*Original Article*

**Microbial Biosystems 6(1) (2021) 2021.1036**
**10.21608/mb.2021.91637.1036**

Contents lists available at Egyptian Knowledge Bank
Microbial Biosystems
Journal homepage: http://mb.journals.ekb.eg/

MICROBIAL BIOSYSTEMS
INTERNATIONAL SCIENTIFIC JOURNAL OF MICROBIAL BIOLOGY

# Genomic DNA extraction from minimal amount of dried mushroom samples

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**ARTICLE INFO**

***Article history***
Received 19 August 2021
Received revised 29 August 2021
Accepted 29 August 2021
Available online 4 September 2021


Corresponding Editor:
Khalifa E.
Sheir D.

***Keywords***
Affordable
DNA extraction
Dried mushroom samples
Rapid
Student friendly

**ABSTRACT**

**Aims**: To develop a simple and rapid genomic DNA extraction technique for dried (≈ 1 year old) mushroom fruiting bodies that yields high-quality DNA, suitable for use by post-graduate institutions.

**Method**: Small amounts (0.04 g) of pulverized dried mushroom sample were incubated in a Tris /EDTA/SDS lysis buffer (100mM:10mM: 2%) at 65°C to lyse the chitinous fungal cell walls. Genomic DNA purification was performed using chloroform isoamyl alcohol (24:1), and DNA was precipitated using 100% ethanol.

**Results**: Genomic DNA was successfully extracted under 70 minutes from 16 samples morphologically identified as *Panaeolus*, *Copelandia*, *Gymnopilus*, *Pluteus* and *Favolus* species. DNA concentrations were on average of 696.9ng/µL. PCR successfully amplified the ITS-5.8S region. The protocol has been successfully used by numerous post-graduate students in our research programme.

**Conclusion**: The rapid and easy protocol produced high-quality genomic DNA void of any inhibitors that is suitable for downstream molecular implications across multiple mushroom genera. Noticeably, this method requires only minute quantities (0.04g) of starting material and is ideal for student training in higher academic institutions.

**Published by Arab Society for Fungal Conservation**

## Introduction

Fungi play essential roles in all components of ecosystems as nutritional sources, decomposers, mutualists and pathogens, as well as in economic sectors where they are sources of food, medicine, biological agents, and bioactive compounds (Cheng, 2021; de Mattos-Shipley et al., 2016; Enow Andrew et al., 2013; Gryzenhout et al., 2012). DNA extraction is aiding research institutions, universities and biology students to identify fungi during biodiversity, ecological or applied studies, complimenting morphological methods (Badotti et al., 2017; Mullineux and Hausner, 2009; Zhang et al., 2016). When morphological characteristics are not available (e.g., a specimen is damaged and incomplete) or the amount of sample is limited, comparing sequences obtained from unknown mushrooms against those of previously sequenced species is an incredibly useful tool to identify species (Wesselink, 2018).

In recent years, higher institutions have been implementing more laboratory orientated research by students, including performing DNA extraction from fungi for various types of studies. Fungal material is collected during fieldwork or retrieved from civilian scientists and is usually stored at room temperature for extended periods or stored erratically, especially for macrofungal samples. As a result, students perform DNA extraction on fungal material that is no longer living. Many extraction protocols require fresh samples or the cultivation of pure isolates for extraction (Aamir et al., 2015; Al-Samarrai and Schmid, 2000; Cenis, 1992; Chi et al., 2009; Feng et al., 2010; Moller et al., 1992; Rodrigues et al., 2018; Wingfield and Atcharawiriyakul, 2021; Yang et al., 2016a; Zhang et al., 2010). Culturing is a skill set not frequently found among biology students and non-taxonomy orientated researchers, while these cultures also need to be maintained following specialist protocols.

