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# Mycobiome sequencing – can we use it for diagnosing fungal infection?

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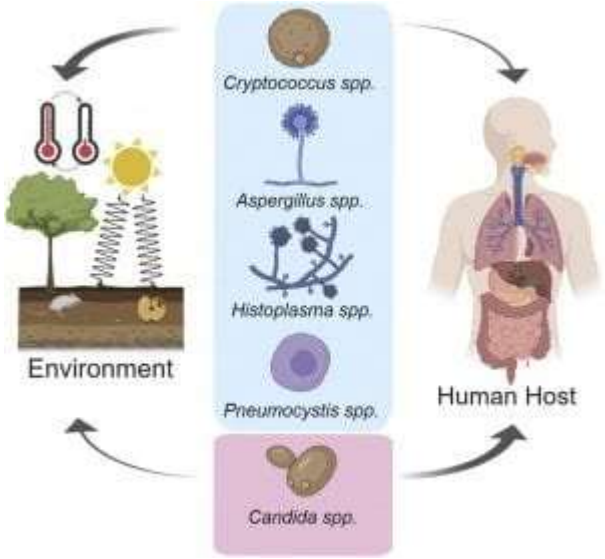
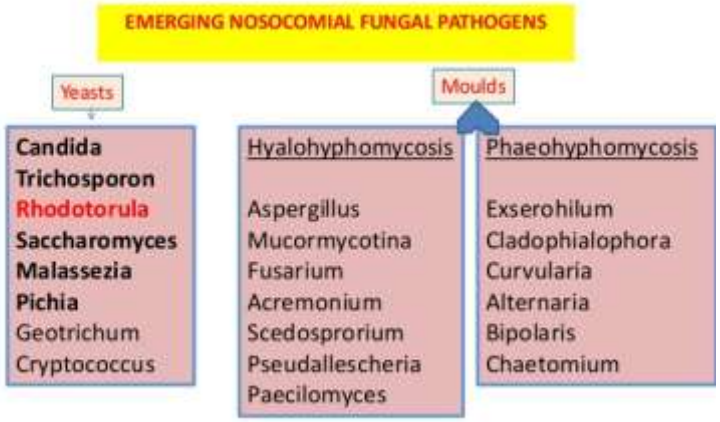
# Diagnosing fungal infections

- Direct microscopy and culture methods
  - time consuming
  - require technical expertise
- GM and β-D-Glucan testing
  - fail to detect some fungal pathogens
  - don't identify the organism causing the positive result

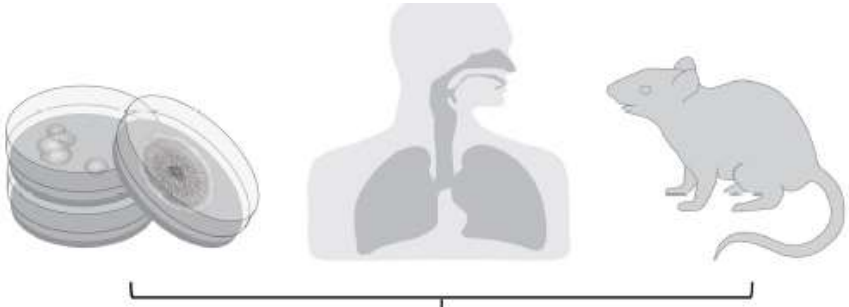



- New diagnostics with broad species coverage & good speciation are warranted



# Mycobiome sequencing

- Mycobiome – the fungal community in and on an organism
- A few targets have been reported in the literature – all are ribosomal RNA regions
- The Internal transcribed spacer (ITS) regions have become standard, particularly ITS1



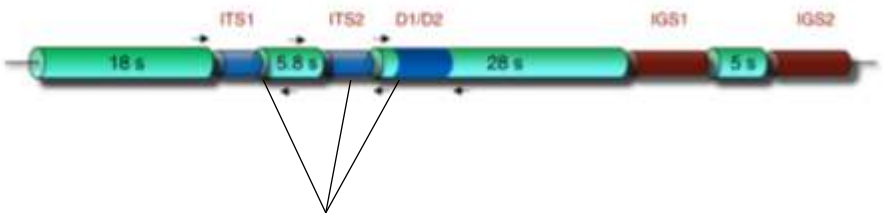
**PCR amplification**
SSU (Euk1a - Euk516r)
ITS1 (ITS1F - ITS2)
ITS2 (ITS3 - ITS4)
LSU (NL1 - NL4)

