Target genes, primer sets, and thermocycler settings for fungal DNA amplification

This document describes some of the target genes and primers that can be used for DNA sequence-based identification of fungi and the PCR conditions with which to use those primers. Other primer sets have been used for other genes, but those described below are the most consistently available in databases for the identification of yeasts and molds that are most likely to be identified in a clinical microbiology laboratory.

Universal primer sets exist, but they often do not have enough discriminatory power to identify species, or they do not have the discriminatory power to identify species within a species complex, often giving 100% match to multiple species.

This tool assumes that fungal DNA already exists; it does not describe the procedure for purification of fungal DNA.

Table of fungal amplification targets for sequence-based identification

Fungal genera	**Gene target**
All fungi	ITS
All yeasts	D1D2
Fusarium	Elongation Factor 1α
Scedosporium, Aspergillus, Penicillium	B-tubulin
Trichosporon	IGS
Dermatophytes	Modified ITS

PCR primers and purposes

ITS

In general, for unknown molds, the ITS region of the rDNA is used as the primary target with primers ITS-1 and ITS-4 as the most general primer set. In some cases, these primers may not provide sufficient identification, and a protein coding region may be required.

For the forward primer there are two options. ITS-5 gives a slightly longer PCR product than ITS-1, but both are good.

Forward: ITS-1 5’-TCCGTAGGTGAACCTGCGG
(or) ITS-5 5’-GGAAGTAAAAGTCGTAACAAGG

Reverse: ITS-4 5’-TCCTCCGCTTATTGATATGC

Annealing temperature: 52°C

These primers amplify approximately 600 basepairs of the ITS1-5.8S-ITS2 region of the ribosomal cistron.

D1-D2 region of large ribosomal subunit

Although the ITS primers are universal for fungi, the D1D2 region of the large ribosomal subunit has better discrimination for yeasts, with primers NL-1 and NL-4.

Forward: NL-1 5’-GCATATCAATAAGCGGAGGA

Reverse: NL-4 5’-TTGGTCCGTGTTTCAAGACG

Annealing temperature: 52°C

These primers amplify approximately 620 basepairs of the 28S region of the ribosomal cistron.

Elongation Factor-1α (for sequencing *Fusarium* species)

The ITS primer set generally only discriminates *Fusarium* species into the various species complexes but does not discriminate cryptic species. For identification of *Fusarium* isolates within species complexes, the EF-1α primers should be used (O’Donnell, 2009).

PCR Forward: EF-1 5’-ATGGGTAAGGARGACAAGAC

PCR Reverse: EF-2 5’-GGARGTACCAGTSATCATG

Annealing temperature: 52°C

These primers amplify approximately 717 bp of the coding region of the EF-1α gene.

β-tubulin (for sequencing *Scedosporium*, *Aspergillus* and *Penicillium* species)

Similar to *Fusarium*, the ITS primer set generally only discriminates *Scedosporium*, *Apsergillus*, and *Penicillium* species into the various species complexes but does not discriminate cryptic species. For identification of *Scedosporium*, *Aspergillus*, and *Penicillium* isolates within species complexes, the β-tubulin primers should be used (Glass, 1995).

Forward: Bt2a 5’-GGTAACCAAATCGGTGCTGCTTTC

Reverse: Bt2b 5’-ACCCTCAGTGTAGTGACCCTTGGC

Annealing temperature: 54°C

These primers amplify approximately 495 bp of exons and introns at the 5’ end of the β-tubulin gene.

Dermatophyte primers (amplify the ribosomal ITS region of dermatophytes)

Trichophyton species DNA is amplified very poorly by the ITS primer set used for most other molds. There is a special set of ITS primers specifically for amplification of the ITS region of dermatophytes, especially *Trichophyton* (Gräser, 2000).

Forward: LR1 5’-GGTTGGTTTCTTTTCCT

Reverse: SR6R 5’-AAGTAAAAGTCGTAACAAGG

Annealing temp: 52°C

These primers amplify approximately 630 basepairs of the ITS1-5.8S-ITS2 region of the ribosomal cistron.

***Trichosporon* primers (amplify IGS)**

Individual species of *Trichosporon* are not well discriminated using either the ITS or D1D2 primer sets. For identification of *Trichosporon* species, the intergenic region of the ribosomal cistron is used (Sugita, 2002).

Forward: 26SF 5′-ATCCTTTGCAGACGACTTGA

Reverse: 5SR 5′-AGCTTGACTTCGCAGATCGG

Annealing temp: 56°C

These primers amplify a section of the intergenic spacer in the ribosomal cistron. The sequence lengths vary greatly from approximately 200 basepairs to up to 700 basepairs depending on the species.

Interpretation of Results

There are several databases through which the sequence results can be searched for matching species. Each of these databases must be validated in individual laboratories before the results can be used for patient records.

National Center for Biotechnology Information (NCBI) — BLAST:

The largest database is the National Center for Biotechnology Information (NCBI) run by the National Institutes of Health.

Westerdiijk Institute (CBS)—MycoBank:

The Westerdiijk Institute (formerly the Centraal Bureau voor Shimmelcultures or CBS) maintains a curated fungal database that is excellent for rare species identification.

The *Fusarium* ID database:

The *Fusarium* ID database contains EF1-α sequences for many species of *Fusarium*.

References

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