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Extraction of genomic DNA is typically done in two steps, firstly by lysing the cell wall and secondly extracting and purifying the genomic DNA (Zhang et al., 2010). Mechanical lysis is done through grinding samples (e.g. mortar and pestle or freeze drying (Al-Samarrai and Schmid, 2000; Yang et al., 2016a)), while chemical lysis is done by treating cells with a lysis buffer (Dairawan and Shetty, 2020). Popular lysis buffers include Cetyltrimethylammonium Bromide (CTAB), sodium dodecyl sulfate (SDS), and ethylenediaminetetraacetic acid (EDTA) (Dairawan and Shetty, 2020; Tripathy et al., 2017). DNA is typically purified through phenol/chloroform extraction and precipitated using ethanol (Aamir et al., 2015; Butler, 2012; Dairawan and Shetty, 2020). These protocols have been optimised over time to reduce time constraints and increase DNA yield and purity (Butler, 2012; Dairawan and Shetty, 2020; Griffin et al., 2002; Kumar and Mugunthan, 2018; Rittenour et al., 2012). However, most techniques are laborious and use toxic reagents such as phenol (Natarajan et al., 2016; Zhang et al., 2010).

Commercial extraction kits are an alternative method of extracting DNA and are intended to streamline extraction (Yang et al., 2016). Fungal DNA extraction kits are not yet standardised for many fungal species and tend not to work well for some groups (Karakousis et al., 2006; Manian et al., 2001). Furthermore, many kits in use by students are designed to extract DNA from plant material (Feng et al., 2010) and include steps and reagents that are unnecessary for fungal DNA extractions (Yang et al., 2016). Kits are also more expensive than conventional approaches (Feng et al., 2010), especially when many extractions are needed.

The current work described a simple, time-efficient, student-friendly DNA extraction protocol optimised for 16 dried mushroom samples. The protocol has been developed and extensively used in the Genetics Department at the University of the Free State (UFS), Bloemfontein, South Africa to extract genomic DNA from minute amounts of 16 dried fungal sample. The efficiency of this method was examined by PCR amplification of the Internal Transcribed Spacer (ITS) region.

## Materials and methods

### *Biological materials*

A total number of 16 samples representing species of *Panaeolus*, *Copelandia*, *Gymnopilus*, *Pluteus* and *Favolus* were collected and identified morphologically using online resources such a Global Biodiversity Information Facility (GBIF, 2021) (Table 1). The samples were collected in South Africa from January to May of 2019. The samples were air-dried at room temperature stored in the Herbarium (Fungarium) of Marieka Gryzenhout (HMG), Genetics Department, University of the Free State.

### *Genomic DNA Extraction*

Reagents needed for the extraction included Tris (100 mM), EDTA (10 mM), SDS (2%), Chloroform:Isoamyl alcohol (24:1) and Ethanol (100%). The method was based on Avin et al. (2012) but with modifications. The dried fungal mass was homogenised with a tissue homogeniser (©QIAGEN, TissueLyserll, Germany). Only 1.5mL Eppendorf tubes were used and the lysis buffer was prepared in a mass master mixture from which was allocated to each sample. A minute amount of pulverised subsample (0.04g) was added to 900 µL of Tris (100mM)-EDTA (10 mM)-SDS (2%) buffer and briefly mixed by inversion and sharp probes. The lysis buffer was then incubated for 30 minutes at 65°C. Centrifugation (13,000 rpm at 4°C for 5 min) of the lysis buffer resulted in cellular debris that was discarded, and supernatant, which was carefully pipetted out. This step was repeated to reduce cellular debris. To the supernatant, 600 µL Chloroform: Isoamyl alcohol (24:1) was added and centrifuged at 13,000 rpm at 4°C for 5 min. The aqueous phase containing DNA was carefully pipetted out. The Chloroform: Isoamyl alcohol step was repeated as a modification to ensure the further removal of cellular debris and PCR inhibiting chemicals. Finally, the DNA was precipitated with cold ethanol (100%) after centrifugation at 16.000 rpm for 30 min at 4°C. The DNA pellet was resuspended in 40µL of sterile pre-warmed nuclease-free water.

