INTEGRATED BIOREPOSITORY OF H3AFRICA UGANDA

MAKERERE UNIVERSITY

COLLEGE OF HEALTH SCIENCES

STANDARD OPERATING PROCEDURE

TITLE: **Isolation of Microbial genomic DNA using the PowerSoil® DNA Isolation Kit**		**PAGE 1 of 6**
SOP #: **IBRH$_3$AU-SOP-BSP-017.1**	Effective Date: **01/08/2014**	Next Rev: **AUG 2021**
Prepared by :	Reviewed by:	Approved by:
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VALIDATION AND RETIREMENT

	NAME	DATE
Validated by:		
Retired by:		

ACKNOWLEDGEMENT OF READING AND UNDERSTANDING

I have received and understood the training on this SOP. If I have not understood the training I have asked the trainer to retrain me to ensure that I completely understand all the requirements.

	NAME	SIGNATURE	DATE
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1. PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to describe the Procedure for novel and proprietary method for isolating genomic DNA from environmental samples utilizing patented Inhibitor Removal Technology® (IRT). It is intended for use with environmental samples like faecal matter.

2. SCOPE

This SOP applies to all MBL personnel performing any assays that require the use of this procedure.

3. PRINCIPLE OF OPERATION

The PowerSoil® DNA Isolation Kit is effective at removing PCR inhibitors from samples. Samples are added to a bead beating tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane. DNA is then ready for PCR analysis and other downstream applications.

TASK, RESPONSIBILITIES AND ACCOUNTABILITIES

Task	**Responsible**	**Accountable**
Cleaning	All trained lab personnel with competence	Lab manager/designee
Operating	All trained Lab personnel with competence	Lab manager
Maintaining all records	Equipment officer	Lab manager
Adaptation of SOP	All trained lab personnel	Lab manager
Repair	Biomed Engineer	Lab manager
Regular review of related doc	Quality officer	Lab manger

4. ABBREVIATIONS AND DEFINITIONS

SOP- standard operating procedure

DNA-deoxyribose nucleic acid

5. EQUIPMENT

Micro centrifuge tubes (2 ml)

Overhead shaker

Shaker-incubator

Tissue Lyser

6. MATERIALS

6.1 Sterile pipet tips with aerosol barriers

6.2 Ethanol (96-100%)

6.3 Phosphate-buffered saline

7. SAFETY AND ENVIRONMENT

Please wear gloves when using this product. Avoid all skin contact with reagents in this kit. In case of contact, wash thoroughly with water. Do not ingest.

Solution C5 contains ethanol. It is flammable.

8. PROCEDURE

8.1 Add 0.25 grams of soil sample to the PowerBead Tubes provided and gently vortex to mix.

8.2 Check Solution C1. If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.

8.3 Add 60 µl of Solution C1 and invert several times or vortex briefly.

8.4 Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1-24) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

8.5 Centrifuge tubes at 10,000 x g for 30 seconds at room temperature.

CAUTION: Be sure not to exceed 10,000 x g or tubes may break.

Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing.

8.6 Transfer the supernatant to a clean 2 ml Collection Tube (provided).

8.7 Add 250 µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.

8.8 Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.

8.9 Avoiding the pellet, transfer up to, but no more than, 600 µl of supernatant to a clean 2 ml Collection Tube (provided).

8.10 Add 200 µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes

8.11 Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.

8.12 Avoiding the pellet, transfer up to, but no more than, 750 µl of supernatant into a clean 2 ml Collection Tube (provided).

8.13 Shake to mix Solution C4 before use. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 seconds.

8.14 Load approximately 675 µl onto a Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through and add an additional 675 µl of supernatant to the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature.

8.15 Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature.

