Alkaline Extraction Process

Note: This protocol has been handed down from lab to lab. The Wilson Lab received this version, with protocols adjusted to fit our lab, from Dr. Brian Perry. October 2019.

This "Alkaline Extraction Process" protocol is derived from: Vandepol, N., Liber, J., Desirò, A., Na, H., Kennedy, M., Barry, K., Grigoriev, I.V., Miller, A.N., O'Donnell, K., Stajich, J.E. and Bonito, G., 2020. Resolving the Mortierellaceae phylogeny through synthesis of multi-gene phylogenetics and phylogenomics. *Fungal Diversity*, 104(1), pp.267-289.

Extraction Solution (ES)

Stocks: 1 M Tris solution (pH=8.0)

1) add 10 ml of 1 M Tris stock into clean 100 ml vessel
2) add 1.86 g KCl
3) add 0.37 g EDTA
4) add 80 ml DI or ultrapure H_2O and shake until solutes dissolve
5) Titrate with 1 M NaOH to pH = ~ 9.5-10.0
6) top up to 100 ml with DI H_2O
7) filter sterilize and aliquot into sterile 1.7 mL eppendorf tubes (filtering is recommended but optional)

Dilution Solution (BSA 3%)

1) add 3 g of BSA (e.g. Sigma or Omni – 98-99% purity, heat shock fractionated) into clean vessel
2) top up to 100 ml with DI/Ultrapure H_2O
3) shake BSA into solution
4) filter sterilize into sterilized 2 ml eppies (same as above here)

Procedure

1. Take a **small piece** of fungal material, not greater than one kernel of long-grain rice, and place that into an appropriately labeled 1.5 mL microcentrifuge tube.
2. Add a small pinch of **sterile sand** (Fisher Sci S25-500) to each tube.
3. Pipette out **100µL of ES** into each tube. Let tissue absorb ES for 5 minutes.
4. Place tubes into -20°C **freezer** until tissue is frozen (~15 min). This softens the tissue for grinding. Remove and thaw.
5. **Grind** with sterilized micropestle (Fisher Sci 12-141-368) until all visible chunks of tissue are broken down and homoginized.
6. **Incubate** at room temp for 10+ minutes then incubate for 10 minutes at 95˚ C.
7. Add an equal volume of **Dilution Solution** (DS) (3% BSA).
8. **Vortex** and **Spin** briefly (max for 30 sec).
9. Samples are now ready for PCR. 1-2 µL is usually sufficient for PCR (from a 20µL ES + 20µL DS = 40µL volume extraction). For herbarium specimens we always do a 1/10 to 1/1000 dilution series, and use 2 µL of each for PCR.
10. Store DNA extractions in freezer.

NOTE: It's currently not clear how long these DNA extractions will last in the freezer for. Current discussions have suggested that after 6 months, DNA that was extracted using this method breaks down is no longer viable. Further exploration and testing is needed.