**Sequencing**
Illumina MiSeq (2 x 250 bp)

**Bioinformatics**

**Taxonomic classification**
SSU (SILVA)
ITS1 (UNITE; RDP)
ITS2 (UNITE; RDP)
LSU (SILVA; RDP)

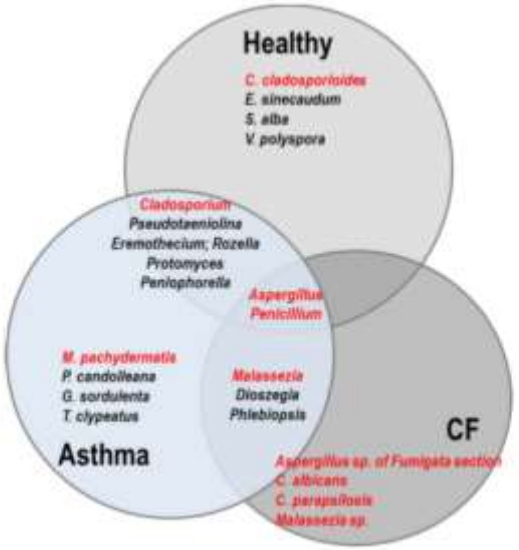
Fungal ribosomal RNA

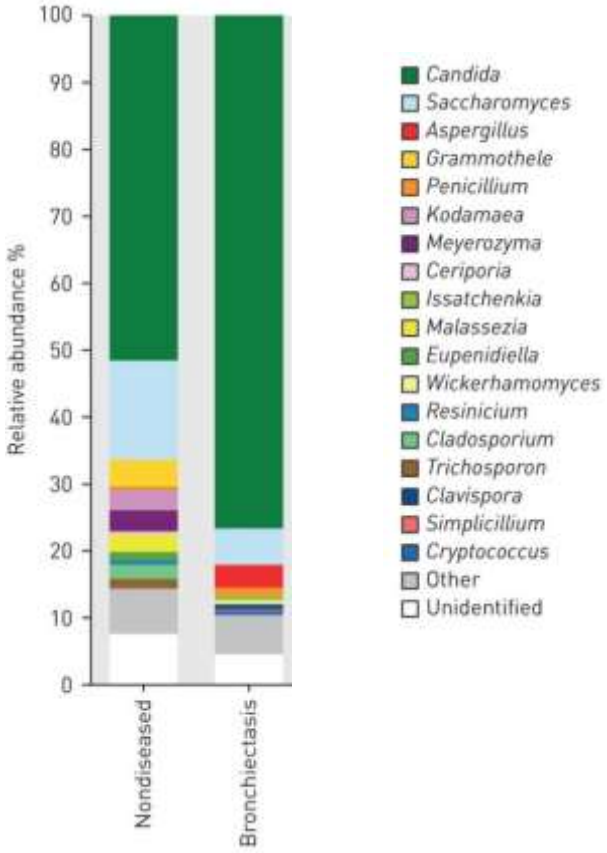



Variable regions

# Mycobiome varies in health & disease – Can we harness it for diagnostics?

- High throughput sample processing
- Sensitive = good limit of detection
- Specificity – depends on chosen primers
- Potential to provide a complete picture of all species within a sample in a quantitative manner

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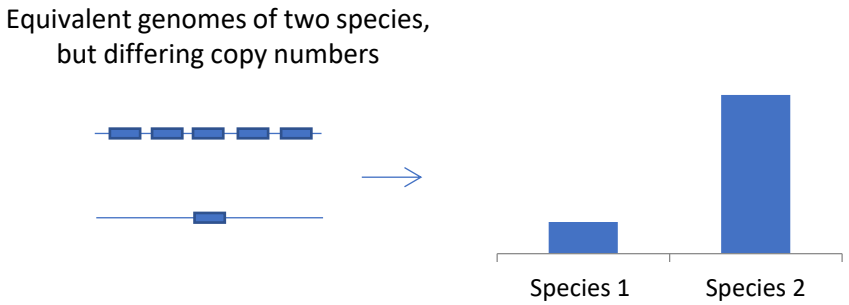
# Pitfalls of ITS for use in an NGS fungal diagnostic