8.16 Add 500 µl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x g.

8.17 Discard the flow through and centrifuge again at room temperature for 1 minute at 10,000 x g.

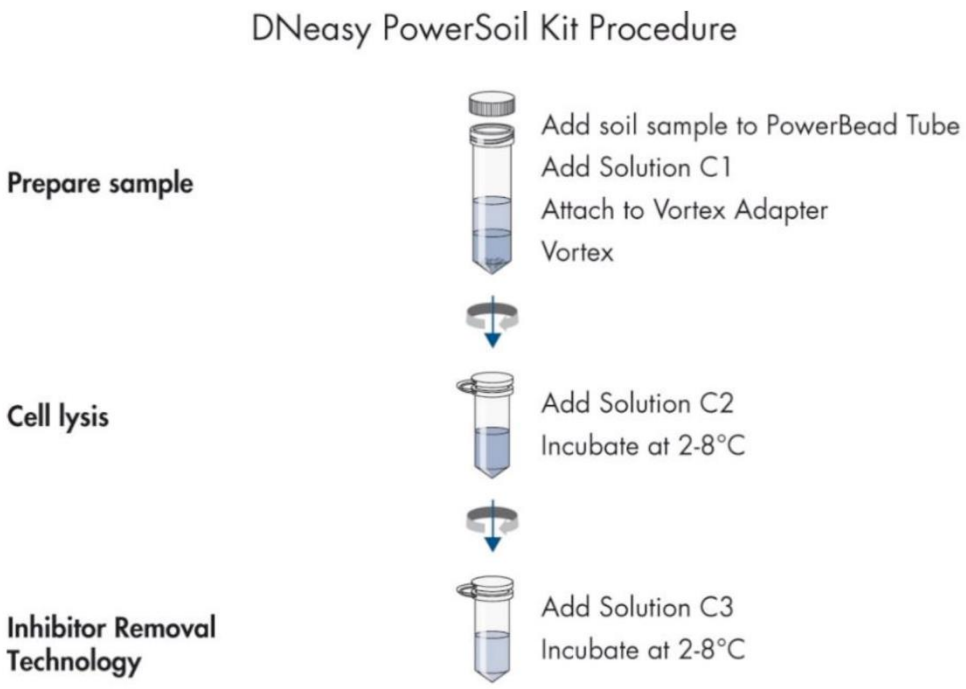
8.18 Carefully place spin filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.

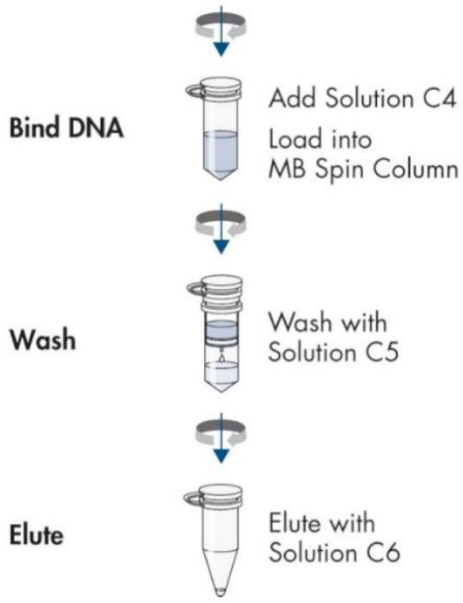
8.19 Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).

8.20 Centrifuge at room temperature for 30 seconds at 10,000 x g.

8.21 Discard the Spin Filter. The DNA in the tube is now ready for any downstream application.

8.22 Store DNA frozen (-20° to -80°C)





9. INTERNAL QUALITY CONTROL

9.1 **Nano drop**

9.2 **Qubit**

9.3 **Agarose Gel electrophoresis**

10. REFERENCES

https://mobio.com/media/wysiwyg/pdfs/protocols/12888.pdf

11. REVISION HISTORY

Revision No	Effective Date	Description of Changes Made from Preceding Revision	Approved by/ Date

ANNEX 1: DOCUMENTATION OF SUGGESTED CHANGES TO THIS SOP

CLAUSE	SUGGESTION	BY	DATE