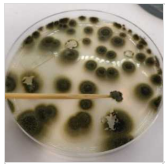

Version 2

Jul 21, 2021

Quick Fungal DNA extraction from colonies on plates V.2

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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 concerend about high molecular weight or purity.
This protocol is quick and can be used to process several samples at the same time.

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PROTOCOL CITATION

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fungal DNA extraction

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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_2O
Adjust pH	concentrated HCl

2M KCl (Potassium chloride)	200 ml
15.11 g	KCl (Potassium chloride)
up to 200 ml	deionised H_2O

A	B
500 mM NaEDTA (pH 8.0)	**100 ml**
18.6 g	EDTA disodium salt dihydrate
up to 100 ml	deionised H_2O

Adjust pH to 8.0, otherwise the EDTA won't go into solution

Autoclave stock solutions at 121°C

Make Extraction Buffer

2

Extraction Buffer	100 ml
2M KCl	50 ml
1M Tris-HCl pH 8.0	10 ml
500 mM NaEDTA	2 ml
deionised H_2O	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).

Try not to use a piece that is too big, as that might lead to carryover of too many PCR inhibitors.
I usually use my toothpick like a sewing machine needle, punching out a piece of mycelium all the way to the bottom of the agar plate. Then I use the toothpick to slice underneath the actual mycelial mat to try to cut off as much of the agar as possible.

Homogenise fungal material

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

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SPEX SP 1600
MiniG 1600 Automated Tissue Homogenizer and Cell Lyser

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 **50-100 ul H_2O or 1x TE buffer**
- Measure DNA concentration (e.g. Nanodrop)
- store DNA at -20°C

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