PureLink Protocol

1. Weigh out 100 mg of plant material.
2. Drop into mortar containing liquid N2, and allow liquid N2 to evaporate.
3. Grind to a powder using a pestle that has been pre-chilled with liquid N2.
4. Repeat 3 times.
5. Scrape powder into a microcentrifuge tube containing 250 µl R2 buffer using a spatula prechilled with liquid N2. Don’t be greedy! Add a bit of powder, cap the tube and mix well, then add a bit more. **It is essential that the powder thaws in buffer!**
6. Add 15 µl 20% SDS and 15 µl RNase A (20 mg/ml) and mix.
7. Leave15 minutes at 55˚ C.
8. Spin 5’ at 14000 rpm.
9. Transfer supernatant to fresh tube and add 100 µl N2 buffer.
10. Mix well and leave 5 minutes on ice.
11. Spin 5’ at 14000 rpm.
12. Transfer 250 µl of supernatant to a fresh tube, then add 375 µl of B4 buffer and mix well.
13. Transfer solution to a PureLink spin column and spin 30” at 14,000 rpm.
14. Transfer spin column to fresh collection tube, then add 500 µl W4 buffer and spin 30” at 14,000 rpm.
15. Discard flowthrough, add 500µl W5 buffer and spin 30” at 14,000 rpm.
16. Discard flowthrough, add 500µl W5 buffer and spin 30” at 14,000 rpm.
17. Transfer spin column to a fresh microcentrifuge tube and spin 2’ at 14,000 rpm to dry the membrane.
18. Transfer spin column to a fresh microcentrifuge tube and pipet 100 µl molecular grade water onto the membrane.
19. Leave 1’ at room temperature, then spin 1’ at 14,000 rpm.
20. Pipet 100 µl molecular grade water onto the membrane.
21. Leave 1’ at room temperature, then spin 1’ at 14,000 rpm.
22. Measure the concentration of DNA in the solution using the Nanodrop.