PCR FOR DETECTION AND IDENTIFICATION OF FUNGI IN CLINICAL SAMPLES

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NOTES

The following protocol is for the detection and identification of fungi in clinical samples. The region 2 of the internal transcribed (ITS) found on the fungal ribosome DNA (rDNA) will be amplified (Figure 1). Sequencing of the ITS region is now considered the gold standard for mycobiome (i.e fungal population in a specific locale) studies due to the availability of the sequences in public databases and its superior discrimination between species compared to other conserved region in the genome of fungi. The length of the amplicon following the PCR is variable between species and will fluctuate between ~200 and 400 base pairs (Turenne et al. 1999).

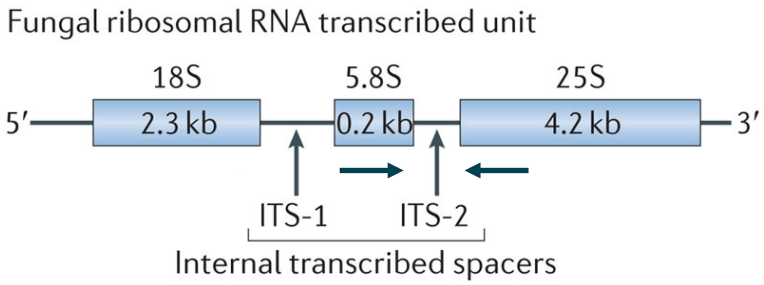

Figure 1: Location of the internal transcribed spacers of fungal rRNA gene
(Underhill & Iliev 2014)

EQUIPMENT

- Thermocycler
- PCR tubes or 96 well plate
- PCR standard reagents
- Primers

PROTOCOL

Primers (Findley et al. 2013)
5.8S_fwd **(MS1) –** GTGAATCATCGARTCTTTGAAC
25S_rev **(MS2) –** TATGCTTAAGTTCAGCGGGTA

Reaction setup	**1X**
10X PCR Buffer (Invitrogen)	2.5 μl
$MgCl_2$ (50mM)	0.75 μl
dNTPs (10mM)	0.5 μl
Forward primer (10μM)	0.5 μl
Reverse primer (10μM)	0.5 μl
Template, genomic DNA	0.5 μl
Taq polymerase (Invitrogen)	0.125 μl
Water	19.625 μl
Total	25 μl*

Prepare a master mix with everything except the template (mix well then spin down to avoid variability). Aliquot the mastermix into all wells then pipette the template to the bottom of each well. No mixing of the tubes/plate is necessary. Following the amplification, run 5 μl on a 1% agarose gel.

*** For sequencing, I double reagents volumes and template to do the reaction in 50 μl.**

Thermocycler set up

1) 94°C – 2min
2) 94°C – 30sec
3) 55°C – 30sec
4) 72°C – 45sec
5) Go to 2 for 34 cycles
6) 72°C – 10min

REFERENCES

Findley, K. et al., 2013. Topographic diversity of fungal and bacterial communities in human skin. *Nature*, 498(7454), pp.367–370.

Turenne, C.Y. et al., 1999. Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. *Journal of Clinical Microbiology*, 37(6), pp.1846–1851.

Underhill, D.M. & Iliev, I.D., 2014. The mycobiota: interactions between commensal fungi and the host immune system. *Nature Reviews Immunology*, 14(6), pp.405–416.