

## Why Nuclear Ribosomal Internal Transcribed Spacer (ITS) has been Selected as the DNA Barcode for Fungi?

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### Abstract

The application of DNA sequences of standardized genetic markers for the identification of eukaryotic organisms is known as DNA barcoding. Based on a recent study by a multinational and multilaboratory fungal barcoding consortium, the nuclear ribosomal internal transcribed spacer (ITS) has been selected as the DNA barcode for fungi. DNA barcoding shows tremendous promise for the organisms rapid identification at the species level. Recently, DNA barcodes are used for identification of fungal species in marine sediments.

**Keywords:** Fungi; Barcoding; Internal transcribed spacer (ITS); Translation elongation factor 1A (tef1a); Ribosomal polymerase B2 (rpb2); Cytochrome oxidase 1 (cox1, OI)

### Abbreviations:

rDNA: Ribosomal DNA; ITS: Internal Transcribed Spacer; LSU: Large Subunit; AFTOL :Fungal Tree of Life; RPB1: RNA Polymerase II; SSU: Small Subunit; IBOL: The International Barcode of Life; MCM: Mini-Chromosomal Maintenance

### Introduction

Fungi are highly diverse group of organisms, with large numbers of species which are not yet described. The accumulative sequencing technologies became crucial for investigating fungal communities in different habitats [1].

Many researchers have been contributing to the effort, with a concerted attempt to obtain representative sequences for all major lineages of the Eumycota [2]. Four markers were selected for different group's comparison; three regions of the nuclear ribosomal DNA and one protein coding gene. The internal transcribed spacer (ITS) of rDNA has gained much attention, where it has a wide utility as marker in different studies

Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi

All fungi ITS sequences were deposited in GenBank, where the major bulk came from Latin binomial of fungi and the smaller numbers from environmental studies [3,4]. ITS has been used in combination with the large subunit (LSU) of ribosomal RNA in small numbers of environmental studies [5,6]. ITS is also used to measure the genetic distances between fungal different groups [7]. Phylogenetic studies have been used in identification and comparing of fungi units [8,9]. Protein-coding genes have proved its supremacy over *rRNA*

genes for resolving relationships at various taxonomic levels [10]. Using specific markers with available primers for translation elongation factor 1- $\alpha$  for *Fusarium* [11] and  $\beta$ -*tubulin* for *Penicillium* [12], usually amplified to a narrow taxonomic value. Among protein-coding genes, the largest subunit of RNA polymerase II (RPB1) gene might have potential as a fungal barcode [13]. The phylogenetic use of the largest subunit of RNA in studies of *Basidiomycota*, *zygomycota*, *Microsporidia* [13-17], and in some protists indicated a promising barcode tool [18]. RPB1 is ubiquitous and single copy, and it has a slow rate of sequence divergence. However, the use of the primers which developed by assembling the fungal tree of Life (AFTOL) project for proposed locus as a barcode remains untested [19]. Schoch et al. [2] have compared the sequences of all rDNA, in addition to representative protein-coding gene, RPB1 regions to be considered as barcode for kingdom Fungi. It is advisable to sequencing the largest subunit of ribosomal polymerase II (RPB1) for fungal species identification. It has been chosen as a protein representative coding gene due to the general good PCR success with this gene and its utilization as phylogenetic marker in AFTOL project. For some fungi, the second largest subunit of ribosomal polymerase II (RPB2) and another recently introduced marker mini-chromosome maintenance proteins (MCM7) was also utilized in the second AFTOL project that were sequenced and analyzed.

To sum up, RPB1 produced more resolving distinction ability than ITS, but the latter remained the preferred choice for species barcode across all that are comparable to the success rate of the two barcode system adopted for plants [2]. ITS PCR performance success rate is a crucial point for the possible utility of the ITS as a barcode. Although the identification success for RPB1 was higher up to 81 %, however poor amplification of candidate gene/DNA fragment PCR success rate is of a serious limitation. Almost no data could be generated for the basal fungal lineages and the only data set generated for those groups came from DNA samples. Although the utility of other protein-coding genes were discussed by some researchers, and it has been concluded that the fungi identification tools efficiency came in that order: ITS and RPB1>LSU>SSU [2,5,6].

Thereafter, a discussion of whether a two gene barcode system should be considered was held, especially for yeasts, where both genes (ITS and LSU) sequencing was carried out. One critical view that should be taken into consideration is the high cost of genes sequencing versus the expected benefit of the second barcode. During barcode database development, both markers would have to be sequenced. Although it is clear that LSU is superior to the ITS for recognizing species in some groups of yeasts, a combined LSU/ITS system gave only a modest increase in identification success. Considering the assent of participating yeast taxonomists, consensus built favour of

one gene barcoding system and ITS was proposed to be the best candidate for the same [2].

Mycologists have shown interest in participating in the DNA barcoding movement, with projects on quarantine-relevant fungi, medically important fungi and indoor moulds already underway. Researchers have developed interest towards sequencing fungal herbarium specimens, and pursuing an international research coordination network (perhaps affiliated with, the International Barcode of Life network; IBOL) for fungal DNA barcoding. DNA regions were evaluated as potential DNA barcodes for fungi, the second largest kingdom of eukaryotic life, by a multinational, multilaboratory consortium (Fungal barcoding consortium: ([www.fungalbarcoding.org](http://www.fungalbarcoding.org) - <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3341068/>). The region of the mitochondrial cytochrome C, and oxidase subunit 1 used as the animal barcode was excluded as a potential marker, because it is difficult to be amplified in fungi, often includes large introns, and can be insufficiently variable [20].

The process of fungal barcode is basically short sections of DNA sequence that can be used to identify fungal species. This barcode area is shared across the majority of fungi, but also has enough small changes between different species that it can be used to distinguish one from another.

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