Zen DNA Extraction Protocol

This protocol is a modified version of the MoBio PowerSoil® DNA Isolation Kit protocol. The modifications are for time-saving and Zen-specific extraction optimization.

Before starting:

- Set heat block to 60°C

1. After following the **Zen Pre-Extraction protocol**, all samples should be in **PowerBead** tubes.
2. **Check Solution C1**. If **Solution C1** is precipitated, heat solution to 60°C until dissolved before use.
3. Add 60 μl of **Solution C1** and vortex briefly. *Note: You will see a white precipitate formed as a result of the cold temperature of the tubes and C1 mixing with the Zymo Stabilization buffer.*
4. Place **PowerBead tubes** in heat block at 60°C for 5 minutes.
5. Place **PowerBead tubes** in bead-beater for 2-3 minutes.
6. While bead-beating, prepare **2ml Collection tubes**: Add 250 μl of **Solution C2** to each.
7. Make sure the **PowerBead Tubes** rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 1 minute at room temperature. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.
8. Transfer 420 μl of the supernatant to **2 ml Collection Tub**e with C2. *Note: Expect between 400 to 500 of supernatant. Left-over supernatent may be stored at -20° C for later extraction. Supernatant may still contain some soil particles.*
9. Vortex for 5 seconds. Incubate at 4°C for 5 minutes.
10. While C2 tubes are incubating, prepare **2ml Collection tubes**: Add 200 μl of **Solution C3** to each.
11. Centrifuge the tubes containing C2 at room temperature for 1 minute at 10,000 x g.
12. Avoiding the pellet, transfer up to, but no more than, 600 μl of supernatant to a C3-filled **2 ml Collection Tube**.
13. Vortex C3 tubes for 5 seconds. Incubate at 4°C for 5 minutes.
14. While C3 tubes are incubating, prepare **2ml Collection tubes** and **Spin Filters**:
    - Organize open **2ml Collection tubes** and open **Spin Filters** opposite each other on a rack. (See diagram)
    - Shake to mix **Solution C4** before use. Add 1200 μl of **Solution C4** to the **2ml Collection tubes**.

      Note: This may take longer than 5 minutes to accomplish. If so, remove incubating tubes and finish task while centrifuging.

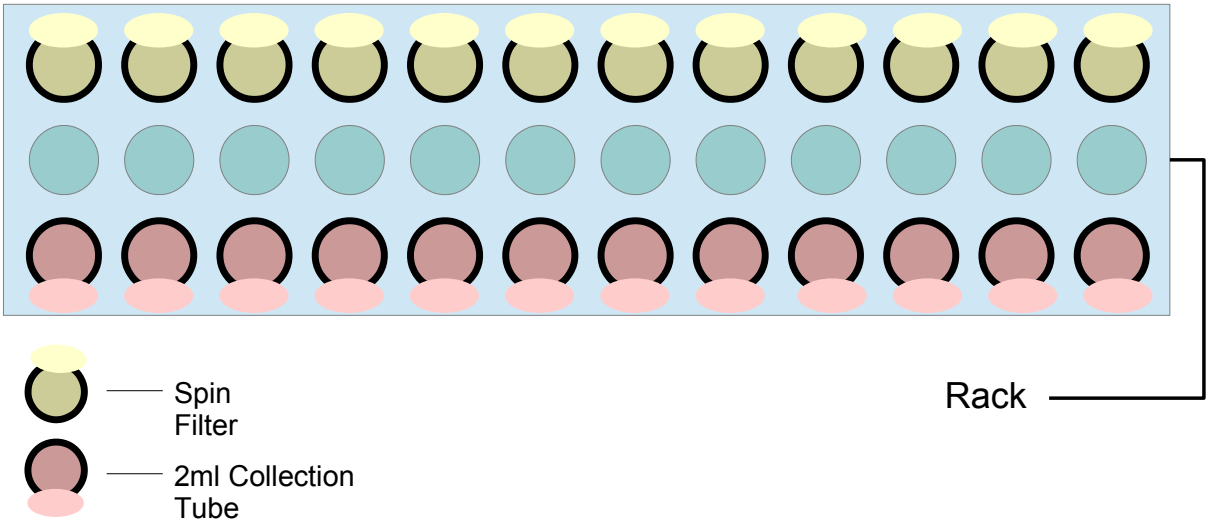

Figure 1: Collection tube and Spin Filter arrangement.

15. Centrifuge the tubes containing C3 at room temperature for 1 minute at 10,000 x g.

16. Set pipette to 800 μl. Carefully avoiding the pellet remove **all supernatant** from tube. In C4-filled **2ml Collection tube** carefully pipette up and down 4-8 times to mix, then fill open **Spin Filter** with mixed liquid. Dispense remaining liquid back into the 2ml Collection tube and *carefully* eject pipette tip into open 2ml Collection tube.

    Note: Avoid over-filling spin filter or it will over-flow when capped. If working with a pipette which violently ejects tips, discard tip in trash instead of ejecting into 2ml Collection tube.

17. Centrifuge **Spin Filters** at 10,000 x g for 1 minute at room temperature. Discard flow through and refill with C4-supernatant mixture using previous pipette tips. Repeat 3 times or until C4-supernatant mix is used up.

18. Add 500 μl of **Solution C5** and centrifuge at room temperature for 1 minute at 10,000 x g. Discard the flow through.

19. Centrifuge again at room temperature for 1 minute at 10,000 x g.

20. While centrifuging, prepare **2ml Collection tubes** to receive spin filters.

21. Carefully place spin filter in clean **2 ml Collection Tubes**. Avoid splashing any **Solution C5** onto the **Spin Filter**.

22. Add 100 μl of **sterile DNA-Free PCR Grade Water** to the center of the white filter membrane.

23. Centrifuge at room temperature for 1 minute at 10,000 x g.

24. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required. Store between -20°to -80°C.