Thacker Lab: PowerSoil Pro DNA Extraction Protocol

Qiagen DNeasy PowerSoil Pro DNA Isolation Kit, Catalogue #: 47016

https://www.qiagen.com/us/resources/resourcedetail?id=9bb59b74-e493-4aeb-b6c1-f660852e8d97&lang=en

Starting Notes: This version of the protocol requires the PowerLyzer to shake the tubes. Be sure to practice how to **lock** the PowerLyzer, especially the small latch on the tube holder. You sometimes need to have a tiny rotation of the tube holder to fully **lock** it. Note that the PowerLyzer rubs off labels, so be sure to note the position of each sample in the rotor.

□ **Checkboxes can help you stay on track through this protocol**

⏱ **Some steps require an incubation time**

⚠ **This symbol indicates points where you can stop and store your materials in the refrigerator. When you start again, make sure your materials have reached room temperature before proceeding.**

***Mise en place*:** Make sure you read through this whole protocol and locate all specimens and reagents before starting.

□ Be sure to make aliquots of all of the reagents to prevent contamination. It is easiest to pour 25 to 30 ml into a 50 ml tube for **Solutions CD1, CD2, CD3, EA, and C5**. Note: we will use warm PCR-quality water instead of solution C6.

□ **Solution CD2** is kept in the refrigerator.

□ If **Solution CD3** has precipitated, heat at 60°C until precipitate dissolves.

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□ Take samples out and warm to room temperature

□ **Heat 120µl of PCR-quality water for each sample to 50-60°C in the dry bath before starting the second lab period.**

1. □ Locate all specimens to be extracted.
    a. Be sure to note in your lab notebook and in an XL spreadsheet the complete sample identification numbers and any short-hand numbers you might be using.
    b. Be sure to compile all the metadata associated with a specimen before beginning extraction.
2. □ Don your gloves and label tubes. You will need the following tubes, in order, per sample:
    a. □ 1 labeled PowerBead tube
    b. □ 2 labeled 2.0 mL microcentrifuge tubes
    c. □ 1 labeled MB Spin Column
    d. □ 1 labeled 2.0 mL Collection tube (without a lid, provided in the kit) for each specimen
    e. □ 1 labeled 1.5 mL Elution tube (label this for DNA storage)

Sometimes it is easier to use a short-hand number on the lid and side of the PowerBead tube – there is not much space to write. ***Be sure to label both the lid and the side of every tube.*** The PowerLyzer might remove the lid label, in particular. It is also good to

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plan out a consistent PowerLyzer and Centrifuge slot for each tube, just in case labels are removed by ethanol-based solutions.

3. **If you are using the BeadBeater instead of the PowerLyzer**, first transfer the contents of your PowerBead Tubes to the special BeadBug 1.5mL tubes found in the BeadBeater supply cabinet. Beat for 180 seconds at speed 300 to separate the beads. Be sure to label these 1.5mL BeadBug tubes.

4. □ Subsample tissue from the sponge or other organism. If you are making several of these, you might need an additional labeled 1.5 ml tube to hold each subsample prior to processing. You can also place subsamples in a multi-well plate prior to processing. *If you are extracting a water filter, cut one quarter of the filter to be extracted.*

    a. Clean tweezers, scalpel, and/or razor with ethanol. Use tweezers and/or blade to remove a VERY small chocolate-chip-sized piece of tissue from each sample. Put piece in labeled tube. If you have a lot to do, this tube could contain additional 95% ethanol or sterile water (if the sample was previously in RNAlater and needs to be rinsed) to prevent the tissue from drying out. ***Note: Do not rinse the water filters.***

    b. Be sure to dip tweezers and razor blades in ethanol and clean between each sample to avoid contamination. We used to flame sterilize these tools, but that is not recommended because of the risk of fire and general safety.

    c. For general purpose sponges, take a piece just below surface of sponge; however, include the external layer for anything with a photosymbiont.

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d. If you have many samples to process, this is a good stopping point. All the subsamples can go back in the freezer at this point. If you freeze everything, be sure to thaw back to room temperature before proceeding.

e. For some specimens, it is better to homogenize the specimen with a mortar and pestle. In these cases, use 10% bleach to clean the mortar and pestle (soak for 5 minutes), then rinse in DI water (soak for 5 minutes), then clean with ethanol.

