Mo Bio PowerMag Soil DNA Isolation Kit on Biomek 4000 Liquid Handling Automation

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Introduction

This document describes the automation of Mo Bio PowerMag Soil DNA isolation kit (27100-4) for purification of microbial DNA from soil, compost, sediment etc. using the magnetic bead based separation technique on the Biomek 4000 liquid handling platform. The kit comes as 4 X 96 plate samples; it is highly recommended to be run as a full 96 well plate. The Biomek 4000 is a cost effective, user-friendly liquid handling solution that is ideal for less complex, lower throughput workflows. It provides the flexibility to use single channel or 8-channel pipetting tools for method development. The system includes a gripper to move the labware on and off the ALPs (Automated Labware Positioners) for temperature control, shaking and magnetic bead operations.

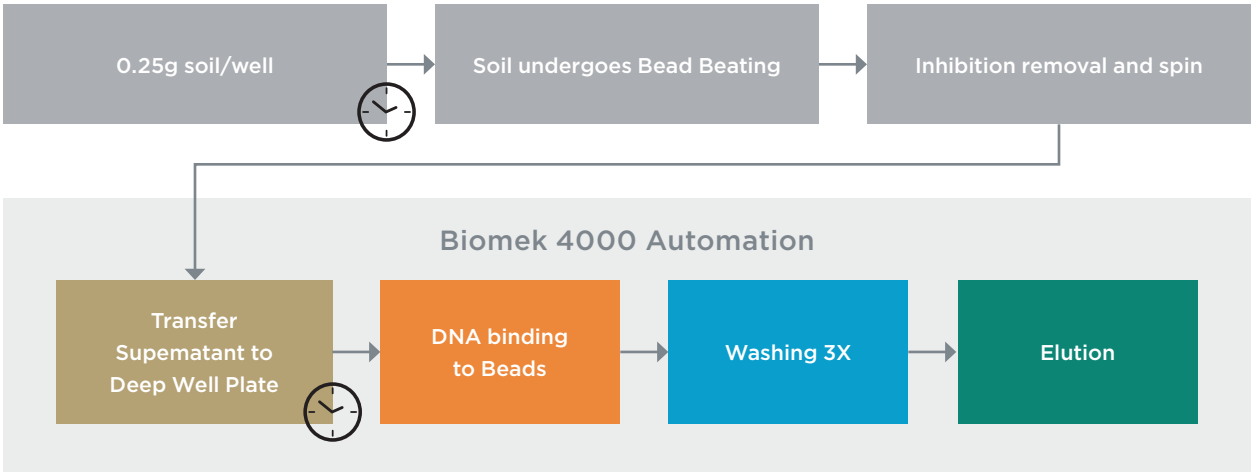

Figure 1. Workflow of the PowerMag DNA Isolation Kit

Figure 2. Biomek 4000 Liquid Handler

Benefits for Automating:

- Improve results and reproducibility: The ready-to-run method is designed with optimized techniques to help the users achieve accurate and reproducible results with maximum yield.
- Reduce Errors and increase walk-away time: The ready-to-run method is built with Guided Labware Setup which provides interactive and user-friendly instructions for quick deck setup and amount of reagents required.

Description of Automated Method

The method guides the user to set the deck correctly along with step by step interactive walk through for each labware position and how much reagent is required based on the sample number information provided by the user. The figures and tables below show examples of deck layout

and the time required for the completion of the method. If desired, the method can be run through the Biomek Method Launcher run time interface for a simplified run time experience.

