**DNA Extraction Using CTAB with RNAse A**

**Notes**

* CTAB is stored as a 2X solution (2% hexadecyltrimethylammonium bromide, 100 mM Tris-Cl [pH 8.0], 20 mM EDTA, 0.2% 2-mercaptoethanol, 1.4M NaCl) in the freezer. It must be thawed to room temperature before use (which takes up to an hour).
* Heat the thermomixer to **56°C**.
* Make sure cold 95-100% and 70% ethanol are available in the -20°C freezer.
* Start cooling the refrigerated centrifuge about 30 minutes before it will be needed.

**Protocol**

1. Add **300 µl** of **2X CTAB** and **300 µl** of **pure water** to a 2 mL tube for each extraction.
2. Chop 1-2 mm3 of tissue up with a sterile razor blade on a sterile surface (e.g., aluminum foil or a petri dish) and add to a 1.5 mL microfuge tube.
3. If needed, briefly homogenize. Clean the probe between samples.
4. Add and **2.5-10 µl** of **proteinase K** (20mg / mL). If you plan to do an RNAse A treatment, use as little proteinase K as necessary.
5. Incubate half an hour to overnight at **56°C** while mixing at 500 RPM on the thermomixer. Occasionally invert the tube ~10 times to mix (do this minimally one time about 30 minutes after starting the digestion if you plan to incubate overnight).
6. Cool heat block to **37°C**.
7. Briefly spin down (just get centrifuge up to 3,000 RPM) to collect all liquid at the bottom of the tube.
8. Optional: Add **10 µl** of **RNAse A** (10 mg / ml), mix briefly by inverting the tube ~10 times, incubate at **37°C** for **10 minutes**, and briefly spin down to collect all liquid at the bottom of the tube.
9. Add **600 µl** of **phenol/chloroform/isoamyl alcohol** (25:24:1; stored in the fridge), mix briefly by inverting the tube ~10 times, and allow to stand 3 minutes. NOTE: In the jar of phenol/chloroform/isoamyl alcohol, there is an upper aqueous layer and a lower organic layer. You should transfer 600µl from the **lower** layer.
10. Spin at full speed (**14,000 rpm**)at **4°C** for **5 minutes.**
11. Transfer the aqueous (**upper**) phase to a new tube.
12. Add **600 µl** of **chloroform/isoamyl alcohol** (24:1; also stored in the fridge). Mix briefly by inverting the tube ~10 times and spin at full speed (**14,000 rpm**) at **4°C** for **3 minutes**.
13. Carefully transfer the aqueous (upper) phase to a new tube being careful not to transfer any of the lower organic phase.
14. Add **60 µl** of **3 M sodium acetate** (NaOAc) and **1000 µl** of **cold 100% ethanol**.
15. Mix briefly by inverting the tube ~10 times and **place in the -20°C freezer for 10 minutes**.
16. Centrifuge at full speed (**14,000 rpm**) at **4°C** for **10 minutes**.
17. Decant or pipet off the supernatant and add **600 µl** of cold **70% ethanol**.
18. Centrifuge at full speed (**14,000 rpm**)at **4°C** for **5 minutes** and remove the supernatant. Remove as much of the liquid by pipetting as possible.
19. Dry the pellet until no ethanol remains (close lid and flick to check).
20. Resuspend in **100 µl** (or an appropriate amount) of **Elution Buffer** (Omega Bio-Tek)orequivalent.
21. Check purity, concentration, and quality by Qubit, NanoDrop, and running ~20-300 ng on a gel.