Procedures: DNA Extraction from fungi

Depending of the nature of the starting material (mycelium, fruiting bodies, spores), the basic extraction protocols presented here might require some modifications. Remember that very little DNA is needed for PCR amplification, and crude extracts can be analyzed if used immediately and not stored for any length of time.
DNA was extracted from approximately 50 mg of either fresh or frozen vegetative mycelium, fresh or frozen hymenium of fruiting bodies, or hymenium of fruiting bodies fixed in glycerol:ethanol:water (30:30:40) (about 100 mg in 1 ml fixative). For fruiting bodies, tissue was carefully sampled from the central part of the cap or stipe to avoid any contamination by other microorganisms or spore DNA.

DNA was routinely extracted by the CTAB protocol (Method 1) according to Gardes & Bruns (1993) with slight modifications (Henrion *et al.* 1994). For recalcitrant samples leading to no amplification or low amplification yield, DNA was extracted using a guanidinium buffer followed by DNA purification using GeneClean glassbeads (Method 2) (Grube et al. 1995)

CTAB Extraction (Method 1)

1. Collect 10-50 mg mycelium. Transfer the sample to an Eppendorf plastic tube (optional: deep the bottom of the tube in liquid N_2).
2. Crush the frozen mycelium with a plastic micropestle specially designed to grind small samples in 1.5 ml Eppendorf tubes.
3. Immediately, add 300-500 ul CTAB buffer containing 0.1 mg proteinase K (Bioprobe PROK02) to ground mycelium and vortex briefly.
4. Incubate for 1 hour at 65deg.C (tubes must be securely capped). A plastic or styrofoam floating rack is useful for this and subsequent manipulations. Centrifuge this extract for 5-10 min at 13 000g to remove cell debris. Remove the supernatant to a new 1.5 ml Eppendorf tube.
5. Remove the proteins from the suspension by sequential extractions with 500 ul of Tris-saturated phenol:chloroform:isoamyl alcohol (25:24:1, v:v:v). Centrifuge this emulsion for 5-10 min at 13 000g. Remove the upper aqueous layer (containing the DNA) to a new 1.5 ml Eppendorf tube. Extract the aqueous supernatant with 500 ul of chloroform:isoamyl alcohol (24:1), centrifuge as above, remove the upper aqueous layer to a new 1.5 ml Eppendorf tube as above.
6. Add 1 ml of isopropanol to the aqueous phase and mix gently. Incubate for 1 h at -70deg.C or overnight at -20 °C.
7. Centrifuge at 13000 g for 30 min to pellet the DNA. Pipette off the supernatant and retain the pellet. Wash the DNA pellet with 150 ul of 70% (v/v) ethanol, pellet again. Pipette off the supernatant, retain the pellet and dried at room temperature.
8. Finally, solubilize the DNA pellet in 30 to 50 ul of sterile ultrapure water or Tris-EDTA buffer. Proceed immediately with PCR. The DNA solution should be stored at -20 °C if not used immediately.

Guanidinium Extraction (Method 2)

1. Collect 10-50 mg mycelium. Transfer the sample to an Eppendorf tube (optional: deep the bottom of the tube in liquid N_2).

2. Crush the mycelium with a plastic micropestle specially designed to grind small samples in 1.5 ml Eppendorf tubes.
3. Immediately, add 300-500 ul guanidinium isothiocyanate buffer and vortex briefly to suspend the crushed material.
4. Incubate for 1 hour at 65deg.C, mixing well at least once during the incubation.
5. Centrifuge the suspension for 2 min at 13 000g. Retain the pellet and re-extract with 50-200 ul guanidinium isothiocyanate buffer, and centrifuge as above. Pool the supernatants.
6. Remove the proteins from pooled supernatants by sequential extractions with 500 ul Tris-saturated phenol:chloroform:isoamyl alcohol (25:24:1, v:v:v). Centrifuge this emulsion for 5-10 min at 13 000g. Remove the upper aqueous layer (containing the DNA) to a new 1.5 ml Eppendorf tube. Extract the aqueous supernatant with 500 ul of chloroform:isoamyl alcohol (24:1), remove the upper aqueous layer to a new 1.5 ml Eppendorf tube as above.
7. Add 1.0 ml saturated 6 M NaI (Sigma #9538) solution and 5 ul well-suspended glassmilk to the aqueous supernatant. Mix well. *Note:* DNA precipitation onto glassmilk reduces the loss of DNA from dilute solution by providing an excess of surfaces for DNA binding. This DNA binding does not co-precipitate interfering polysaccharides.
8. Incubate for 5 min on ice. This step could be extended overnight.
9. Centrifuge a few seconds to pellet the DNA-glassmilk. Pipette off the supernatant. Retain the glassmilk pellet, which has the bound DNA.
10. Resuspend the glassmilk pellet in 200-500 ul of ice cold (- 20 °C) 80% ethanol. Centrifuge 5 s to pellet the glassmilk. Pipette off the supernatant. Repeat the washing step twice. Dry the washed glassmilk pellet.
11. Re-suspend the washed glassmilk pellet, which has the bound DNA, in 30 ul ultrapure water. Centrifuge 5 s to pellet the glassmilk before removing the DNA solution. Proceed immediately with PCR. The DNA solution should be stored at -20 °C if not used immediately.

Buffers

CTAB buffer

- 100 mM TrisHCl, pH 9.0
- 20 mM NaEDTA, pH 8.0
- 1.4 M NaCl
- 2% cetyltrimethylammonium bromide (CTAB)
- 0.2% ß-mercaptoethanol, freshly added

Guanidinium isothiocyanate buffer (Sambrook et al. 1989)

- To a 100 g bottle of guanidinium isothiocyanate (Bioprobe, #GUAT03) add 100 ml of ultrapure water, 10.6 ml of 1 M TrisHCl (pH 7.6), and 10.6 ml of 0.2 M NaEDTA. Stir overnight at room temperature.
- Add 21.2 ml of 20% Sarkosyl (sodium lauryl sarkosinate) (BDH #44275) and 2.1 ml of ß-mercaptoethanol to the supernatant and bring the volume to 212 ml with ultrapure (MilliQ, Millipore) H2O.
- Filter through a disposable 0.2 µm Nalgene filter and store at 4°C in a tightly sealed, brown glass bottle.

TE buffer

- 10 mM TrisHCl, pH 8.0
- 1 mM EDTA

Materials

- Polypropylene micropestles (Kontes #749520), to fit in 1.5 ml microcentrifuge Eppendorf tubes

- Glassmilk (ground SiO_2) for DNA precipitation (GenecleanII, Bio 101, La Jolla, CA, #1001-400)

References

1. **Grube M, Depriest PT, Gargas A, Hafellner J (1995)** DNA isolation from lichen ascomata. *Mycol. Res.* **99**: 1321-1324
2. **Henrion B, Chevalier G, Martin F (1994)** Typing truffle species by PCR amplification of the ribosomal DNA spacers. *Mycol Res* **98**:37-43
3. **Sambrook J, Fritsch EF, Maniatis T (1989)** Molecular Cloning; A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York