**Qiagen Plant Tissue DNA Extraction Kit**

Before Starting:

Use filter tips.

Keep samples on ice.

Clean bench space with 70% ethanol.

Prepare 3 sterilized microcentrifuge (1.5mL) tubes, 1 collection tube found in kit, and 1 mini microcentrifuge (.5mL) tube for each sample. Use autoclaved tubes and sit them under the UV lamp for 15 minutes before starting the extraction.

\*\*\*Confirm the Qiagen solutions have had ethanol added (if not, use molecular grade ethanol when diluting).

Most of the protocol will stay the same as the Plant Tissue protocol in the Qiagen handbook (page 24). Revisions are noted with an asterisk (\*)

Protocol:

1. Turn on the heat block to 65°C.
2. Add 400µl of Buffer AP1to each sample that is filled with approximately 50 uL of acid-cleaned glass beads.
   1. Note: If AP1 has visible precipitate, shake the bottle. If the precipitate persists place the bottle in a beaker with water and set it on the hot plate to bring it to 65°C. Swirl until precipitant goes into solution.
3. Vortex to displace cells from filter.
4. \*Remove the filters with tweezers and clean with ethanol in between samples.
5. Place the tube into the bead beater and turn on at 48 rpm for 1 minute (6).
6. Centrifuge for 5 mins at 14,000 rpm to collect the shell material and beads at the bottom of the tube.
7. Add the supernatant to a fresh tube.
8. Add 4µl of RNase A (located in the 4°C fridge in lab). Mix the vile before use by flicking. Place back in the fridge after use.
9. Invert the samples, quick spin and place them on the heat block for 10 minutes.
10. Every 3 minutes, invert the samples and give them a quick spin.
11. Add 130 µL Buffer P3 to the lysate, vortex and quick spin, and incubate for 5 minutes on ice.
12. Centrifuge for 5 minutes at 14,000 rpm.
13. Transfer the supernatant to the purple QIAshredder Mini spin column placed in a collection tube. Avoid disturbing the pellet.
14. Centrifuge for 2 minutes at 14,000 rpm.
15. Transfer all of the flow-through fraction into a new clean tube (~600 µL)
16. Add 1.5 volumes of Buffer AW1 and mix by pipetting. (For example, for a 600 µL sample, add 900 µL of Buffer AW1.)
17. Label the white capped DNeasy Mini spin columns.
18. Pipet 650 µL of the mixture (the maximum that it holds) into a spin column with collection tube. Centrifuge for 1 minute at 8000 rpm.
19. Discard the flow through into a beaker.
20. Repeat with the remainder of the sample.
21. Place the DNeasy Mini spin column in a new ***collection tube***. Add 500 µL Buffer AW2 (wash buffer) and centrifuge for 1 minute at 8000 rpm. Take note of tube orientation in the centrifuge and orient them all the same way. Discard the flow through.
22. Rotate the tube 180 degrees in the centrifuge from its previous orientation in step 20 to ensure all ethanol is extracted. Be careful not to invert. Add another 500 µL of Buffer AW2 to the column and centrifuge for 2 minutes at 14,000 rpm. Discard the flow-through.
23. Transfer the DNeasy Mini spin column to a microcentrifuge tube and pipet 40 µL sterile water (MiliQ-H20) directly onto the membrane. Let the column sit for 5 minutes, then centrifuge for 1 minute at 8000 rpm. Then add an additional 20 µl of elution buffer (MiliQ- H20) and centrifuge once more.
24. Store DNA in the -20°C freezer.
25. Place the beaker with waste under the fume hood to evaporate.