5. □ Spin the PowerBead Pro Tubes briefly to ensure that the beads have settled at the bottom of the tube.

6. □ Place the specimen tissue (about 0.25 grams) or quarter water filter in the tube with the beads.

7. □ Add 800 µl of **Solution CD1** to each tube. Vortex the tube for 5 seconds to mix.

8. Homogenize the samples with beads using a BeadBeater or a PowerLyzer.

□ **BeadBeater**: Place tubes 3 at a time in the BeadBeater and beat for 180 seconds at speed 300. If less than 3 tubes, it must be balanced, like a centrifuge.

□ **PowerLyzer**: Place up to 24 tubes into the PowerLyzer. It must be balanced, like a centrifuge. Be sure to secure the locking ring to hold the tubes in place. There is a small latch on the locking ring; you might need to make a tiny rotation to the locking ring to fully secure the latch. Make sure it is secure and **locked**. **Set the timer to 7 minutes, 30 seconds x 2 for a total of 15 minutes. Set the speed to 2000.** Press run, but be ready to press stop if the latch is not secure. Note: the PowerLyzer rubs off labels, so be sure to note the position of each sample in the rotor.

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Note: if your DNA is too fragmented, try this same speed, but for less time, approximately 5 minutes. Alternatively, switch to the proteinase-K based extraction method.

9. ☐ After beating, centrifuge at 13,000 x *g* for 1 minute. If you do not have a good pellet, centrifuge for 3 more minutes.

10. ☐ Transfer the supernatant (400-500 µl) to a **new**, labeled 2.0 mL microcentrifuge tube. ⚠

11. ☐ Obtain **Solution CD2** from the refrigerator. Use the aliquot from a 50 mL tube first. This solution ***must*** be kept in the refrigerator.

12. ☐ Add 200 µl of **Solution CD2** to each tube. Vortex for 5 seconds to mix, then incubate at 5°C (in refrigerator) for 5 minutes. ⏱

13. ☐ Centrifuge at 13,000 x *g* for 1 minute.

14. ☐ Transfer the supernatant (up to 600 µl) to a **new**, labeled 2.0 mL Microcentrifuge Tube. Do not disturb the pellet. ⚠

15. ☐ Add 600 µl of **Solution CD3** to each tube. ☐ Vortex for 5 seconds to mix. ☐ Incubate at 5°C (in refrigerator) for 5 minutes. ⏱

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16. ☐ Load the lysate (up to 650 µl) onto a labeled **MB Spin Column** and centrifuge at 13,000 x *g* for 1 minute.

17. ☐ Discard the flow-through and repeat loading and centrifuging until all of the lysate has been passed through the MB Spin Column.

18. ☐ Carefully place the MB Spin Column into a labeled 2 ml Collection Tube.

19. ☐ Add 500 µl of **Solution EA** to each MB Spin Column. Centrifuge at 13,000 x *g* for 1 minute.

20. ☐ Discard the flow through and place the MB Spin Column back into the **same** 2 ml Collection Tube. ⚠

21. ☐ Add 500µl of **Solution C5** to each MB Spin Column. Centrifuge at 13,000 x *g* for 1 minute.

22. ☐ Discard the flow through and place the MB Spin Column into the **same** 2 ml Collection Tube.

23. ☐ Centrifuge at 13,000 x *g* for 2 minutes.

24. ☐ Carefully place the MB Spin Column into **a new, labeled 1.5 mL elution tube.** Discard collection tube.

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25. □ Add 100 µl of Solution C6 or **PCR-quality water [50-60°C]** to each filter.

    Incubate at room temperature for **15 minutes.** ⏱

    IMPORTANT: Instead of C6, the Thacker Lab uses **50-60°C PCR-quality water** to elute the DNA. For many downstream applications, C6 is a problem. Warm water can enhance elution.

26. □ Centrifuge at 15,000 x *g* for 1 minute. Discard the MB Spin Column and keep the solution in the elution tube.

27. □ Store the DNA in the refrigerator or freezer. It is helpful to carefully label one of the square cardboard boxes on the top and side.

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