

Protocol for DNA extractions from dried plant tissue using the Promega Wizard DNA Extraction Kit

Protocol:

1. Turn on the 65 °C and 37 °C water baths.
2. Place 20 to 40 mg of dried plant tissue in a 1.7 ml Eppendorf tube.
3. Use pestle to compact leaf tissue at bottom of tube.
4. Add **600 µl of Nuclei Lysis** solution.
5. Grind on ice until the sample is well ground--no large pieces of tissue remaining.
6. Place sample into the 65 °C Water bath.
7. Grind the next sample. Continue until all samples are ground. Samples can remain in the water bath for 15 to 60 min. I generally wait 15 min after the last sample is ground to proceed.
8. **Optional:** Add 3 µl of 1 mg/ml RNase to the sample and stir with the pipette tip. Place the samples in the 37 °C water bath for 15 min.
9. Cool the samples to room temp.
10. Add **200 µl of Protein Precipitation** solution.
11. Mix the sample well by inverting and flicking the tube.
12. Centrifuge at max speed for 3 min.
 - 12.1. To aid in compacting the pellet, once the centrifuge stops, centrifuge for 1 additional min.
13. Prepare a new set of tubes for each sample and add **600 µl of isopropanol** to these **new tubes.**
14. Pour the supernatant into the new tubes containing 600 µl of isopropanol and mix well.
15. Centrifuge at max speed for 1 min.
16. Pour off and discard the supernatant.
17. Add **600 µl of 70% ethanol** to wash the pellet, mixing well by inversion.
18. Centrifuge at max speed for 1 min.
19. Pour off and discard the supernatant.
20. Dry the pellet.
21. Add 100 µl of TE buffer to resuspend the DNA.
22. Allow the DNA to resuspend overnight at 4 °C, or 1 hr at 65 °C.

General notes:

This protocol is adapted from the draft protocol obtained from Promega. I use dried leaf tissue for the extractions. I grind the tissue using Kontes Pellet Pestles (Fisher Sci. Cat#: K749520-0000). These can be reused by washing after use and soaking in a 10% bleach solution at least overnight. Prior to use rinse 6-8 times in distilled water to wash off bleach. To aid in grinding, I use a hand drill attached to the shaft of the pestles. Samples should be kept on ice while grinding, especially when using the drill.