



DNA EXTRACTION FROM THE FUNGAL ORGANISMS *COPRINOPSIS CINEREA* AND *TALAROMYCES EMERSONII*.

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/ CONTEXT

DNA extraction is an essential tool in the molecular analysis of fungi. The architecture of the fungal cell wall renders many fungi resistant to standard DNA extraction procedures employed for yeast and bacteria. Fungal DNA extraction protocols tend to be time consuming and/or result in a poor yield of DNA. While commercial kits are available for the extraction of fungal DNA, they do require a pre step of grinding (with or without liquid N₂) for the initial breaking up of mycelia. Grinding can be consuming, taking up to 30 minutes per sample depending on the fungal species. For high throughput screening and analysis, a rapid DNA extraction method is required for fungal samples. This method was tested on two different classes of fungal species; an ascomycete (*T. emersonii*) and a basidiomycete (*C. cinerea*).

/ MATERIALS

- Sample: *C. cinerea* and *T. emersonii* were grown in both solid and liquid state cultures following standard protocols (Binnering *et al.*, 1987; Gupta *et al.*, 2012). 0.5g of mycelia scrapped from solid culture and 0.4g of mycelia harvested from liquid culture were used for DNA extractions.
- Precellys® Lysing Kit, VK05 Cat n° KT03961-1-004.2
- Minilys® personal homogenizer
- Omega bio-tek E.N.Z.A SP Fungal DNA Mini kit D5542-01

/ PROTOCOL

Mycelia samples were placed into the Precellys® vial containing ceramic/glass beads, using a sterile pipette tip to help push mycelia into contact with the tubes contents. Samples were homogenize for 60 seconds in the Minilys® bead mill homogenizer at the low/medium setting until the contents were seen to be blitzed. 600 µL SFG1 Buffer and 4 µL RNase A (E.N.Z.A SP Fungal DNA Mini kit D5542-01) were added to the vial, and vortexed to ensure that samples were suspended and that no clumps remained. The extraction was carried out from step 3 of the E.N.Z.A SP Fungal DNA Mini kit, according to manufactures instructions.

/ CONCLUSION

Minilys® personal homogenizer along with the appropriate lysing kit is a suitable, simple and convenient homogenization system to break open the cell wall of fungal species. Minilys® personal homogenizer combined with a suitable fungal extraction kit provides a rapid method for DNA extraction when compared with previous published protocols.

/ RESULTS

DNA extractions were performed on both *T. emersonii* and *C. cinerea* mycelia harvested from liquid culture. The combination of the Minilys® personal homogenizer with the E.N.Z.A fungal DNA extraction kit resulted in a successful and rapid DNA extraction from both organisms. In order to increase efficiency for high throughput screening, the method was then tested on mycelia scraped from solid state cultures. DNA was successfully extracted from both organisms thus eliminating the need for liquid cultivation.

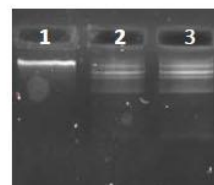


Figure 1: DNA extraction from plate mycelia performed using Minilys® bead mill, Precellys® lysing kit tubes and E.N.Z.A. fungal extraction kit on *Coprinopsis cinerea* FA2222. 1% agarose gel with gel red, lane 1: *C. cinerea* DNA, lane 2&3: 1kb molecular weight ladder.

References:

- Binnering, D. M., Skrzynia, C., Pukkila, P. J. and Casselton, L. A. (1987) DNA-mediated transformation of the basidiomycete *Coprinus cinereus*. *Embo j*, 6 (4), 835-40.
- Gupta, V.K., Tuohy, M.G., Ayyachamy, M., Turner, K.M. and O'Donovan, A.(2012) *Laboratory Protocols in Fungal Biology: Current Methods in Fungal Biology*. Springer Science & Business Media. p. 490-491.

/ CUSTOMER

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SPPP-810-126



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