

SHORT CTAB DNA EXTRACTION PROTOCOL

[This protocol was successful for extracting DNA for the *Suillus brevipes* and *Wilcoxina mikolei* genomes]

Grind tissue in liquid N with fine beads (glass beads 50-100 microns). Harvest the powder into 2ml screw cap tubes. Keep everything cold - I grind over dry ice.

1. Add **500µl 2% CTAB** extraction buffer + **2% PVP-40** + **2.5µl β-mercaptoethanol** to freshly ground tissue. Mix well.
2x CTAB buffer and 2% PVP-40 can be mixed in advanced and stored at room temperature. Upon using, mix with appropriate amount of β-mercaptoethanol
2. Incubate at 65°C for 30 minutes, shake sample every 10 minutes.
For difficult or poorly preserved samples, incubate for at least 1 hour.
3. Add **500µl isoamyl-chloroform**, shake well and shake on the Nutator for 20 minutes.
Phenol-isoamyl-chloroform can also be used at this step.
4. Spin down at max speed for 5 minutes.
5. Transfer the top phase to a fresh tube and repeat steps 3 & 4 if samples were unclear.
6. Add **15µl 5M potassium acetate** and **500µl ice-cold isopropanol**. Gently mix the sample.
Any acetate salt can be used for this step. Cold incubation from -20°C to -80°C for 10 minutes is optional. I usually skip the cold incubation.
7. Spin down 3 minutes at max speed. Discard isopropanol, being careful to not loose the pellet.
Adding acetate salt usually causes the pellet be goopy. If this happens you need to pipe out the iso-propanol wash to not loose the pellet.
8. Wash the pellet with **500µl ice-cold 70% ethanol**, making sure the pellet is loose and has been washed throughout. Incubate at room temperature for at least 5 minutes. If pellet is thick, dislodge the pellet from the side of the tube and flatten it out to allow ethanol to permeate.
9. Spin down for 1 minute and discard solution.
10. Wash the pellet with **500µl 100% ice-cold ethanol**.
11. Spin down for 1 minute and discard solution.
12. Completely dry the pellet at 65°C, either with a dry heater or vacuum centrifuge.
13. Reconstitute with **30-50µl TE buffer or ddH₂O**.