VERSION 1

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 We use this protocol and it's working

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Quick Fungal DNA extraction from colonies on plates V.1

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ABSTRACT

Adapted from [Chi, M. H., Park, S. Y., & Lee, Y. H. \(2009\).](#)

Colony PCR on fungal colonies grown on plates does not work as well as it does for bacteria (it usually doesn't work at all). DNA therefore needs to be extracted first. As this DNA will only be used as a PCR template to check for presence / absence of individual genes I am not too concerned about high molecular weight or purity. This protocol is quick and can be used to process several samples at the same time.

Make stock solutions

1

1M TrisHCl pH 8.0	100 ml
12.14 g	Tris base or Trizma base
up to 100 ml	deionised H ₂ O
Adjust pH	concentrated HCl

2M KCl (Potassium chloride)	200 ml
15.11 g	KCl (Potassium chloride)
up to 200 ml	deionised H ₂ O

500 mM NaEDTA	100 ml
18.6 g	EDTA disodium salt dihydrate
up to 100 ml	deionised H ₂ O

Autoclave stock solutions at 121°C

Make Extraction Buffer

2

E x t r a c t i o n B u f f e r	100 ml
2 M K C l	50 ml

1 M Tris-HCl pH 8.0	10 ml
500 mM NaEDTA	2 ml
deionised H ₂ O	up to 100 ml

Preparation

- 3** Per sample prepare **2 x 1.5 ml Eppendorf tubes**. Label them with sample number and add:

Tube 1: **500 ul Extraction Buffer + 1 stainless steel bead**

Tube 2: **300 ul Isopropanol**

Harvest fungal material

- 4** With a **sterile toothpick or pipette tip** remove a fungal colony or a piece of mycelia of about 3-5 mm x 3-5 mm in size from a plate and put it into **Tube 1** (containing the Extraction Buffer and steel bead).

Homogenise fungal material

- 5** Homogenise samples for **5 min at 1500 rpm** in the MiniG 1600.

Equipment

	NAME
SPEX	BRAND
SP 1600	SKU
MiniG 1600 Automated Tissue Homogenizer and Cell Lyser	SPECIFICATIONS

Pellet Cell Debris

- 6 Pellet cell debris by centrifugation at **17.000 x g for 5 min.**

DNA Precipitation

- 7
- Transfer supernatant to **Tube 2** (Containing 300 ul Isopropanol)
 - Vortex for 5 - 10 seconds
 - Pellet DNA by centrifugation for **5 min at 17.000 x g**

Wash DNA

- 8
- Discard supernatant
 - Add **800 ul 70% Ethanol**
 - Vortex mix sample, make sure pellet comes loose from bottom of the tube
 - Pellet DNA by centrifugation for **5 min at 17.000 x g**

Dissolve DNA

- 9
- Discard supernatant
 - Remove as much of the ethanol as possible by drawing drops out with a pipette tip
 - Let leftover ethanol evaporate for 5-10 min
 - Dissolve pellet in **50 ul H₂O or 1x TE buffer**
 - Measure DNA concentration (e.g. Nanodrop)
 - store DNA at -20°C