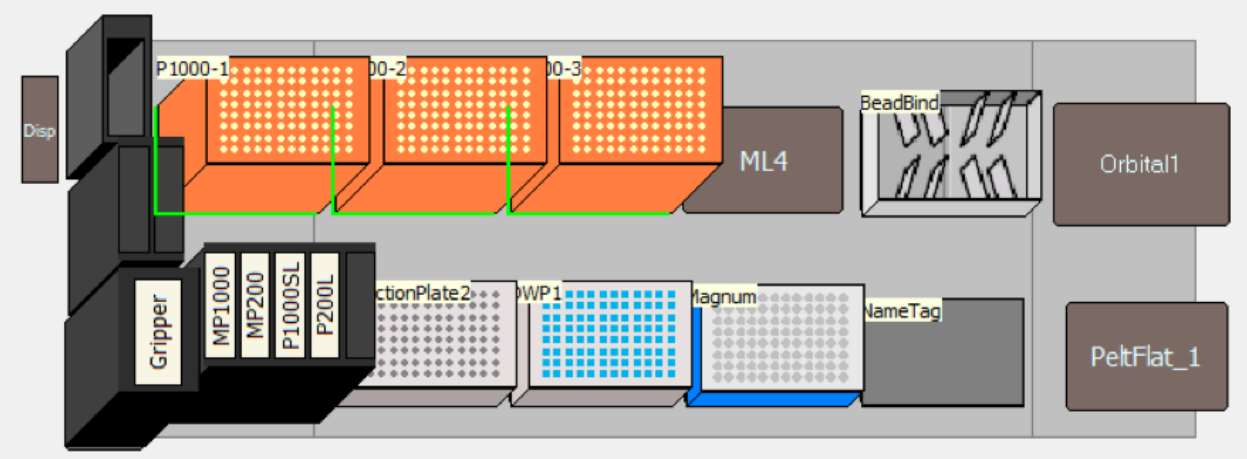

Figure 3. Deck Layout for 1st Guided Labware Setup

Name on Deck	Manufacturer	Labware Name in Biomek	Description	Manufacturer Part #	Per 96 Run
P1000-1, P1000-2, P1000-3	Beckman	Span_8_1000uL _Barrier	Biomek Span-8 P1000 Tips, Pre-sterile with Barrier	B01124	3
ElutionTips1, ElutionTips2	Beckman	AP96_200ul _Barrier	Biomek AP96 P250 Tips, Pre-sterile with Barrier	717253	2
BeadBind	Beckman	BCFullReservoir	Full reservoir with baffles	372784	1
ElutionBuffer	Beckman	BCI_QtrL	Quarter Reservoir, Divided by Length	372788	1
Modular Reservoir Frame	Beckman		Frame for Reservoirs	372795	1
Magnum	Alpaqua	Magnum_EX	Alpaqua Magnum EX Universal Magnet Plate	A000380	1
CollectionPlate2	Costar	Costar96_1ml_395x	Comes in the kit as 27100-4-EP-1CP	3959	1
DWP1	Greiner Bio-One	Greiner96Round DeepSquare	Comes in the kit as 27100-4-EP-DWP	780280	1
MTP1	Geiner Bio-One	Greiner96RoundPP	Comes in the kit as 27100-4-EP-MTP	650201	1

Table 1. Consumables required for the method

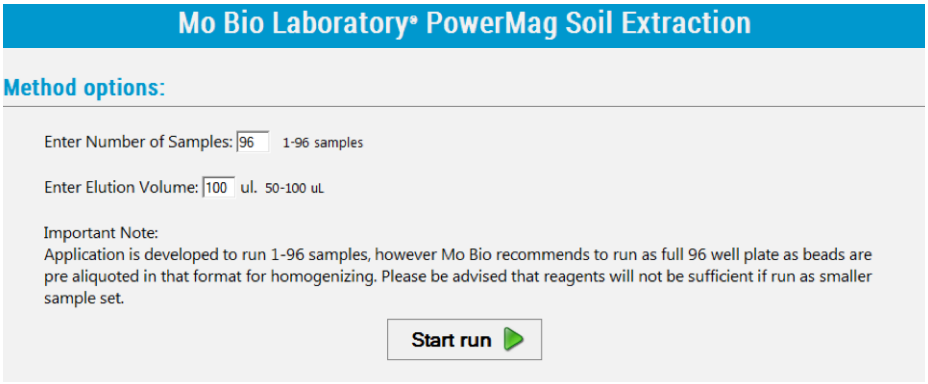

Figure 4. Method UI – User selects number of samples to be processed and desired elution volume. Required reagent volumes will be calculated automatically.

