Protocol for DNA Extraction from CCZ Sediment Samples

MP Bio FastDNA SPIN Kit for Soil **protocol is modified to obtain higher yield (Vonnahme et al., 2020); changed steps are bold on Laboratory Procedure part.**

General equipment and supplies:

- Racks for tubes (more the merrier)
- Lysing Matrix E (provided with kit)
- Precision scale
- Water-bath or heating block (water-bath is preferable)
- Micro-centrifuge (max. 14000X G is required)
- 2ml sterile micro-centrifuge tubes.
- 15 ml sterile centrifuge tubes.
- SPIN Filters (provided by kit)
- 2 ml sterile catch tubes (provided by kit)
- Set of pipettes and tips (10-1000 µl)

Reagents and Solutions:

NOTE: Solutions and reagents are provided by kit, but SEWS-M solution should be diluted by using 100% Ethanol when kit is opened. All solutions are stored at room temperature.

- Sodium Phosphate Buffer
- MT Buffer
- PPS (Protein Precipitation Solution)
- Binding Matrix suspension
- SEWS-M
- DES (DNAse/Pyrogen-Free Water)
- 70% Ethanol for cleaning
- 100% Ethanol for dilution of SEWS-M

DNA Extraction from Muddy Sediment with

1) Add 500 mg of sample to Lysing Matrix E Tube

2) Add 978 µL Sodium Phosphate buffer to sample in Lysing Matrix E Tube

3) Add 122 µL MT Buffer

4) Incubate sample and buffers for 15 minutes at room temperature (optional)

5) Homogenize sample in the FastPrep instrument for 60 seconds at a speed setting of 4.0 m/s

6) Incubate the sample at 65°C for 10 minutes

7) Centrifuge at 14000X G for 10 minutes

8) Transfer Supernatant (ca. 1250 µL is expected) to a 2.0 mL microcentrifuge tube (use autoclaved tubes from house).

9) Add 250 µL PPS (Protein precipitation solution), inverse the tube **20 times.**

10) 5 Minutes incubation on ice

11) Centrifuge at 14000X G for 5 minutes.

12) Take supernatant into a new tube.

13) Repeat steps 9, 10.

14) Centrifuge at 14000X G for 5 minutes.

15) Transfer Supernatant to a 15 ml microcentrifuge tube.

16) Resuspend the binding matrix suspension and add 1.0 mL to the supernatant in the 15 mL tube.

17) Invert sample and binding matrix for 4 minutes, after inversion wait another 5 minutes for resettling.

18) Remove and discard 550 µL of upper phase. Be careful to not to disturb settled matrix.

19) Gently resuspend Binding matrix in the remaining amount of upper phase.

20) Transfer **780 µL** of the mixture to a SPIN filter.

21) Centrifuge at 14000X G for 1 minute.

22) Discard the flow-through, and replace the SPIN filter.

23) Repeat steps 19 to 21 until all mixture is passed through the column. **(780, 680, 680 µL)**

24) Add 500 µL prepared SEWS-M and gently resuspend by using flow of the liquid.

25) Centrifuge at 14000X G for 1 minute.

26) Discard the flow-through, and replace the SPIN filter.

27) Centrifuge at 14000X G for 2 minutes.

28) Discard the catch tube and put the tube in a new catch tube (it will be the final tube with sample, beware centrifuge tubes from the house is not suitable for that part, use tubes provided by kit).

29) Air dry the SPIN filter for 5 minutes at room temperature.

30) Resuspend the binding matrix (which is the SPIN filter tube) with 50 µL of DES (Dnase/Pyrogen free Water)

31) Incubate at 55°C for 5 minutes.

32) Centrifuge at 140000X G for 2 minutes.

33) Place flow-thorugh on filter again, resuspend gently. Replace the SPIN filter in the same tube.

34) Incubate in RT for 5 Minutes

35) Centrifuge at 140000X G for 2 minutes.

36) Keep DNA at -20°C for downstream applications.