Becca Maher notes

Added some of my own notes

High Throughput DNA Extraction Protocol

Modified from DNeasy PowerSoil Pro Kit (50) (QIAGEN® catalogue: 47014)
Rebecca Maher
September 26, 2023
Notes/questions added by Grace: July 29th, 2026

Items NOT provided in the extraction kit:

1. RNase AWAY™ surface decontaminant (Thermo Scientific™ catalogue: 700511)
    a. I don't think we have any of this… but we have Eliminase that gets rid of RNAse and DNAse and DNA contamination
2. 100% molecular biology grade ethanol (Koptec, Decon Labs catalogue: V1001)
    a. Have a ton from OG from FHL
3. Large volume pipette (100-1000 µl)
4. Medium volume pipette (50 µl)
5. Appropriate tips for pipette
6. Water bath at 65˚C
7. Microcentrifuge
8. Vortex-Genie® 2
9. Vortex Adapter for 24 (1.5–2 ml) tubes (cat. no. 13000-V1-24)
10. Laboratory quality permanent marker (Sharpie)
11. Tweezers
12. Bunsen burner and tubing Don't have this, but I have a lighter

Items included in the extraction kit (for 50 preps):

1. PowerBead Pro Tubes
2. MB Spin Columns
3. Labeled solutions
    a. Solution CD1
    b. Solution CD2
    c. Solution CD3
    d. Solution EA
    e. Solution CD5
    f. Solution CD6
4. Microcentrifuge Tubes (2ml) x 100
5. Elution Tubes (1.5 ml) x 50
6. Collection Tubes (2 ml) x 100
7. Quick Start Protocol

Before you start:

1. Wear gloves and the required personal protective equipment (PPE) at all times
2. Clean all work surfaces, pens, tools, and pipettes to be used with RNase Away followed by ethanol to remove DNA and other contaminants
3. Record date, time, kit batch number, and sample names in lab notebook.

Procedure:

1. Ensure the work area and necessary objects are clean, use both RNase Away and ethanol for cleaning.

2. ~~Fill 15 mL falcon tube with MilliQ water. I don't think we have MilliQ… I think we have Nanopure water?~~

3. Label / prefill all tubes
    a. Bead tubes (label 1-24)
    b. –2 : 200ul C2 (label 1-24)
    c. –3 : 600ul C3 (label 1-24)
    d. Spin tubes (label 1-24)
    e. Final tubes (label with sample names)

4. Centrifuge the PowerBead tubes at 2500 x g for 1 min to pellet the beads.

5. Retrieve samples from the -80C freezer.

6. Transfer sample to PowerBead tubes. All initial transfers of samples into PowerBead tubes should be performed **in the presence of a flame**.
    a. Swab: Sterilize tweezers with 70% ethanol and flame. Use tweezers to transfer swab from sample tube to PowerBead tube.
    b. Filled PowerBead tubes **may be stored at -20C for up to 24 hours** before adding CD1.

7. Add 800 µl of CD1 to each PowerBead tube. Vortex briefly to mix.

8. Incubate tubes for 10 mins in a 65˚C water bath.
    a. Added water to tube holders in the incubator thing

9. Immediately, secure the PowerBead Pro Tubes horizontally on a Vortex Adapter for 1.5–2 ml tubes.
    a. Kit: Vortex at maximum speed for 10 min. If using the Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time by 5-10 mins.

10. Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 min.

11. Transfer the supernatant (500-600 µl) to Microcentrifuge Tube (provided) with 200 ul C2 solution. **Vortex for 5 s.**

12. Centrifuge at 15,000 x g for 1 min.

13. Avoiding the pellet, transfer up to 700 µl of supernatant to Microcentrifuge Tube (provided) with 600 ul C3 solution. Vortex for 5 s.

14. Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15,000 x g for 1 min.

15. Discard the flow-through and repeat step 14 to ensure that all of the lysate has passed through the MB Spin Column.

16. Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.

17. Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.

18. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.

19. Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.

20. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube.

21. Centrifuge at up to 16,000 x g for 2 min.
    a. This step removes the residual C5 solution.

22. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).

23. Add 50 µl of Solution C6 (kit) or Nuclease-free water (Davis) to the center of the white filter membrane.

24. Incubate at room temperature for 10 mins.

25. Centrifuge at 15,000 x g for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    a. Store DNA at -20C or lower. Will store at -80C

Aug 9, 2026

DNA eelgrass swab extractions → try with one sample per treatment plus blanks in order to test protocol → after protocol run 1ul of the eluted DNA on nanodrop to make sure it worked before doing all the other swabs!!

Set up benchtop:
11:30-1151am
Wipe down with dilute bleach and then Eliminase then ethanol
Wipe down P1000 and P200

Gather supplies:

- Microcentrifuge
- Vortex (with ability to vortex multiple tubes horizontally)
- Water bath (able to be 65C)
- Molecular grade water
- P1000 tips
- P200 tips
- Sharpie
- Tweezer
- Sterile setup
    - Dilute bleach
    - 70% ethanol
    - Flame

#	Sample Name
1	S_ESW_01
2	S_EVP_03
3	S_EM_03
4	S_EMVP_02
5	S_Blank_01
6	S_Blank_02

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9. MB Spin Columns
10. Labeled solutions
    a. Solution CD1
    b. Solution CD2
    c. Solution CD3
    d. Solution EA

    e. Solution CD5
    f. Solution CD6
11. Microcentrifuge Tubes (2ml) x 100
12. Elution Tubes (1.5 ml) x 50
13. Collection Tubes (2 ml) x 100
14. Quick Start Protocol

PROTOCOL:
FORGOT TO TURN HEATER TO 65C!! NEXT TIME SET BEFORE CENTRIFUGING

Add 800ul of molecular grade H20 to each well that will be used

1. Centrifuge powerbead tubes 2500g for 1 min
2. Transfer swabs to powerbead tubes
    a. Use Sterile technique
    b. For blanks… do nothing but open the caps (no swab)
3. ADD 800UL CD1 to powerbead tubes and vortex briefly
    a. Forgot to turn on 65c heater… so samples are sitting for about 10min
4. Incubate at 65c for 10min
5. Tape horizontally to a tube rack and vortex at top speed for 10mins
6. Centrifuge for 1min at 15,000 g
7. Transfer 500ul of liquid to microcentrifuge with 200ul of C2 solution
    a. Have to use sterile tweezers to remove swab so that can access liquid
8. Centrifuge 15,000g for 1 min
    a. FORGOT TO VORTEX FIRST
        i. NEXT TIME VORTEX 5 SEC BEFORE CENTRIFUGE
9. Avoiding the pellet, transfer up to 700 µl of supernatant to Microcentrifuge Tube (provided) with 600 ul C3 solution. Vortex for 5 s.
10. Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15,000 x g for 1 min.
11. Discard the flow-through and repeat step 11 to ensure that all of the lysate has passed through the MB Spin Column.
12. Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.
13. Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.
14. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.
15. Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.
16. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube.
17. Centrifuge at up to 16,000 x g for 2 min.
    a. This step removes the residual C5 solution.
18. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).
19. Add 50 µl of Solution C6 (kit) to the center of the white filter membrane.
20. Incubate at room temperature for 10 mins.
21. Centrifuge at 15,000 x g for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    a. Store DNA at -80

i. Run 1ul of each sample on nanodrop to ensure that there is actually DNA!!

b.