**DNA Extraction Protocol Qiagen Kit**

**Materials per sample:**

* 3 – 1.5ml eppendorf tubes per sample
* Polyvinylpyrrolidone (PVP)
* Ice-cold 100% Ethanol
* Ice-cold 70% Ethanol
* DNase-free H2O
* T “low” E Buffer
  + 10 mM Tris pH 8.0, 0.1 mM EDTA

**Procedure:**

1. Use ~100 mg of plant tissue. Place sample in sterile mortar, bathe in liquid nitrogen (N2)
   * \*\*Store in -80ºC freezer for 4 hours, or -20ºC freezer overnight in a 1.5mL microcentrifuge tube if liquid N2 is not an option\*\*
2. Add 15mg of PVP, grind tissue into powder (will be a slurry if tissue is not flash frozen)
   * \*\*If grinding in 1.5mL tube skip this step\*\*
3. Using ice-cold pestle, grind frozen tissue into powder
   * \*\* If grinding in 1.5mL tube, complete step 4 first (500 µL of CTAB buffer), then grind with an ice-cold **micropestle**\*\*
4. Pipet 500µL of slurry into a sterile 1.5ml microcentrifuge tube, vortex, use microfuge to get all liquid to bottom of tube
5. Add 400 µL of Buffer AP1 to tissue homogenate
6. Add 4µL RNase A to to tissue homogenate
7. Vortze and incubate tubes at 65°C for 10 minutes (water bath or heat block).
8. Add 130 µL Buffer P3, vortex, incubate on ice for 5 minutes.
9. Centrifuge at 5 min @ 20,000 x g (or top speed, whichever is lower)
10. Pipet the cell lysate, avoiding any large clumps of tissue into a QIAshredder spin column (purple ones)
11. Centrifuge for 2 minutes @ 20,000 x g

**If performing Ethanol Precipitation:**

1. Pipet flow-through to new 1.5 mL microcentrifuge tube
2. Add 2x volumes of ice-cold 100% Ethanol, proceed to step 22

**If following remainder of Qiagen protocol:**

1. Add 1.5 volumes of Buffer AW1 to flow through, mix by pipeting
2. Transfer 650 µL of the mixture into Dneasy Mini spin column (white column), centrifuge for 1 minute @ 6,000 x g
3. Discard flow through, repeat with remainder of mixture
4. Add 500 µL Buffer AW2 to spin column, centrifuge for 1 minute @ 6,000 x g
5. Discard flow-through, add another 500 µL Buffer AW2, centrifuge for 2 minutes @ 20,000 x g
6. Transfer spin column to new 1.5 mL microcentrifuge tube (1.5 mL tube will have cap open)
7. Add 25 µL T “low” E buffer (or DNase free H2O), incubate at room temperature for 5 minutes
8. Centrifuge, with caps facing the center of the centrifuge, for 1 minute @ 6,000 x g
9. Repeat steps 18 and 19.
10. Store DNA at 4ºC until quality control step

**Ethanol Precipitation of DNA**

1. Remove samples from previous day from the -20ºC freezer
2. Centrifuge samples at 14,000xg at 4ºC for 15 minutes. Pipet off the supernatant without disturbing the pellet.
3. Add 1 mL of ice cold 70% ethanol. Centrifuge at 14,000xg for 10 minutes at 4ºC. Pipet off the supernatant without disturbing the pellet.
4. Repeat step 14, leave a small bit of liquid (~10-20µl) at bottom of tube.
5. Quick centrifuge for 30 seconds. Carefully pipet off the remaining supernatant (usually < 20 µl, use a P200). Let samples sit open at room temperature for 10-20 minutes, examine the tubes carefully to ensure that no liquid ethanol remains. DO NOT let the pellet get over dried.
6. Suspend samples in 30-60ul of “T low E” buffer. Gently move the pellet off of the tube wall into the liquid with the pipet tip to aid suspension. Put samples in 4ºC refrigerator to keep until DNA quality control step.