Qualitative assessment of the DNA was done with gel electrophoresis in a 1.5% agarose gel (Cleaver Scientific Ltd, UK) containing Condasafe (Condalab, Madrid, Spain), visualised with a geldoc (Vacutec, Roosevelt Park, South Africa). The DNA concentration (ng/µL) was quantitatively measured using a NanoDrop® Spectrophotometer ND-1000 (ThermoFisher Scientific). The purity of the DNA was assessed at 260/280 nm absorbance. Measurements were performed in duplicates, and the averages of the two measurements were calculated. The DNA was stored at -20°C for subsequent use.

### *PCR amplification of 5.8S-ITS region*

In order to evaluate the quality of DNA, PCR was employed to amplify the ITS-5.8S region. The two universal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') (19bp) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (20bp) were used for this study (Romanelli et al., 2014; White et al., 1990). Each 25 µL PCR reaction included 1µL of template DNA (≈ 100-200ng), 1.25µL of each primer (10 µM), 12.5µL One Tag® 2X MM w/standard buffer (New England BioLabs, inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa) and 9µL nuclease free water.

**Table 1** Samples collected in Bothas Hill, South Africa

| Code | Identification | Collection Date |
|---|---|---|
| HMG35 | *Panaeolus africanus* Ola'h | 22 January 2019 |
| HMG36 | *Panaeolus semiovatus* (Sowerby) S. Lundell & Nannf. | 23 January 2019 |
| HMG37 | *Panaeolus africanus* Ola'h | 13 May 2019 |
| HMG41 | *Gymnopilus purpureosquamulosus* Høil. | 13 May 2019 |
| HMG43 | *Gymnopilus purpureosquamulosus* Høil. | 22 May 2019 |
| HMG44 | *Copelandia mexicana* Guzmán | 04 February 2019 |
| HMG45 | *Copelandia mexicana* Guzmán | 22 May 2019 |
| HMG46 | *Gymnopilus purpureosquamulosus* Høil | 03 April 2019 |
| HMG47 | *Panaeolus cinctulus* (Bolton) Sacc. | 19 March 2019 |
| HMG48 | *Gymnopilus purpureosquamulosus* Høil. | 19 March 2019 |
| HMG49 | *Pluteus longistriatus* (Peck) Peck | 26 March 2019 |
| HMG50 | *Pluteus longistriatus* (Peck) Peck | 04 February 2019 |
| HMG51 | *Pluteus longistriatus* (Peck) Peck | 28 March 2019 |
| HMG53 | *Pluteus longistriatus* (Peck) Peck | 03 April 2019 |
| HMG54 | *Favolus brasiliensis* Fr (Fr.) | 23 January 2019 |
| HMG57 | *Pluteus longistriatus* (Peck) Peck | 03 April 2019 |

PCR was performed in a BioRad T100 Thermal Cycler (BIO-RAD, Johannesburg, South Africa). The thermal cycling conditions were set with an initial denaturation temperature at 94°C for 2 min 30 s, followed by 40 cycles of denaturation at 94°C for 30s, annealing at 54°C for 30s and extension at 72°C for 40 s. A final extension was performed at 72°C for 10 min. The amplicons obtained were resolved on 1.5% agarose gels.

## Results

Results for this paper included fungal samples of *Panaeolus africanus*, *Panaeolus semiovatus*, *Panaeolus cintulus*, *Copelandia mexicana*, *Gymnopilus purpureosquamulosis*, *Pluteus longistriatus*, and *Favolus brasiliensis*. The total genomic DNA yield after extraction was recorded to be in the range of 273.7ng/µL – 1538.1ng/µL, with one sample having a concentration of 69.7ng/µL (Table 2, Fig.1).

Protein contamination in the pure gDNA measured between 1,80¬ – 2,14 using the ratio A260/A280, and one sample measured 1,59 (Table 2). Salt and amino acid contamination was measured in a range of 0,84 – 1,90 using the ratio A260/A230 (Table 2).

PCR amplification of the ITS region was successful for all samples (Fig. 2) without any need of optimization.

## Discussion

Genomic DNA was successfully isolated from 16 dried mushroom samples using the developed protocol. PCR successfully amplified the ITS region for all of the DNA samples proving that there were no inhibiting substance that could complicate downstream experiments.

