Modified Qiagen DNeasy Plant Mini Kit Protocol

We used a modified Qiagen prep to extract high quality DNA from silica dried Rhinanthus leaf tissue. All steps which were modified are indicated with a asterix(\*).

1. Flash freeze ~20mg of silica dried leaf tissue in a 2mL microcentrifugre tube. Disrupt for 45 sec. Although Qiagen states that freezing is unnecessary for lyophilized tissue, we chose to freeze tissue to ensure DNAses remained inhibited.

**Step 7 in Qiagen Handbook**

1. \*Add 500uL of AP1 mix by shaking tube and vortexing immediately after adding buffer. Add 4uL RNase. Vortex lightly
2. Incubate in waterbath at 65C for 10min. Mix by gently inversion 2-3 times during this period
3. \*Remove from bath. Add 162.5uL of buffer P3. Incubate on ice for 5 min.
4. \*Centrifuge for 7-10min on max. This is important to precipitate floating plant debris and other contaminants that can interfere with downstream processes.
5. Transfer lysate into QIAshredder mini spin column. Approximately 500-550 uL will be transferred. It is crucial to avoid touching the plant debris pellet and avoid transferring any plant debris or foam from top of liquid phase. Centrifuge on max for 2 min.
6. Transfer flow-through into a new tube. Avoid touching the cell-debris pellet. It will be faint. It’s important to spin all your tubes with the hinges turned out. This will ensure that you know where in the tube pellets are formed, and thus you can avoid pelletsthat are difficult to see.
7. Add 1.5 X volume of AW1. Typically 750uL of AW1 will be needed Mix immediately by pipetting. Pipette slowly 5-7 times. This step mixes binding agent with DNA.
8. Transfer 650 uL to spin column. Spin on 12000 rpm for 1 min. Discard flow through. Tap edge of tube onto clean kim wipe.
9. Repeat with remaining sample.
10. Place tube into new 2 mL tube add 500uL AW2. Centrifuge for 1 min at 10000rpm. Discard flow-though. AW2 is a wash buffer and removes impurities. Check the colour of the column at these steps. If the column is coloured do extra wash steps.
11. Add 500 uL of AW2. Spin on max for 2 min. Discard flow-through and tap/wipe edge of tube on kim wipe.
12. Spin for 1 min on max to dry column. Ethanol can interfere with downstream steps, so you must ensure it is removed.
13. \*Transfer spin column to new tube and pipette 30uL of water –RNAse, DNase free. Incubate for 7-9 minutues.