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

April 2022

Version 1

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Document Revisions

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| GL-SOP-3.4 | 1 | April 2022 | Original |
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# 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
2. Lysing Matrix E
3. Sodium Phosphate Buffer
4. MT Buffer
5. PPS Solution
6. Binding Matrix
7. SPIN Modules
8. Catch Tubes
9. Concentrated SEWS-M
10. 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.

1. 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.
2. Centrifuge samples in Lysing Matrix E tubes at 14,000 x g at RT for 5-10 mins to pellet debris.
3. To precipitate proteins, transfer supernatant to a clean 2 mL microcentrifuge tube, and

add 250 μL PPS and pipet 10 times to mix.

1. Centrifuge at 14,000 x g for 5 mins to pellet precipitate.
2. 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.
3. Discard 500 μL of supernatant (in a separate clean tube in case needed to recover more DNA).
4. To bind the DNA, transfer max 600 μL of DNA Solution to a SPIN Filter Tube.
5. Centrifuge at 14,000 x g at RT for 1 min and empty catch tube.
6. Repeat steps 8-9 if the volume of the mixture is higher than 600 μL.
7. To wash the spin filter, add 500 μL SEWS-M Solution.
8. Centrifuge at 14,000 x g at RT for 1 min and empty catch tube.
9. To dry the spin filter, centrifuge again at 14,000 x g at RT for 2 mins.
10. Air dry SPIN Filter for 5 mins at room temperature in new catch tube.
11. Pre-heat DES Elution Solution at 55 C.
12. To elute DNA, add 50 μL DES Elution Solution and place spin filter tubes on heat block at 55°C for 5 mins.
13. Centrifuge at 14,000 x g at RT for 1 min.
14. DNA in the catch tube is ready-to-use.
15. Measure DNA concentration by Qubit 1X dsDNA HS Assay Kit (Refer to SOP #4.1 DNA quantification using Qubit Fluorimeter).
16. Measure gDNA size by TapeStation 4200 using Genomic DNA ScreenTape (Refer to SOP #4.2 QC genomic DNA).
17. Store the DNA in -80°C until use.