

# DNA minipreps from fungi

*Vilgalys Lab*

## Reagents:

2X CTAB extraction buffer: 2% (w/v) CTAB (Sigma H5882 or M7635), 100 mM Tris, pH 8.0, 20 mM Na<sub>2</sub>EDTA, 1.4 M NaCl. (optional: b-mercaptoethanol may be added to 1% final volume right before use).

recipe for 50 ml 2x CTAB: 1 g CTAB, 4.09 g NaCl, 2.5 ml Tris stock (1M, pH 8), 1 ml Na-EDTA stock (.5 M, pH 8).

Chloroform:isoamyl alcohol (24:1)

Isopropyl alcohol: store at -20 C.

TE buffer: 10mM Tris, 1 mM EDTA, pH 8.0.

RNAse A: 1 µg/µl stock solution in H<sub>2</sub>O (pre-treated to deactivate DNAses by heating to 100 C for 10 min. Store frozen at -20 C).

Plasticware, grinders, spatulas as necessary. Should be autoclaved before use, and acid-washed if reused.

## Sample preparation:

**lyophilized tissue** (< 100 mg dry wt) is ground to a powder in liquid nitrogen using a pre-chilled mortar and pestle, and small amounts dispensed into 1.5 ml eppendorf microfuge tubes (up to 100 ul level).

**fresh tissues** (up to 100-300 mg, less is usually better) can be placed directly into 500 µl extraction buffer (use 2X concentrated buffer is best). Tissues may be stored this way until use (we've stored field-collected tissues this way up to a year. refrigeration not always necessary). Before proceeding with extraction, grind tissues using a spatula or special pestle (little blue grinders can be purchased from Kontes). Alternatively, add a dozen or so glass or zirconium beads and place in a Savant FastPrep machine at max speed for 1.5 minutes (use screw capped tubes).

**mycorrhiza samples:** small amounts of fungal tissue from environmental samples (10-20 mg max) may be placed directly into 250 µl 2xCTAB buffer (right in the field or soon after collection). Mycorrhizal root tips are collected with sterile forceps and rinsed in water if necessary; rhizomorphs, mycelial mats and cords may also be sampled this way.

The volumes given below may be scaled up or down according to the actual amounts of sample available. It is best not to overdilute your samples in the initial phases of the extraction.

1. Add 250  $\mu$ l 2X CTAB extraction buffer to each sample. Use a Kontes pestle and homogenize the sample completely.
2. Add 250  $\mu$ l additional 2X CTAB extraction buffer to each sample and mix by vortexing.
3. Incubate samples at 65 C for 60 min in a water bath or incubator. This helps extract and hydrate the nucleic acids. Mix at least once by vortexing during the incubation period.
4. Chloroform:isoamyl alcohol extraction step: Add an equal volume (500  $\mu$ l) of chloroform-isoamyl alcohol to the samples. Cap tightly and shake vigorously to form an emulsion. Continue extraction for several minutes by periodic shaking to maintain emulsion.
5. Spin tubes in a microfuge at 13,000 rpm for 12 min (long enough to produce a clear supernatant). Using a disposable pipette tip, carefully remove upper aqueous layer to a clean, labeled eppendorf tube (avoid taking up any of the interface material). Discard contents of the old tube as organic waste into a waste bucket (don't put it down the sink).
6. Optional RNase step: Add 50  $\mu$ l of RNase stock to the aqueous contents of each tube and incubate at 37 C for 30 min.
7. Re-extract samples a second time, using an equal volume of chloroform:isoamyl alcohol. This time, there will be less material emulsified. Collect the upper aqueous fraction as before by centrifugation. (If extra-clean DNA is desired, repeat the extraction step a third and even fourth time, until no interface material remains and the aqueous portion is nice and clear). In any case, be careful to avoid any of the proteinaceous interface material after your final extraction- *its always better to leave some of the interface behind if necessary to avoid protein contamination*.
8. Estimate volume of your DNA sample. Add 2/3 volume of ice cold isopropyl alcohol, cap and invert/mix. You may see a white, stringy precipitate (DNA as well as polysaccharides when present). Place samples in the freezer overnight. This should bring down lots of other stuff as well as any DNA which is present. Collect precipitate the next day by centrifugation at 13,000 rpm for 7 min.
9. Pour off the alcohol supernatant, spin the tubes quickly in a table top centrifuge and remove the rest of the liquid using a pipetteman. Add 1 ml of cold 70% EtOH to each tube and shake gently. Spin the tubes again at 13,000 for 2min. Pour off the alcohol supernatant, spin the tubes quickly in a table top centrifuge and remove the rest of the liquid using a pipette. Dry the sample completely in the laminar flow hood or Speedvac. For regular minipreps, resuspend DNA pellet in 50  $\mu$ l TE. Store frozen at -20 C.
10. Quality of DNA samples may be checked by loading 2-3  $\mu$ l on a 0.8% agarose gel. Good DNA extractions are characterized by the presence of a distinct high molecular weight DNA band with little or no evidence of lower molecular weight degradation products.