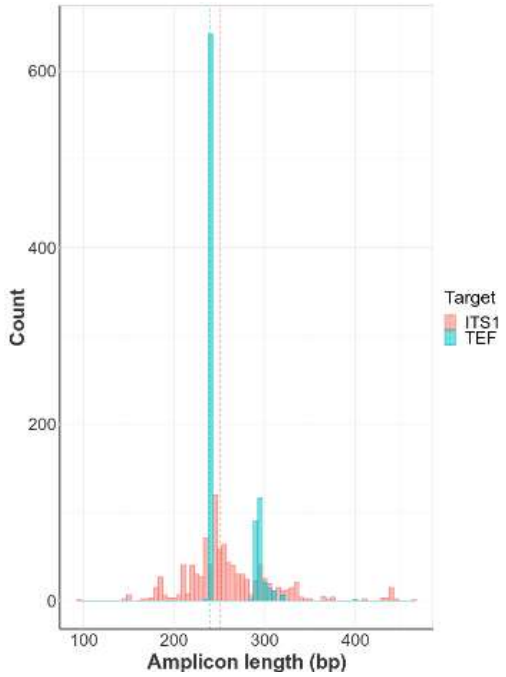
- Amplicon not consistent in length – PCR bias against some species
- Variable copy number in fungal genomes (13-fold between *A. fumigatus* isolates!)

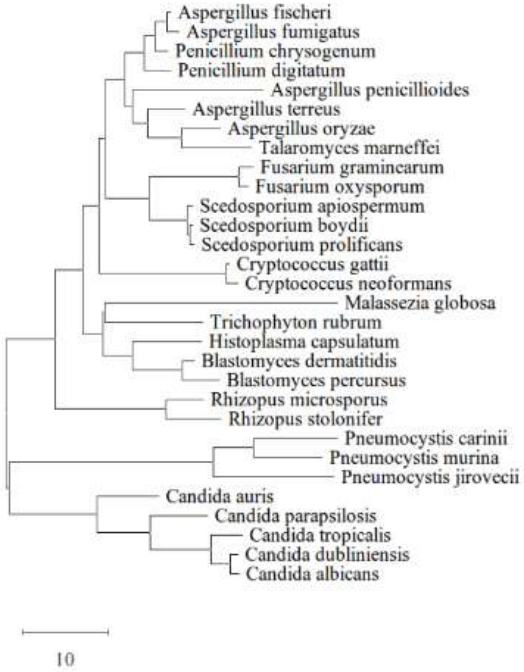



**Accurate quantification is key to differentiate between members of mycobiome which may be transient due to inhalation and those which are infecting**

# A novel amplicon target, TEF

- Single copy gene
- More consistently sized amplicon
- Can distinguish between closely related fungal pathogens
- Small amplicon size means can use Illumina benchtop sequencer: iSeq100