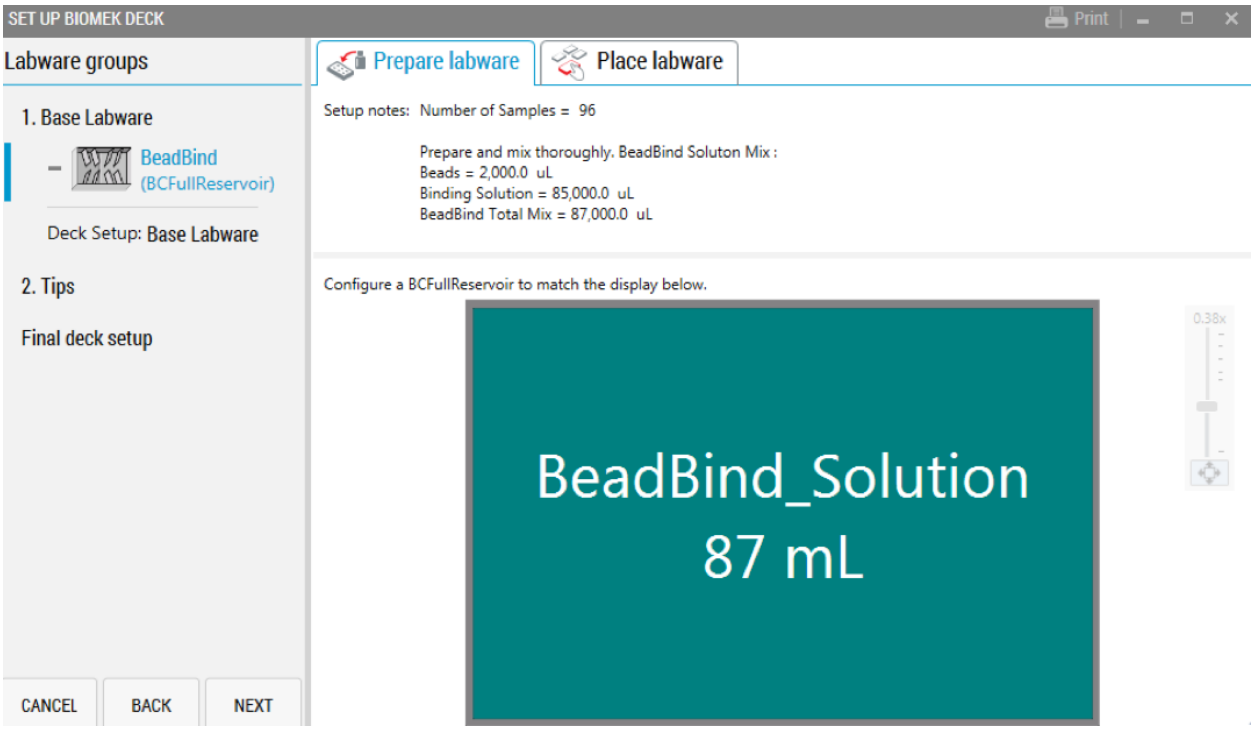

Figure 5. Example of labware depicted through Guided Labware Setup with required reagent and volume needed.

MAJOR PROCESS DESCRIPTION	
PowerMag CleanUp	96 Samples
Weigh out 96 soil samples	1 hour
Homogenizing	30 mins
Spins, Transfers and Inhibitor Removal	30 mins
Prepare Reagents/Set up on Robot	15 min
Method Run	5 hrs
Total	**7 hrs 15 min**

Table 2. Time estimate for running the experiment as full plate
**Timing does not include thawing of reagents

Experimental Design

250mg of Soil samples were taken from different yards and ponds and the experiment was performed following the protocol. Care was taken to weigh soil samples accurately and put into the wells carefully without cross contamination. After initial chemical and mechanical lysing and inhibitor removal, the samples are put on the automated platform for binding, wash and elution steps. In order to achieve good results and maximize yield, the manual steps before the beginning of the automated part of the protocol should be executed as specified in the protocol (for all manual supernatant transfers).

