**DNA Isolation - CTAB protocol**

Reagents: CTAB, β-mercaptoethanol (remains in fridge), 75% EtOH, Chloroform:IAA (24:1), Isopropanol

Glassware: 4 50mL beakers (CTAB, EtOH, Isopropanol and Chloroform), 25mL pipette + pipette controller

Pipettes: 1000uL tips, 200uL tips, P1000, P200

PPE: safety glasses, long pants, long shirt preferably (we have lab coats), close toed shoes, gloves

**\***Before starting: grind freeze dried tissue in 2mL tubes with glass beads using lyser (30freq/s, 2:00min., #24 facing outward, make sure each holder is balanced before starting)

1. Turn on incubator- 65℃, check thermometer
2. Organize 48 samples into two small tube racks (24 each) with respective 1.5mL tubes in two small tube racks organized in the same order

**In fume hood.**

1. For 48 samples: add 34.3mL CTAB + 343uL β-mercaptoethanol (in fridge) into 50mL “CTAB” beaker
2. Add 700uL CTAB mix to each 2mL tube and **Vortex**
3. Incubate at 65℃ for 60-90 minutes; invert every 15 minutes

**In fume hood.**

1. Add 400uL Chloroform:IAA (24:1) to each tube (need ~20 mL in beaker) and **Vortex**
2. Spin for 5 min. -12500rpm. With cap hinge facing outward. (Load 1-12, 16-24, if using larger 30 centrifuge).
3. Transfer top phase (clear) to the first 1.6mL tube (set P1000 to ~800uL)
4. Add 400uL Chloroform:IAA (24:1) again to each tube (need ~20 mL in beaker) and **Vortex**
5. Spin for 5 min. -12500rpm again.
6. Transfer top phase (clear) to the second 1.6mL tube (set P1000 to ~800uL)
7. Add 300uL Isopropanol, **mix by inversion - do not vortex**
8. Spin 5 min., pour off top phase (in sink with paper towels to dab, pour two at a time)
9. Add 450 uL 75% EtOH (need ~ 25mL in beaker) and **Vortex**
10. Spin 5 min. and pour off top phase
11. Spin again for 1 min. and pipette out additional EtOH (use P200 set to ~140uL)
12. Let air dry with caps open
13. Resuspend with 250uL NF H20