The genomic DNA protocol was high-yield and comparable with other fungal studies (Cho, 1996; Faggi et al., 2005; Galliano et al., 2021; Moller et al., 1992). The proposed method produced 260/280 ratios in the range of 1,59 – 2,11 (mean=1,96). Fungal DNA typically has a R260/280 measurement between 1,50 and 2,20 (Galliano et al., 2021; Guo et al., 2005). DNA concentrations ranged between 255.0 ng/µL and 1538.1 ng/µL, with one sample measuring 69.7 ng/µL.

The extraction method is student-friendly and only requires essential equipment such as an incubator, heat-block or water bath and centrifuge machine. To streamline the DNA extraction process, a tissue homogeniser was utilized as a closed system to crush the sample and avoid contamination. Other methods to pulverize and crush samples or to maximise absorption can, however, also be applied, such as freeze drying (Griffin et al., 2002), liquid nitrogen (Tripathy et al., 2017; Wu et al., 2001), magnetic beads (Faggi et al., 2005) or using mini pestles and grinding with sea sand (Yee et al., 2018). This method can be used for large scale DNA extraction because at any given time, samples can be processed in within 70 minutes.

The most considerable advantage of the developed protocol is the ability to extract DNA from dried fruiting bodies as opposed to mycelium. Fungal tissues in amounts as small as 0.04 g is sufficient for extraction, which is often designated as too small for kits (Umesha et al., 2016).

A stronger concentration of SDS was used in the current study in the comparison to the previous reports (Bellemare et al., 2018; Müller et al., 1998; Umesha et al., 2016) in order to solubilise the proteins and lipids in the cell

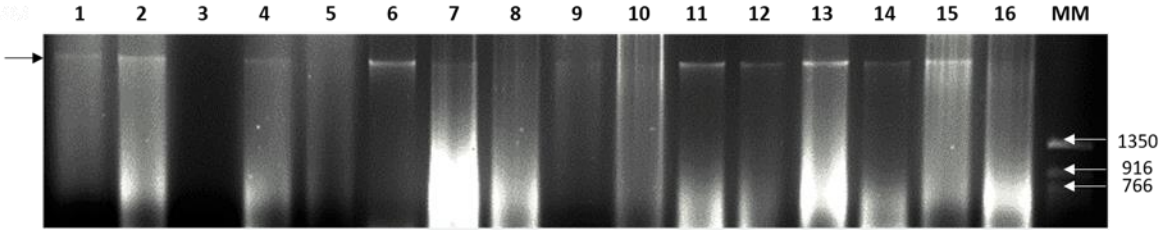


**Fig 1.** Agarose gel (1.5%w/v) of gDNA obtained from 16 mushrooms samples loaded in Lanes 1 -16 in the volumes of 5µL DNA (undiluted) and 2µL GelRed. Lane 17 corresponds to the Quick-Load Purple 50bp DNA ladder (New England Bio Labs Inc., USA), added in the volumes of 2µL DNA ladder (undiluted) and 2µL GelRed. White arrows on the DNA ladder indicated band sizes 1350bp, 916bp and 766bp. The black arrow indicates the presence of genomic DNA

**Table 2** Genomic DNA concentration of 16 mushroom samples measured using a Thermo Scientific NanoDrop 1000 recorded at $\lambda_{260}$

| Sample Number | 260/280 | 260/230 | ng/µL | Agarose Gel Lane |
|---|---|---|---|---|
| HMG35 | 2.01 | 1.02 | 690.4 | 2 |
| HMG37 | 1.97 | 1.16 | 468.9 | 3 |
| HMG38 | 1.91 | 1.31 | 478.1 | 4 |
| HMG39 | 2.14 | 1.90 | 1246.6 | 5 |
| HMG40 | 1.81 | 1.54 | 680.2 | 6 |
| HMG44 | 2.04 | 0.95 | 255.0 | 7 |
| HMG46 | 2.09 | 1.68 | 1411.8 | 8 |
| HMG47 | 2.04 | 1.23 | 371.2 | 9 |
| HMG49 | 2.03 | 1.45 | 859.0 | 10 |
| HMG50 | 2.06 | 1.68 | 1538.1 | 11 |
| HMG51 | 1.80 | 1.11 | 504.3 | 12 |
| HMG53 | 1.89 | 1.14 | 546.5 | 13 |
| HMG54 | 1.88 | 0.84 | 273.7 | 14 |
| HMG55 | 2.08 | 1.72 | 1059.8 | 15 |
| HMG56 | 1.59 | 0.86 | 69.7 | 16 |
| HMG57 | 2.11 | 1.56 | 697.8 | 17 |

