DNeasy 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 400 µl AP1 buffer and 4 µl RNAse A solution 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. Leave10 minutes at 65˚ C.
7. Add 130 µl P3 buffer and leave 5 minutes on ice.
8. Transfer into a Qia shredder column (purple) and spin 2’ at 14000 rpm.
9. Transfer the supernatant to a fresh tube, then add 1.5 volumes of AW1 buffer and mix by pipeting.
10. Transfer 650 µl to a DNeasy spin column (white) and spin 1’ at 10000 rpm. Discard flowthrough, then load another 650 µl until entire lysate is loaded on column.
11. Transfer spin column to fresh 2 ml collection tube, then add 500 µl AW2 buffer and spin 1’ at 10,000 rpm.
12. Discard flowthrough, add 500µl AW2 buffer and spin 1’ at 10,000 rpm.
13. Transfer spin column to a fresh microcentrifuge tube and spin 2’ at 10,000 rpm to dry the membrane.
14. Transfer spin column to a fresh microcentrifuge tube and pipet 100 µl molecular grade water onto the membrane.
15. Leave 5’ at room temperature, then spin 1’ at 10,000 rpm.
16. Pipet 100 µl molecular grade water onto the membrane.
17. Leave 5’ at room temperature, then spin 1’ at 10,000 rpm.
18. Measure the concentration of DNA in the solution using the Nanodrop.