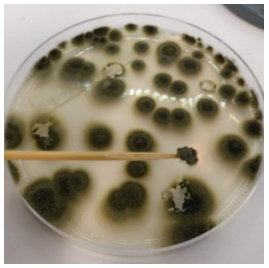
Aug 16, 2018 Version 1

# Quick Fungal DNA extraction from colonies on plates V.1



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**Protocol status:** Working
**We use this protocol and it's working**

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**Keywords:** fungal DNA extraction, quick fungal dna extraction from colony, colony pcr on fungal colony, quick fungal dna extraction, fungal colony, colony pcr, dna, pcr template, pcr

## 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.

## 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 $H_2O$ |

| | 500 mM NaEDTA | 100 ml |
|---|---|---|
| | 18.6 g | EDTA disodium salt dihydrate |
| | up to 100 ml | deionised $H_2O$ |

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 × 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

| | |
|---|---|
| new 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 $H_2O$ or 1x TE buffer**
- Measure DNA concentration (e.g. Nanodrop)
- store DNA at -20°C