BIO's PowerMag® Soil DNA isolation Kit on VERSA 1100 Workstation resulted in consistent yields of high quality DNA from contaminated soil types including garden soil known to contain high levels of humic acids and other inhibitors. This workstation automates the entire process (except centrifugation) from the soil samples to isolated DNA and thus offers merits over the other systems that automate from the soil extract carried off deck. The successful PCR carried on the DNA isolated from these soils indicated high inhibitor-free DNA.

The study, although, carried on limited number of samples but demonstrated that VERSA Workstation and its modules on the deck are optimal in their performance. The system offering 15 deck positions allows processing of 96 samples in a single run minimizing human error.

II. Introduction

Purification of DNA from soil samples is a challenging process since the presence of humic acid, fulvic acid, phenolics, and polysaccharides inhibit the downstream applications including PCR, and NGS[1-2]. Since these inhibitors co-extract with the DNA template and thus DNA isolation technology should be able to remove these inhibitors[3]. Moreover, for achieving effective relative yield and purity, a uniform distribution of magnetic beads is essential[4].

These challenges have been suitably addressed during automation on VERSA 1100 Workstation using MO BIO's PowerMag® Soil DNA isolation kit (Table 1). This automation offers merits while the competitive automations start only from soil extract carried off deck (Table 2). Thus the system offers walk-away workflow by minimizing human error and provides inhibition-free DNA from 96 soil samples.

Table 1: Soil DNA Isolation Challenges and addressing by automation and kit chemistry

#	Soil DNA Isolation Challenges	Requirements	MO BIO Kit Chemistry on Other Workstations	Automation VERSA 1100
1	Presence of humic acid, fulvic acid, phenolics, and polysaccharides produced by soil microflora inhibit PCR and NGS.	Effective inhibitor removal kit chemistry and liquid handling	yes	Yes
2	These soil inhibitors being high molecular weight and polyanionic mimic DNA that most purification methods do not distinguish .	Effective inhibitor removal kit chemistry and automated handling	yes	yes
3	Soil samples vary widely in their pH, amount and composition of organic and inorganic matter that affect relative uniformity.	Effective kit chemistry and automated handling	yes	yes
4	The highly variable composition of different soils affects DNA yield and purity.	Effective kit chemistry and automated handling	yes	yes
5	Microbial cells and spores difficult to break and lyse.	Effective mechanical lysis is an essential first step	Mechanical beads on separate bead beater	Powerful on-deck shaker
6	Manually difficult to remove supernates from pellets of soil materials and precipitates.	Automated removal of supernates	N/A	yes
7	Floating inhibitors tend to stick to the wall of the plate wells during pipet removal of supernates	Aspiration from top surface	N/A	96-tip Aspirator
8	Uniform distribution of magnetic beads	Magnetic bead mixer	N/A	Vortex on deck

III. Objectives

The automation of DNA isolation from soils was carried to validate the following:

- DNA isolation from high humic acid soils
- Efficiency of deck modules
- Automation of whole process from soils samples to DNA
- Optimal size of sample
- Any cross-contamination

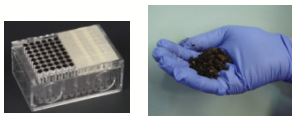


Figure 1: Soil samples in 96 well plate

Table 2: Merits of VERSA 1100 Workstation over others

#	Protocol Steps	Competitors	VERSA 1100
1	**Sample prep of soils**	Manual	On-deck
2	**Needs a powerful shaker**	Yes (off-deck)	On-deck shaker
3	**Centrifugation**	Manual	Manual
4	**Aspirator removes floating inhibitors**	No Aspirator	96-tip Aspirator
5	**Magnetic bead process**	On-deck	On-deck
6	**PCR set up**	On/off - deck	On-deck
7	**PCR inhibitor-free DNA**	Yes	Yes
8	**Only used organic soils?**	Yes	Fertilizer added & organic soils

IV. Materials & Methods

The automation of the PowerMag® Soil DNA Isolation Kit cat # 27100-4 (http://www.mobio.com/soil-dna-isolation/powermag-soil-dna-isolation-kit. html) was performed on VERSA 1100 Workstation (www.aurorabiomed.com) displayed in Figure 2. The entire process was carried on the deck (Figure 3) except centrifugation :

1. **Soil sample preparation**: 250mg of 8 soil samples were added to the wells of a bead-beating 96 deep square well plate supplied in the kit.
   a. Lysis: 750µl of PowerMag® Bead Solution/RNAse solution was added to each well of the PowerMag®Bead Plate placed on the Versa shaker. It was followed by addition of 60µl of PowerMag®Lysis Solution. The plate was sealed before shaking at 2400 rpm for 20 min and then centrifuged at 4000gx6 min.
   b. The clear supernate was transferred to a 2ml 96 round well plate to which 450µl of PowerMag® IRT Solution was added and mixed. The plate was incubated at 4°C for 10 minutes followed by sealing of the plate and centrifugation at 4000gx6 min.
   c. The clear supernate (850 µl) was transferred to a 96 Deepwell round bottom plate followed by sealing of the plate and centrifugation at 4000gx6 min to remove any residual IRT pellet.
   d. The clear supernate (not more than 850µl) was transferred to a 96 Deepwell round bottom plate followed by sealing of the plate and centrifugation at 4000gx6 min.
2. **Purification of DNA**: To each well of a 96 deepwell plate, 40µl of the ClearMag™ beads constantly suspended in ClearMag™ Binding Solution on bead shaker, were added followed by addition of 450µl of ClearMag™ Binding Solution and mixed on shaker.
   a. To this plate, 450µl of lysate was added followed by mixing on shaker for 10 minutes at 60°C. The deep well plate was moved to the magnetic block and incubated for 2 min to collect the beads and remove supernate using 96-tip aspirator.
   b. To the beads, 500µl of the ClearMag™ Wash Buffer was added using ReagentDrop Channel #1 and the plate was moved to shaker for mixing for 10 sec and moved back to the magnet block for removal of supernate using 96-tip aspirator.
   c. Repeated the steps b twice for a total of three washes with 500µl.
   d. To elute the DNA, the beads were suspended in 100µl of PowerMag Elution Buffer and the plate was moved to shaker for mixing at 65°C for 5 minutes. The supernate was collected from the magnet block and saved in a 96 well plate incubated at 4°C.
3. **Analysis:** The DNA samples were analyzed on plate reader (Epoch Microplate Spectrophotometer, www.biotek.com) and for PCR to determine quality of the isolated DNA.