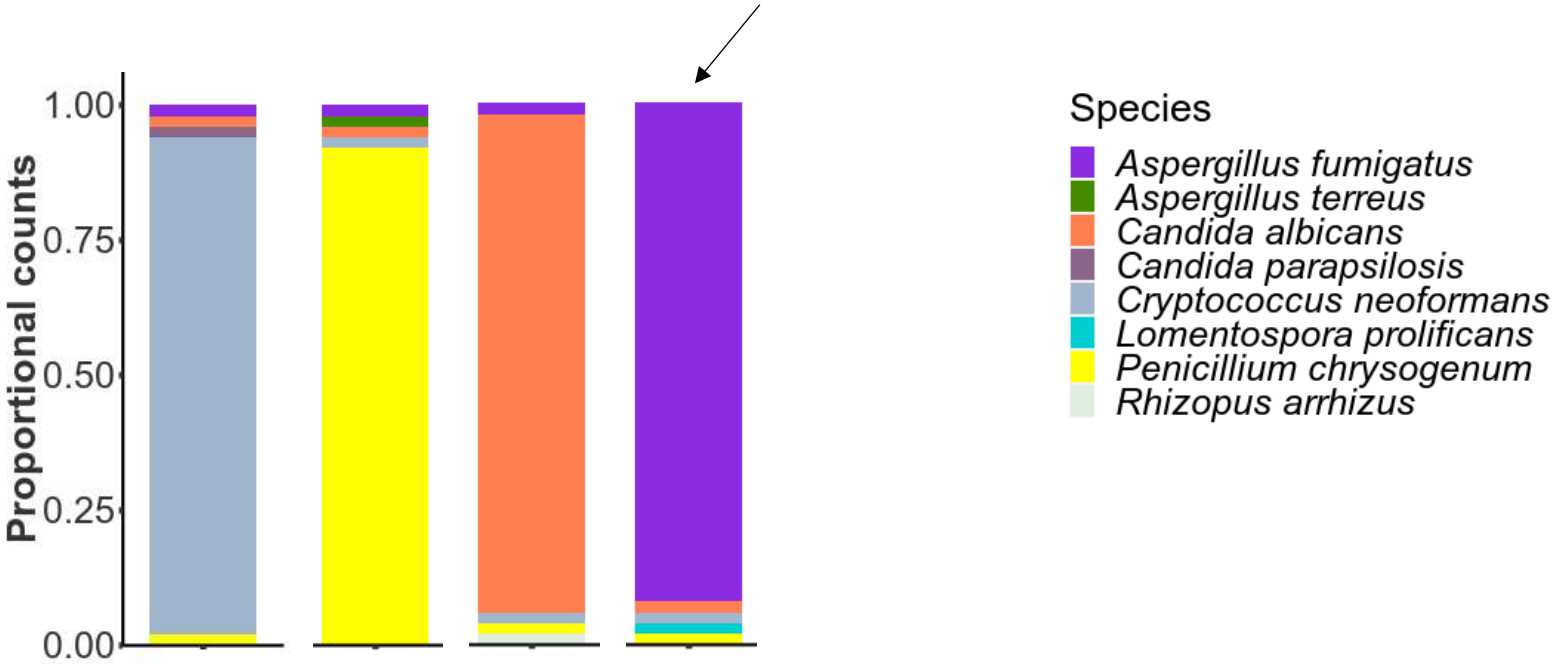



# Mock fungal communities for assay validation

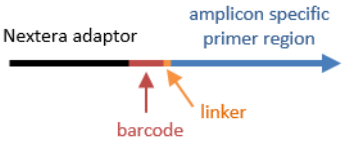
- Mock fungal communities – mixes of genomic DNA from 5 species
- Dilute genomic DNA to haploid genome equivalents & combine
- Each mock has a single species at high abundance and all remaining species are at low abundance (2%)

An *Aspergillus fumigatus* 'high' mix



# Sequencing library preparation

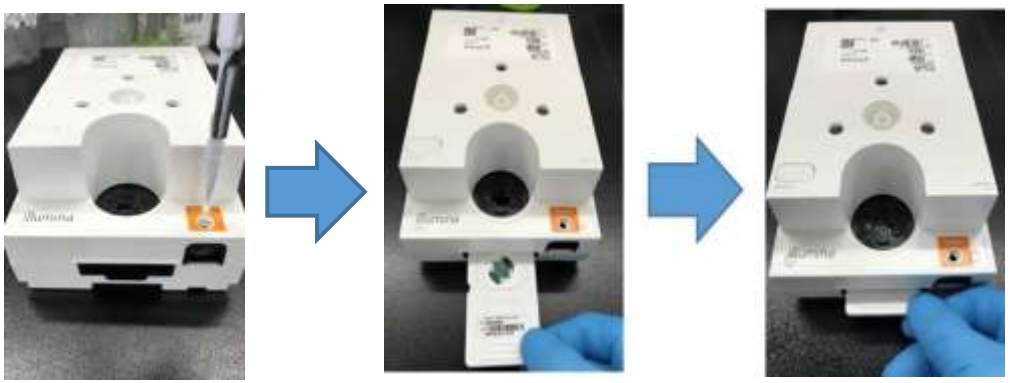
Amplicon PCR - Mock communities used as PCR template



