GeneLab Standard Operating Procedure: DNA isolation using FastDNA SPIN Kit for Soil

April 2022

Version 1

Document Revisions

Document Number	Revision Number	Date	Description of Changes
GL-SOP-3.4	1	April 2022	Original

Scope and Purpose

The procedure below describes the steps required to isolate genomic DNA from soil and other environmental samples using the FastDNA SPIN kit.

Equipment and Consumables

1. Analytical scale
2. Small (2”) weigh boat (VWR, Cat#10770-454 or similar)
3. Microspoon and spatula
4. Eppendorf Centrifuge 5424/5424 R
5. Heat block
6. 2mL Collection tubes
7. 15mL Tubes
8. Table top vortex (VWR, Cat#102091-234 or similar)

Reagents

1. FastDNA SPIN Kit for Soil, MP Biomedicals, Cat# 116560200, 50 preps
    a. Lysing Matrix E
    b. Sodium Phosphate Buffer
    c. MT Buffer
    d. PPS Solution
    e. Binding Matrix
    f. SPIN Modules
    g. Catch Tubes
    h. Concentrated SEWS-M
    i. DES

Procedure

1. To prepare the sample, add up to 500 mg of soil sample, 978 µL Sodium Phosphate Buffer, and 122 µL MT Buffer to Lysing Matrix E tube.
2. Load filled Lysing Matrix E tube in Bullet Blender Gold (Refer to GL-SOP-002.1 for Tissue Homogenization using Bullet Blender Gold) and set to 3 min at speed 12.
3. Centrifuge samples in Lysing Matrix E tubes at 14,000 x g at RT for 5-10 mins to pellet debris.
4. To precipitate proteins, transfer supernatant to a clean 2 mL microcentrifuge tube, and add 250 µL PPS and pipet 10 times to mix.
5. Centrifuge at 14,000 x g for 5 mins to pellet precipitate.
6. To adjust binding conditions, transfer all supernatant to 15 mL tube and add 1 mL Binding Matrix Solution (mix well by vigorous shaking or vortexing). Invert tubes for 2 mins and incubate at RT for 3 mins.
7. Discard 500 µL of supernatant (in a separate clean tube in case needed to recover more DNA).
8. To bind the DNA, transfer max 600 µL of DNA Solution to a SPIN Filter Tube.
9. Centrifuge at 14,000 x g at RT for 1 min and empty catch tube.
10. Repeat steps 8-9 if the volume of the mixture is higher than 600 µL.
11. To wash the spin filter, add 500 µL SEWS-M Solution.
12. Centrifuge at 14,000 x g at RT for 1 min and empty catch tube.
13. To dry the spin filter, centrifuge again at 14,000 x g at RT for 2 mins.
14. Air dry SPIN Filter for 5 mins at room temperature in new catch tube.
15. Pre-heat DES Elution Solution at 55 C.
16. To elute DNA, add 50 µL DES Elution Solution and place spin filter tubes on heat block at 55°C for 5 mins.
17. Centrifuge at 14,000 x g at RT for 1 min.
18. DNA in the catch tube is ready-to-use.
19. Measure DNA concentration by Qubit 1X dsDNA HS Assay Kit (Refer to SOP #4.1 DNA quantification using Qubit Fluorimeter).
20. Measure gDNA size by TapeStation 4200 using Genomic DNA ScreenTape (Refer to SOP #4.2 QC genomic DNA).
21. Store the DNA in -80°C until use.