Results

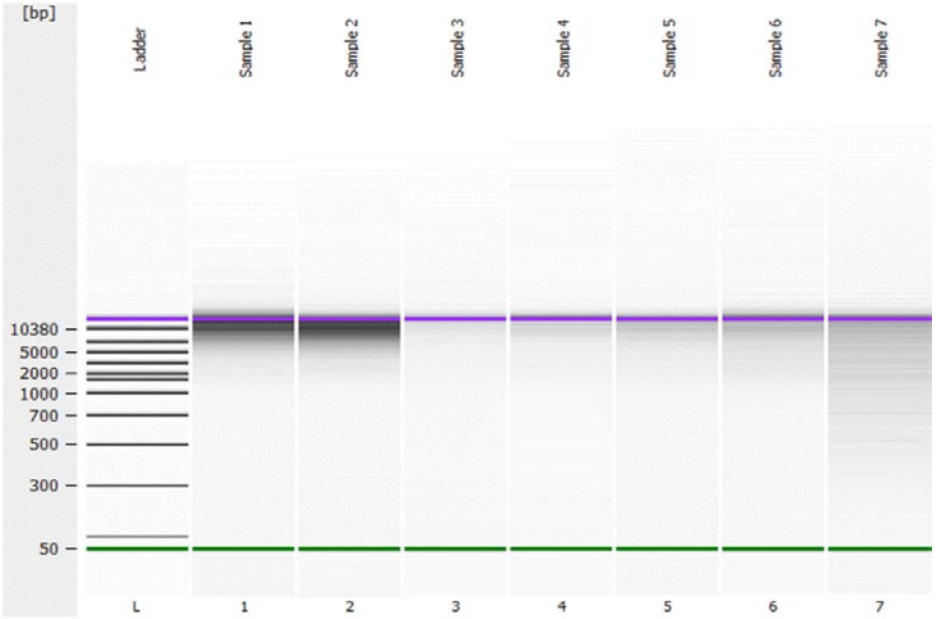

Figure 6. Extracted DNA was analysed with Agilent Bioanalyzer 2100 with Agilent DNA 12000 kit and DNA chip.

Gel Lane	Sample Well	Amount of soil used	Sample Name	Conc (100uL)	Yield ug
1	A1	250mg	ZS Yard	37.7 ng/ul	3.7
2	B1	250mg	ZS Yard	41.4 ng/uL	4.1
3	D1	250mg	BC Pond	11.38 ng/uL	1.1
4	E1	250mg	DownStream 1	14.4 ng/uL	1.4
5	F1	250mg	DownStream 1	15.2 ng/uL	1.5
6	G1	250mg	UpStream 1	20.1 ng/ul	2
7	H1	250mg	UpStream 1	20.3 ng/ul	2

Table 3. Samples loaded on the gel and their yield with 850uL of Lysate quantified by Nanodrop

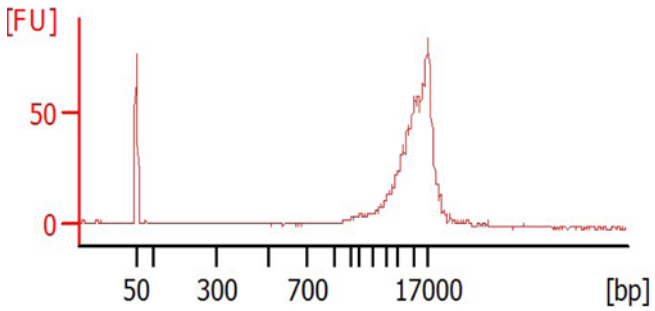

Figure 7. Sample electropherogram of sheared DNA

Conclusion

The method and the data demonstrate that the automated method generates measurable yields of quality DNA on Biomek 4000 platform. The method is also available for extracting DNA from 450uL of the lysate.

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