Modified low-salt CTAB (MoLSC) Benchtop Protocol

- All steps in this protocol must be carried out at room temperature (RT) unless stated otherwise.
- Do not vortex any of the samples in order to avoid shearing the DNA. When necessary, mix tubes by inverting them until homogenous.
- Prepare the following stock reagents:
    - CTAB lysis buffer (composition : TrisHCl = 100mM, EDTA = 20mM, CTAB 2% w/v, NaCl 1.2M) : combine 5 mL of 1M Tris-HCl (pH = 8.0), 2 mL of 0.5M EDTA (pH = 8.0), 1g of CTAB powder and 3.5g of NaCl. Complete to 50 mL with lab grade water.
    - Diluted CTAB buffer (composition : $\mathrm{TrisHCl} = 100\ \mathrm{mM}$, $\mathrm{EDTA} = 20\ \mathrm{mM}$, $\mathrm{CTAB}\ 2\%\ \mathrm{w/v}$) : combine 5 mL of 1M Tris-HCl (pH = 8.0), 2mL of 0.5M EDTA (pH = 8.0) and 1g of CTAB powder. Complete to 50 mL with lab grade water.
    - High-salt TE buffer (composition : EDTA = 2mM, Tris = 10mM, NaCl = 1M) : combine 2.907g of sodium chloride, 200ul of 0.5M EDTA (pH = 8.0), 500ul of 1M Tris-HCl (pH = 8.0). Complete to 50mL with lab grade water. Autoclave, let cool, store at room temperature.
- Finely mince 25 mg of tissue sample using a scalpel blade. For more difficult samples, it may be necessary to freeze the tissue in liquid nitrogen first and use a mortar/pestle to grind up the tissue into a powder instead.
- Aliquot 500 µL of CTAB lysis buffer into 2 mL microcentrifuge tubes and add tissue samples to each tube. Then add 30 µL of Proteinase K (stock conc. = 20 mg/mL) to each sample. Incubate samples at 56-60^{o}C for 3 hours or overnight in a shaking incubator at 150 rpm.
- Cool down tubes to room temperature and add 500 µL of a solution of Chloroform: Isoamyl alcohol (24:1) to each tube. Mix by inverting the tubes and centrifuge at RT for 15 minutes at 3,000g.
- Transfer the aqueous phase to a new 1.5 mL tube. Add 2 volumes of diluted CTAB buffer to the aqueous phase (roughly 800 µL of diluted CTAB for 400 µL of aqueous phase). Mix well by inverting the tubes and place again in a shaking incubator at 56-60^{o}C for ≥30 minutes at 150 rpm until white crystals can be observed floating inside the tubes. This step is crucial for DNA precipitation, and visible CTAB-DNA complexes should form at this point but they may be difficult to observe depending on the sample. If there appears to be nothing at all, incubate for a longer period of time, or try increasing the speed of the shaking platform, as shaking the samples appears to considerably help with the formation of these complexes.
- Centrifuge at 16,000g for 3 minutes at RT to precipitate the CTAB-DNA complex. Discard the supernatant.
- Add 1 mL of 75-80% ethanol and let soak for 15 minutes at RT to remove excess CTAB. Invert the tubes multiple times to try and dislodge the pellet so it can soak in the ethanol. Spin again at 16,000g for 3 minutes to precipitate the DNA and discard the supernatant.
- (Optional) Re-suspend the DNA pellet in 100 µL of High-salt TE buffer and add RNase A to a final concentration of 50 µg/mL. Incubate the samples at 56-60^{o}C for 15 minutes to degrade RNA. Add 900 µL of 75-80% ethanol and mix the tube well by inverting. Spin down the tube at 16,000g for 3 minutes and discard the supernatant. Note: you may alternatively precipitate the DNA onto paramagnetic bead preparations (such as AMPure® XP, MagBio HighPrep™ PCR, or custom preparations) if preferred for high-throughput applications.
- Perform a final wash with 1 mL of 75-80% ethanol. Spin down at 16,000g for 3 minutes and discard the supernatant.

- Place tubes under a fume hood with the lids open to remove all traces of ethanol from the tubes.
- Re-suspend DNA pellets using 100 µL of Tris-HCl (pH = 8.5), low-TE, or sterile lab-grade water. Elution may be improved by the addition of 0.5% v/v of tween-20. Pipet up and down to help re-suspend the DNA and allow ≥ 30minutes for the DNA to dissolve completely before proceeding further.
- Store samples at 4^{o}C for short term storage (≤2 weeks) or at -20^{o}C for long term.