Figure 2: VERSA 1100 Workstation (L 93cm x W 62cm x H 62cm)

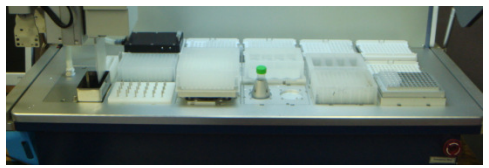
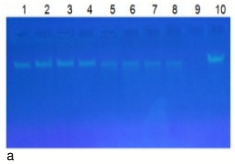
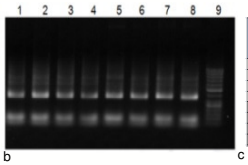


Figure 3: Deck of VERSA 1100 Workstation

V. Results & Discussion

1. **Efficiency of automated process:** The appearing of dark bands in 7% agarose gel (Figure 4a), and yield of isolated DNA (Figure 4c) suggest that the Workstation and its modules worked effectively. While the on-deck shaker effectively homogenized the soil bacterial cells and spores, the bead mixer contributed towards uniform suspension of the beads accounting for uniformity among the replicates of soil samples.
2. **Quality of isolated DNA:** The integrity of the isolated DNA running through 7% agarose gel is reflected from the compact isolated DNA band running close to the 50kb standard indicated a high molecular weight of the DNA. This also suggested the VERSA deck shaker gently handled the DNA from fragmenting (Figure 1a).
3. **PCR inhibitor removal**: The successful run of the PCR on the isolated DNA samples is indicative of the efficient removal of PCR inhibitors both by the kit chemistry evident from pellet of precipitates, and liquid handling modules including aspirator (Figure 1b).
4. **Yield:** Although DNA yield depends upon various factors including the number of microbes and nature of the soil samples, the yield among the replicates of both the soil types had a CV% of 4.6 and 6.2, respectively (Figure 1c).
5. **Throughput**: 96 samples in <3 hours.





c

Soild Samples	OD 260	OD 281	Purity 260/280	Conc µg/ml	Yield µg	Volume µl
Grass lawn	0.599	0.34	1.8	29.95	3.6	120
Grass lawn	0.584	0.315	1.9	29.2	3.5	120
Grass lawn	0.541	0.3	1.8	27.05	3.2	120
Grass lawn	0.595	0.307	1.9	29.75	3.6	120
Flower pot	0.392	0.209	1.9	19.6	2.4	120
Flower pot	0.341	0.2	1.7	17.05	2.0	120
Flower pot	0.376	0.208	1.8	18.8	2.3	120
Flower pot	0.354	0.2	1.8	17.7	2.1	120

Figure: 4: (a). Isolated DNA Lane 1-4, Grass Lawn Soil (10µl), Lane 5-8 Flower Pot Soil (10µl), Lane 10: HMW (50Kb) (b). PCR from DNA samples in respective lanes except LMW ladder (c). Purity and yield of isolated DNA

VI. Why VERSA 1100 Workstation?

This system offers merits over other systems for the following technical reasons:

1. The workstation automated the whole process from soil samples to isolated DNA (except centrifugation) in comparison to other systems that automate only from magnetic bead addition to the manually extracted soil homogenate.
2. The system also automates NGS library prep for metagenomics and other applications.
3. The system offers a throughput of 96 samples within 3.5 hours of the entire process.
4. The 96 tip aspirator removes floating inhibitors resulting in inhibitor–free DNA purification.
5. The ReagentDrop channels save on time, and running cost.
6. The automated removal of supernates from soil and humic acid pellets minimizes manual intervention and human error.
7. The high efficiency air filter hood equivalent to a small ultra clean workbench effectively avoids cross contamination which is highly essential for diagnostic and other applications.

VII. Acknowledgements:

The authors acknowledge the editions of the manuscript by technical staff of MO BIO Laboratories, Inc.

VIII. References

1. Pionzio AM *et al.*,- The effect of internal control sequence and length on the response to PCR inhibition in real-time PCR quantitation. *Forensic Sci Int Genet* 2014;9:55-60.
2. Gunawardana M *et al.*,- Isolation of PCR quality microbial community DNA from heavily contaminated environments. *J Microbiol Methods* 2014; 102:1-7
3. Giampaoli S et al.,-The environmental biological signature: NGS profiling for forensic comparison of soils. *Forensic Sci Int* 2014;240:41-47
4. Gill S *et al.*,- Validation of magnetic bead mixer on an automated next-generation sequencing library preparation system. *American Laboratory* March 2015: 6-9

*Correspondence should be addressed to Aurora Biomed Inc., 1001 E. Pender St., Vancouver, BC, Canada, V6A 1W2, Tel: 1-604-215-8700, Fax: 1-604-215-9700, info@aurorabiomed.com, www.aurorabiomed.com