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 Isolation of DNA from soil samples can be very difficult due to the presence of inhibitors within the samples, this necessitates special kit chemistry and sample processing 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 extraction process (except centrifugation) to isolated DNA and thus offers merits over the other systems that require off deck extraction steps. The successful PCR carried on the DNA isolated from the soil samples demostrates that inhibitor-free DNA, ready for use in downstream applications is isolated by automating MO BIO's PowerMag™ Soil DNA isolation Kit on VERSA 1100 Workstation.

The study, although, carried on limited number of samples,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 as 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, the 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 competitors automation can start only from soil extract carried off deck (Table 2). Thus the Versa 1100 system offers a walk-away workflow, this minimizes human error and provides inhibition-free DNA from 96 soil samples.

Table 1: Soil DNA Isolation Challenges and addressed 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	Yes	Yes
2	These soil inhibitors being high molecular weight and polyanionic mimic DNA that most.	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	Effective kit chemistry and automated handling	Yes	Yes
4	The highly variable composition of different soils affects DNA yield and purity.	Effective mechanical lysis is an essential first step	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

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III. Objectives

The automation of DNA isolation from soil 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
- Any cross-contamination

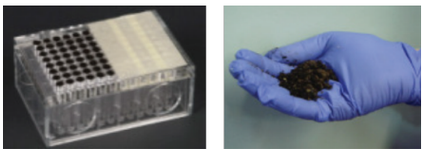

Figure 1: Soil samples loaded into 96-well plate

Table 2: Merits of VERSA 1100 Workstation over competitors platfroms

#	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 use organic soil	Yes	Fertilizer added & organic soils

IV. Materials and Methods

The automation of the PowerMag® Soil DNA Isolation Kit (cat # 27100-4, http://www.mobio.com) 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: Eight 250mg soil samples were added to the wells of the supplied bead-beating 96 deep square well plate.

- **a.** Lysis: 750µl of PowerMag® Bead Solution/RNAse solution was added to each sample well of the PowerMag®Bead plate, followed by addition of 60µl of PowerMag®Lysis Solution. The plate was sealed and shaken at 2400 rpm for 20 min. Next, the plate was manually placed in a centrifuge. The plate was centrifuged for 6min at 4000g.
- **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 4000g for 6 min.
- **c.** The clear supernate (850 µl) was transferred to a 96 Deep well round bottom plate followed by sealing of the plate and centrifugation at 4000g for6 min to remove any residual IRT pellet. This step was repeated one more time

2. Purification of DNA: The bead mixer was used to enusure complete homoginization of the combined ClearMag™ beads and ClearMag™ Binding Solution. 40µl of the homogenized solution was added to eight wells of a new 96 deepwell plate, followed by addition of 450µl 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 tranfered to the magnetic block and incubated for 2 min to pull down the beads. The supernate was removed using 96-tip aspirator.

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IV. Matrials and Methods cont.

b. To the beads, 500µl of the ClearMag™ Wash Buffer was added using the ReagentDrop 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. Step **b** was repeated twice more 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 the shaker and mixed for 5 minutes at 65°C. The supernate was collected from the magnet block and transfered to a 96 well plate incubated at 4°C.

3. Analysis: The DNA samples were resloved on an agarose gel and the quatified using plate reader (Epoch Microplate Spectrophotometer, www.biotek.com) to determine the quality of the isolated DNA. The isolated DNA was used as template in PCR reactions to demostrate the absence of inhibitors.

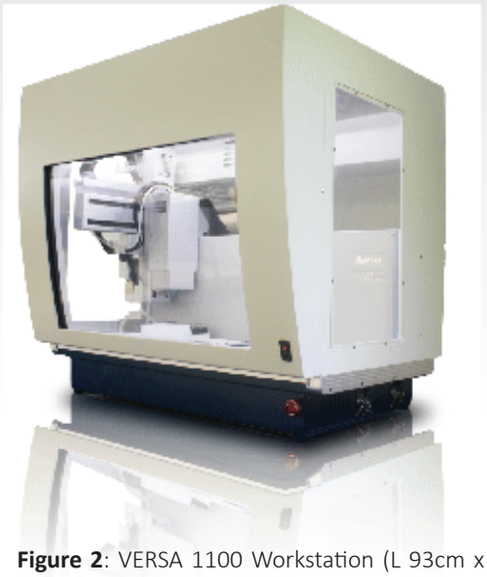

Figure 2: VERSA 1100 Workstation (L 93cm x W 62cm x H 62cm)

Figure 3: The deck layout of VERSA 1100 Workstation

V. Results and Conclusions

- Efficiency of automation process: The ability of the Versa 1100 to automate the isolation of DNA from difficult samples and ensure stable yields of isolated DNA (Figure 4c) demonstrate the efficacy of using this workstation. The on-deck plate shaker and proprietary magnetic bead mixer are central to achieving consistant and reproducible DNA yields

- Quality of isolated DNA: The integrity of the isolated high molecular weight DNA (Figure 4a) is reflected by the compact isolated DNA band running close to 50kb standard on a 7% agarose gel. This also demonstrated that the on-deck shaker did not shear the genomic DNA (Figure 1a).

- PCR inhibitor removal: The successful PCR amplification using the isolated DNA samples as template is indicative of efficient removal of the PCR inhibitors both by the kit chemistry evident from the pellet of precipitiates,and liquid handling modules including the 96-tip aspirator (Figure 1b)

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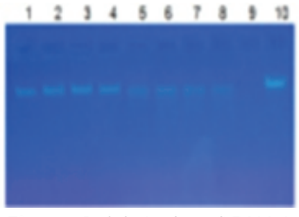
Results and Conclusions cont.

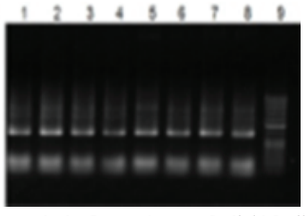
- Yield: Although DNA yield depends upon various factors including the number of microbes and the nature of the soil samples, the yield among the replicates of both soil types had a CV% of 4.6 and 6.3, repsctively (Figure 4c)

- Throughput: 96 samples processed in <3 hours

a



b



c

Soil Sample	OD 260	OD 280	Purity (260/280)	Conc (µg/ml)	Yield (µg)	Elution vol (µl)
Grass Lawn	0.599	0.34	1.8	29.95	3.60	120
Grass Lawn	0.584	0.315	1.9	29.2	3.50	120
Grass Lawn	0.541	0.3	1.8	27.05	3.20	120
Grass Lawn	0.595	0.307	1.9	29.75	3.60	120
Flower pot	0.392	0.209	1.9	19.6	2.40	120
Flower pot	0.341	0.2	1.7	17.05	2.00	120
Flower pot	0.376	0.208	1.8	18.8	2.30	120
Flower pot	0.354	0.2	1.8	17.7	2.10	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. Benefits of Versa 1100 Workstation

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

- The workstation automated the whole process from soil samples to isolate DNA (except centrifugation) in the comparison to other systems that automate only from magnetic bead addition to the manually extracted soil homogenate.
- The system also automates NGS library prep for metagenomics and other applications
- The system offers a throughput of 96 samples within 3.5 hours of the entire process
- The 96-tip aspirator removes floating inhibitors resulting in inhibitor-free DNA purification
- The ReagentDrop channels save on time and running costs
- The automated removal of the supernates from soil and humic acid pellets minimizes manual intervention and human errors
- The high efficiency air fliter hood equivalent to a small ultra clean workbench effectively avoids cross contamination, a prerequisite for diagnostic and genomic applications

VII. Acknowledgements

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