Rhizosphere DNA extraction protocol

Sean R. Schaefer, adapted by Barrilot et al., 2013 (DOI 10.1007/s13213-012-0491-y)

Updated 2/20/2024

1. Aliquot 10-12 ml of 0.9% NaCl solution into 15 ml falcon tubes
2. Tare the tubes containing 0.9% NaCl solution and add 0.5-2 grams of root/ soil into them using sterile tweezers
3. Place the tubes horizontally on a 15 ml vortex adapter and let them shake for 15 minutes
4. After shaking, remove the roots and place them into a new tube.
    a. Roots can be ground and extracted with Qiagen Plant DNA miniprep kit to obtain plant, mycorrhizal, or endophytic DNA
5. Place the 15 ml slurry tubes into a large centrifuge and spin at 10,000 RPM for 15 minutes
6. Remove the “supernatant” from the tube aiming to leave 4-5 ml. Note that the top layers should contain fewer soil particles but should still be darker colored than the NaCl solution
7. Transfer 2 ml from the “pellet” into a 2 ml centrifuge tube and spin at 13,000 g for 2 minutes
8. Remove most of the supernatant until there is about 0.5 ml left
9. Briefly vortex the remaining 0.5 ml slurry to resuspend
10. Repeat steps 7-9 to increase the amount of starting material
11. The remaining 0.5 ml slurry will be used as the starting material in the Qiagen Power Soil Pro kit, follow the kit instructions for the rest of the downstream extraction