Powersoil DNA Extraction Protocol for Root Samples DATE:\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Before starting, bleach and ethanol the entire bench. Do not do this extraction concurrent with other extractions happening in the lab. Use a new checklist for each extraction.

* 1) Label / prefill all tubes

|  |  |
| --- | --- |
| # | Sample Name |
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| 22 |  |
| 23 |  |
| 24 | Blank! |

Bead tubes (label 1-24)

–2 : 250ul C2 (label 1-24)

–3 : 200ul C3 (label 1-24)

–4 : fill during fridge incubation periods. Be sure to shake C4 before use.

Spin tubes (label 1-24)

Final tubes (label with sample names)

* 2) Add 500ul of MilliQ water to each sample tube. Note any anomalies with the samples in the lab notebook.
* 3) Vortex in pairs for 30 seconds (use timer)
* 4) Transfer (pipetting up and down every time) liquid to bead tube. Mix liquid while adding by pipetting up and down.
* 5) add 60ul C1 solution to each sample (if C1 precipitated heat in drying oven until dissolved). Invert several times
* 6) Incubate 5 minutes at 60˚C in the drying oven.
* 7) Bead beat at 30r/s for 3 minutes in bead beater in Schmitt lab. Be sure to balance the bead beater)
* 8) Centrifuge 30 seconds at 10,000 rpm
* 9) Avoiding the pellet, pipet up to but no more than 1000ul of supernatant into tube with 250ul C2 solution. Vortex for 5 seconds.
* 10) Incubate in the fridge at 4˚C for 5 minutes.
* 11) Centrifuge 1 minute at 10,000 rpm
* 12) Avoiding the pellet, pipet up to but no more than 600ul of supernatant into tube with 200ul C3 solution. Vortex briefly.
* 13) Incubate in the fridge at 4˚C for 5 minutes.
* 14) Centrifuge 1 minute at 10,000 rpm
* 15) Avoiding the pellet, pipet up to but no more than 750ul of supernatant into tube with 1200ul C4 solution. Vortex for 5 seconds.
* 16) Add 660ul solution to spin column
* 17) Centrifuge 1 minute at 10,000 rpm. Discard the flow through.
* 18) repeat steps 16 and 17 two more times until all solution has been transferred to spin column/discarded
* 19) Add 500ul solution C5 to the spin column
* 20) Centrifuge 30 seconds at 10,000 rpm. Discard flow through.
* 21) Centrifuge 1 minute at 10,000 rpm
* 22) Carefully transfer spin column to final tubes labeled with sample names.
* 23) Add 50ul of C6 elution buffer directly to the center of white membrane on the spin column (do not touch the membrane)
* 24) Let sit 5 minutes, then centrifuge in batches of 12 for 30 seconds at 10,000 rpm.
* 25) Close tubes and put them in the freezer. Confirm positions in the box/freezer spreadsheet.

Remember please note anything that went weird with complete sample information (sample number and sample name) and step in the lab notebook following this page.