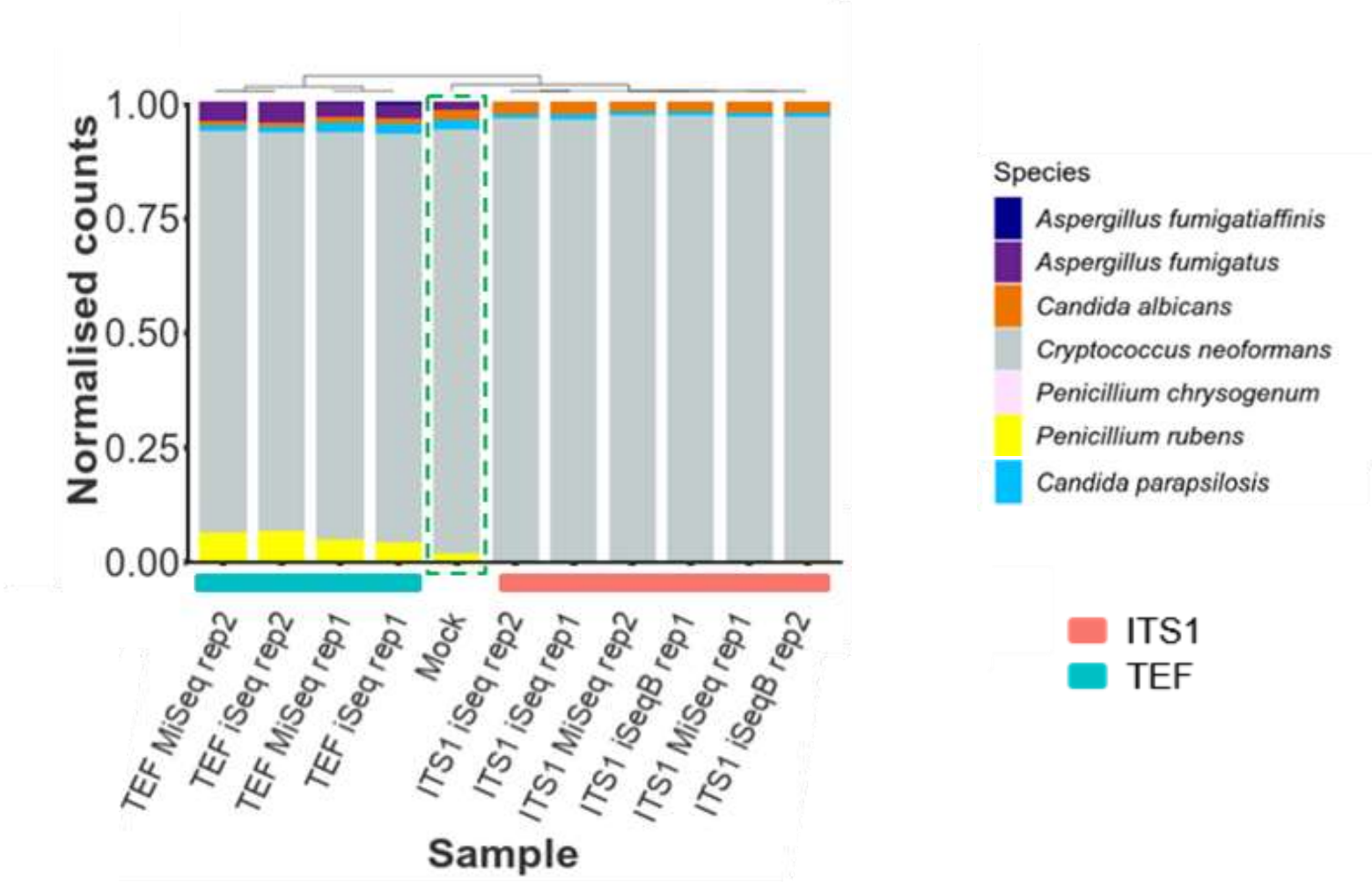

Barcoding allows us to process multiple samples


Index PCR adds sequencing adaptors

