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We use this protocol and it's working

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Extraction of fungal DNA and PCR for identification using the Sigma-Aldrich REDExtract Plant PCR kit

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ABSTRACT

This protocol describes how we use the REDExtract Plant PCR kit (Sigma-Aldrich) to extract DNA from fungal mycelium and PCR from the ITS region.

GUIDELINES

Make sure your fungal plates aren't contaminated!

MATERIALS

Plasticware

	Description	Catalogue Number	Supplier
	PCR tubes	TFI0201	Bio Rad laboratories
	Pipette tips		

Chemicals and Kits

	Description	Catalogue Number	Supplier
	REDExtract, Plant PCR Kit	XNAP	Sigma-Aldrich (New Zealand)
	Ultrapure water	10977015	Life Technologies New Zealand
	Agarose	A-1280-100	pH Scientific Limited
	Sybrsafe DNA Gel Stain	S33102	Thermo Fisher Scientific
	TAE buffer	PMV4271	In vitro technologies
	1kb ladder	SMO313	Life Technologies New Zealand

Primers:

	Primer	Sequence
	ITS1F	5' CTTGGTCATTTAGAGGAAGTAA 3'
	ITS4	5' TCCTCCGCTTATTGATATGC 3'

Equipment:

- Pipettes - various sizes
- Thermocycler PCR machine for cell lysis and colony PCR
- Micro centrifuge
- Powerpack for gel electrophoresis

BEFORE START INSTRUCTIONS

You will need to prepare your fungal cultures so that you have visible mycelium for extraction.

DNA Extraction

1 Place 30 ul extraction solution into PCR tubes. Using a small sterile pipette tip place a small piece of mycelium so its only just visible on the tip into the tube. Vortex and briefly centrifuge.

2 Place PCR tubes into the PCR machine and run a cell break protocol (95 °C for 10 minutes).

Cool down and add 30µl dilution solution.

Dilute the samples as needed. Start with 1:20 and go to 1:10 or 1:5 if needed.

Store the extracted DNA at -20 °C until PCR.

PCR

3 **Make a master mix based on 9 µl per single reaction**

Recipe per PCR sample (single reaction = 9 µl)

	Component	Volume per single reaction	Volume based on 10 samples*
	Reaction mix	5µl	65µl
	Forward primer	0.5µl	6.5µl
	Reverse primer	0.5µl	6.5µl
	Dilution solution	0.5µl	6.5µl
	Extraction solution	0.5µl	6.5µl
	Ultrapure H2O	2µl	26µl

*Total preparation amount = number of samples + 1 negative control + 2 extra to cover pipetting errors.

4 Aliquot 9µl of master mix into individual PCR tubes.

5 Add 1µl of extracted DNA sample to each aliquot of master mix.

6 Centrifuge samples briefly (just a few seconds) in a benchtop centrifuge to ensure they are at the bottom of the tube.

7 Place tubes into PCR machine and run using the following settings:

1x 94 °C - 3 minutes.

35-40x cycles of:

94 °C - 30 seconds

52 °C - 30 seconds

72 °C - 30 seconds (For LSU primers use 72 °C for 40 sec)

1x 72 °C - 7 minutes

Gel electrophoresis

8 Make a 1% molecular grade agarose gel using 1x fresh TAE. Microwave until the agar has just dissolved.
Note. 100ml for running 25 samples, 35ml for 13 samples

9 Add 2µL Sybrsafe stain per 50ml volume of agar

10 Pour into setup gel tray, add comb and leave for half an hour or until set.

11 Place the set gel into the gel tank (make sure the wells are at the negative end).

12 Fill the tank with waste TAE until entire gel is submerged - can top up with fresh TAE if needed.

13 Load 2-5µl of sample into each well including a 1kb ladder and negative controls

14 Set powerpack to run at 90v for 30mins

15 After gel is run gently lift the tray out of the tank and movie it onto a sybersafe tray view bands in the gel viewer.