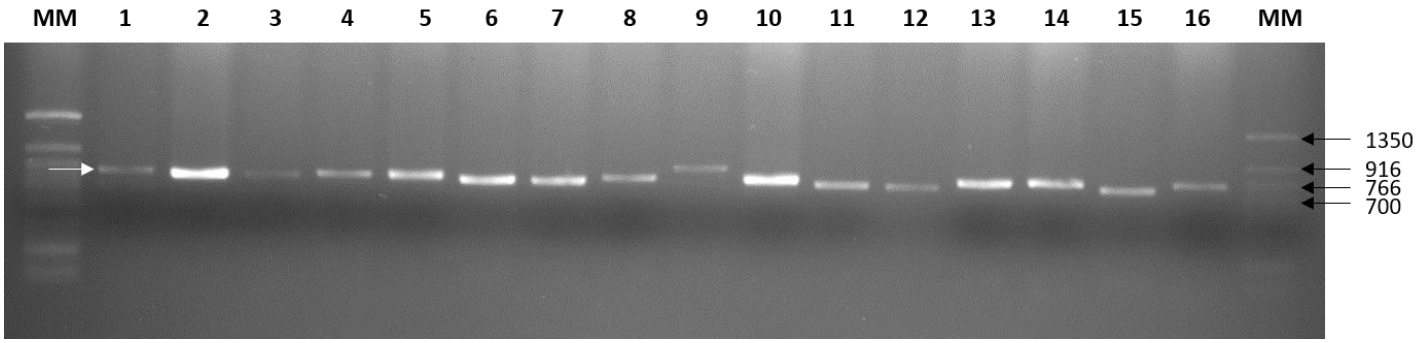


**Fig 2.** Agarose gel (1.5%w/v) of the ITS region amplified in 16 mushrooms samples loaded in the volumes of 5µL DNA (undiluted) and 2µL GelRed. Lane 17 corresponds to the Quick-Load Purple 50bp DNA ladder (New England Bio Labs Inc., USA), added in the volumes of 2 µL DNA ladder (undiluted) and 2 µL GelRed. Black arrows on the DNA ladder indicated band sizes 1350bp, 916bp, 766bp and 700bp. The white arrow indicates the ITS-5.8S-ITS2 regions (700 – 800 bp) ofthe DNA samples.

membranes, while simultaneously denaturing proteins in the cytoplasm at a faster rate. Expensive and toxic reagents were avoided, including liquid nitrogen and hydrogen chloride, which is required by some protocols (Avin et al., 2012; Lahuf et al., 2019; Yang et al., 2016). Lastly, the protocol has been used with great success by postgraduate students with limited experience and varying abilities. In their projects a wide variety of fruiting body types were used, including from brittle to tough, which normally would have yielded problematic and varying results necessitating a great deal of optimization. Because of its low cost, very limited output expenses are needed after which it will serve a research or training programme for an extended period of time.

## Conclusion

The present study embodied an easy student-friendly protocol that has been standardised for the extraction of high-quality DNA from approximately a year-old dry mushroom sample.

## Declaration of interests

This study was supported by the University of the Free State and the Environmental and Biosafety Research Ethics Committee (UFS-ESD2019/0033). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors are grateful to Jon MacGillivray for his work in collecting and drying the samples used in this study.

## Author's contribution

MG obtained the funding for the study. SG conceived the study and designed the experiment. DS conducted all the experiments under the close guidance and supervision of SG. DS and SG analysed the data and drafted the manuscript. SG, MG and ZM read and edited the manuscript.

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