Libraries are ready to sequence



# Mock community analysis identifies ITS1 under-representation of filamentous fungi



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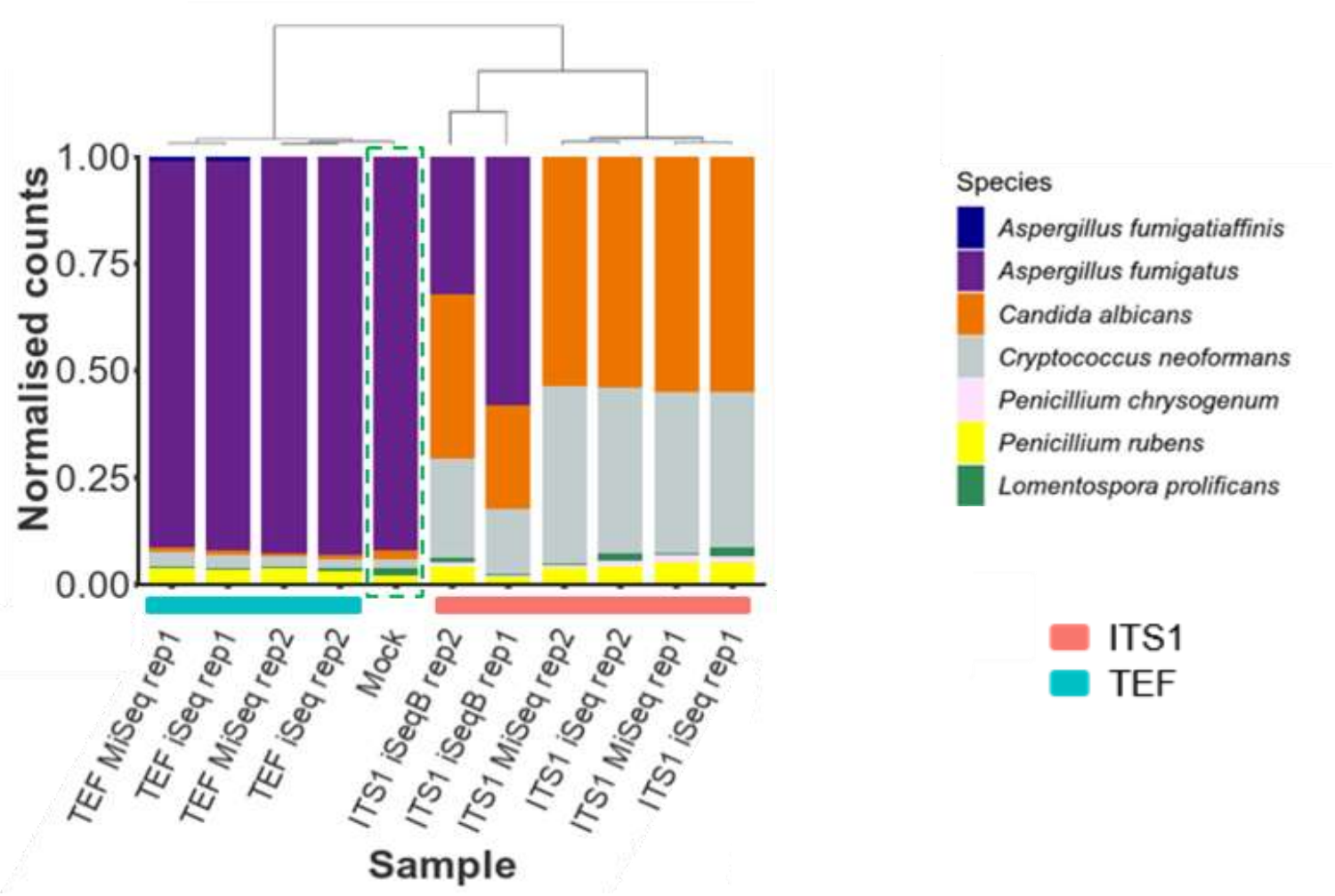
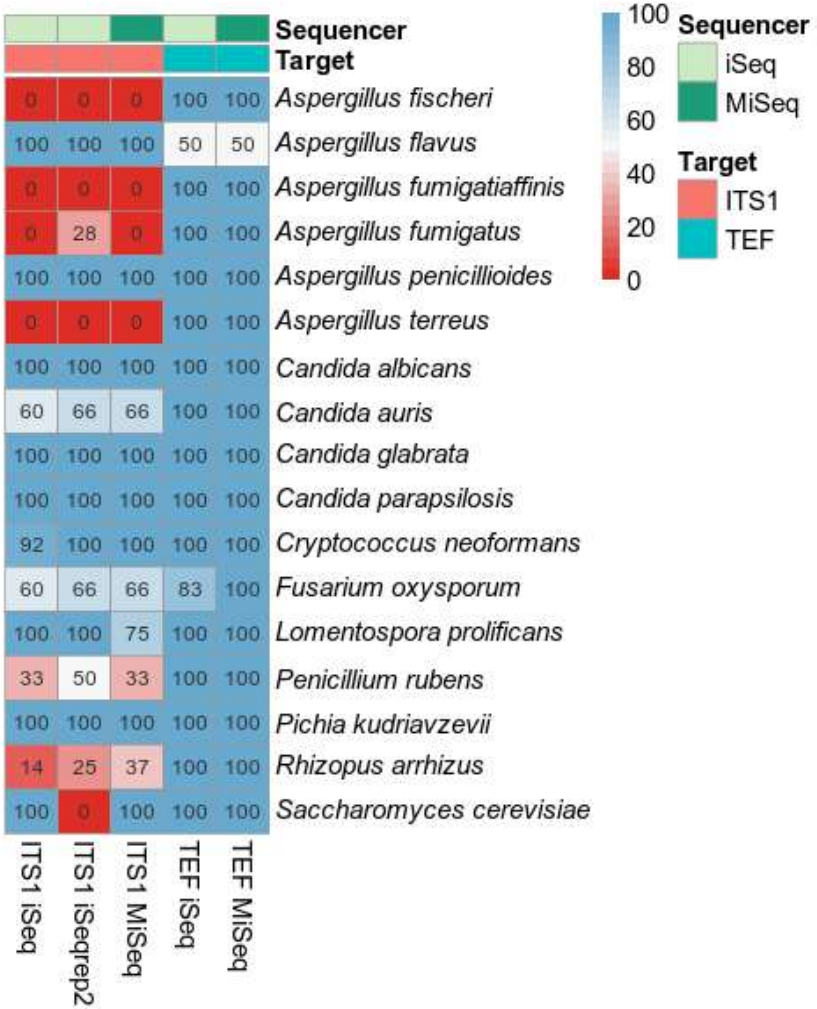
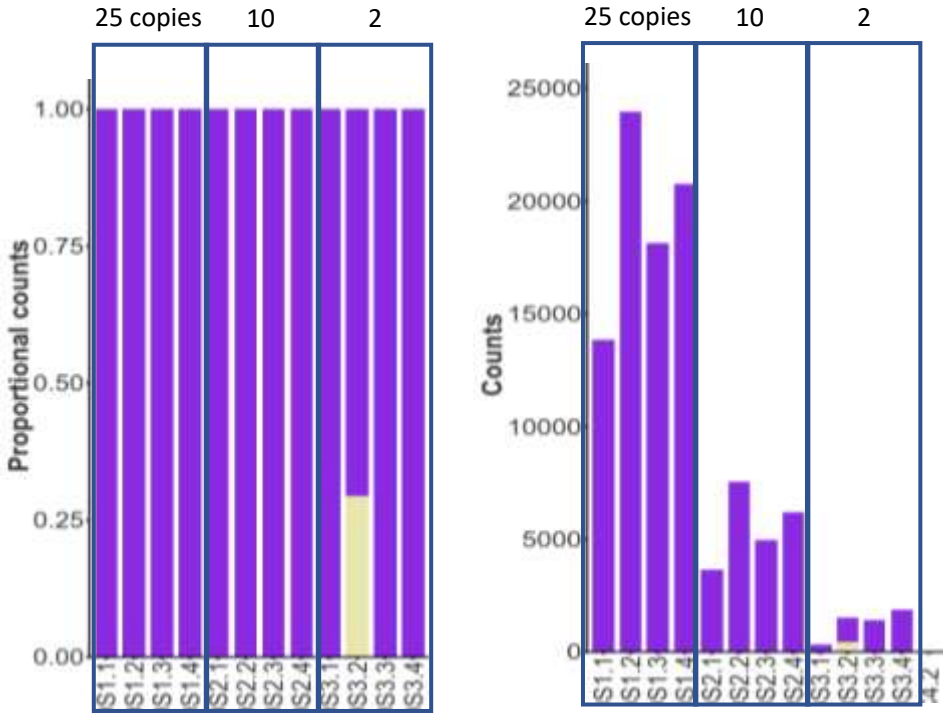


