3X CTAB Protocol for DNA Isolation
Modified from Doyle and Doyle 1987
Last Updated: 5/19/2023 by Jessie Pelosi

Before getting started:
1. Prepare CTAB/BME solution by adding 150µL of beta-mercaptoethanol (BME) to 50 mL of 3X CTAB buffer- this only needs to be done the first time a 50 mL 3X CTAB tube is used. Make sure to write the date on the tube on which BME is added. NOTE: CTAB must stay under the hood once BME is added when being used!

2. Turn on 65°C water bath and put 3X CTAB+BME in water bath for 30 minutes to warm up.

3. Label two sets of tubes. The last set needs to be labeled with the complete sample name and extraction date. Write date on side of tube in there is not enough space on the top.

Homogenization
1. To each tube, add approximately 10mg of tissue of the sample. This may be species or tissue specific, but 10mg is usually a good starting point.

2a. If using silica-dried material, add 2-3 zirconium or metal beads to each tube and pulverize tissue using BeadBlast for 1-2 minutes. The resulting material should be a fine powder. If there are still bits of tissue that are still intact continue to pulverize until all tissue is fully homogenized.

2b. If starting with material flash-frozen in liquid nitrogen, add 2-3 metal beads to each tube and place back in liquid nitrogen. Once they are fully frozen, BeadBlast the tubes for 10 seconds. Then re-freeze for about 20 seconds and repeat until all tissue is a fine powder.

Lysis and Extraction
1. Add 500µL of CTAB/BME solution to each tube and vortex fully to get all plant homogenate into solution.

2. Incubate tubes at 65°C for 1 hour (or slightly longer). Invert frequently during incubation.

3. Add 500µL chloroform:isoamyl alcohol (24:1 C:IA) to each tube. Shake/invert 30-50 times, make sure solution is mixed well. Centrifuge at 8,500g for 5 minutes. Note that a 20-minute incubation period at room temperature may be inserted prior to centrifuging if desired.

4. Remove aqueous phase (top), and transfer to new labeled tube. The DNA is contained in the aqueous phase. Discard bottom organic layer in hazardous waste container in hood.

5. Repeat steps 3 and 4 if the aqueous phase is not clear.

6. Add 500µL cold/chilled isopropanol to the new tube (aqueous phase). Mix gently by hand (invert 20-30 times).

7. Precipitate in -20°C freezer overnight. Place in –80°C freezer if precipitating for 30 minutes to 1 hour (not recommended).

Washing
1. Centrifuge for 30 minutes at 8,500g. Orient tubes so hinge is up. DNA pellet is not always

visible; with this orientation you will know that the pellet is on the hinge side of the tube.

2. Discard supernatant (by pouring out), being very careful not to lose the pellet. Add 500µL 80% EtOH, invert to mix and spin at 13000g for 15 minutes.

3. Discard the supernatant being careful not to lose the pellet. Add 500µL of 95% EtOH, and spin at 13,000g for 15 minutes.

4. Discard the supernatant being careful not to lose the pellet. Spin for 1 minute at full speed and remove the rest of the EtOH with a pipette.

5. Allow the pellets to dry, making sure that no residual EtOH remains. This may take a few minutes to a few hours but be sure not to let the pellets over-dry.

6. Resuspend pellet in 50-100 µL elution buffer (10mM Tris-Cl, pH 8.5), depending on the size of the pellet and initial quantity of material. Incubate at 37°C for 1-2 hours if necessary or let sit at 4°C overnight. Do not re-suspend in TE buffer as this may inhibit downstream enzymatic reactions during library preparation and sequencing.

Quality Assessment

1. Assess the quality of the DNA using a Nanodrop. The 260/280 ratio should be between 1.8-2.0 and the 260/230 ratio should be between 2.0-2.2. Requires 1µL.

2. The concentration of the DNA should be determined by fluorometry using an assay such as a Qubit. Requires 2µL.

3. A 1% agarose gel may also be used to assess the quality of the DNA and check for degradation in the sample. Requires 2-3µL.

High Salt Clean-Up (Optional)

1. If there is evidence of contamination in the DNA (e.g., lower 260/230 ratio than expected), a high salt clean-up may be performed. Add an equal volume of 5M NaCl and mix gently.

2. Centrifuge at 8,500g for 20 minutes and carefully transfer the supernatant to a new tube.

3. Add an equal volume of isopropanol to the transferred liquid and place at -20°C overnight to precipitate DNA.

4. Perform steps 1-6 of the “Washing” section and re-assess the quality of the DNA.