# Issue translates into poor ITS1 species identification rates



# Determining assay limit of detection

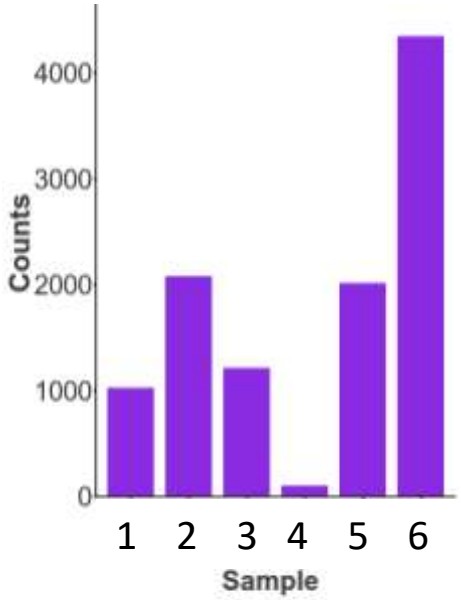



- **Cystic fibrosis sputum sample spiked with *A. fumigatus* DNA**
- **2, 10 or 25 haploid genome equivalents per PCR reaction**

# Assay validation with clinical samples

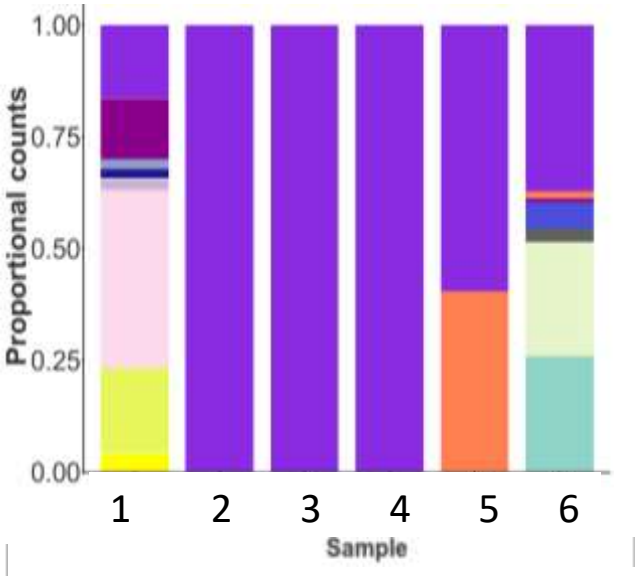
- Patient samples positive by *Aspergillus* qPCR

*A. fumigatus* read counts




Assay can detect *A. fumigatus*

Relative abundance of all Fungi





..and also other clinically relevant species

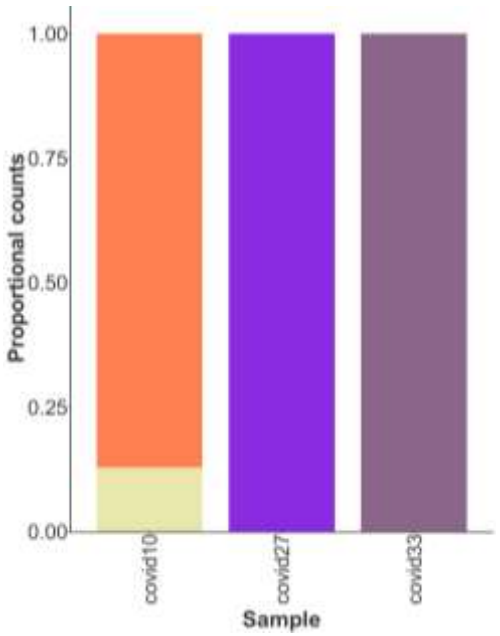
# Searching for fungi in covid patient samples

- Covid-associated pulmonary aspergillosis (CAPA) reported in literature – varying incidence
- 32 covid patient samples
- 4 extraction negative controls
- 6 no template controls

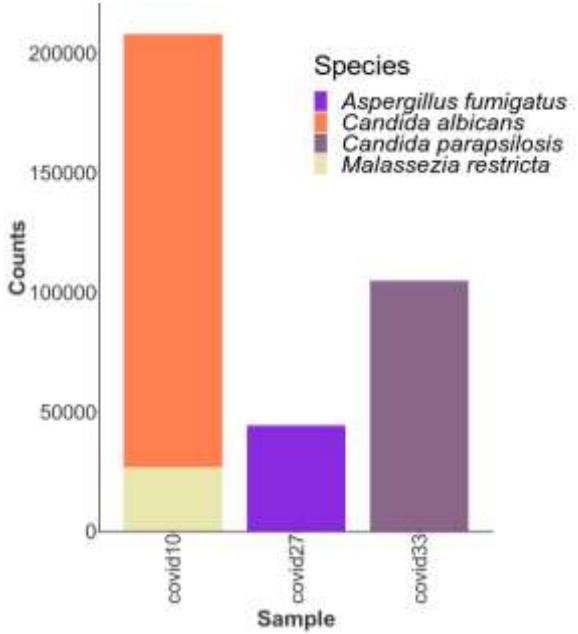
**Output:** 3 samples with significant fungal counts
1) *C. albicans*
*2) A. fumigatus*
*3) C. parapsilosis*

Potential CAPA incidence of ~3%

Relative abundance




Total read counts

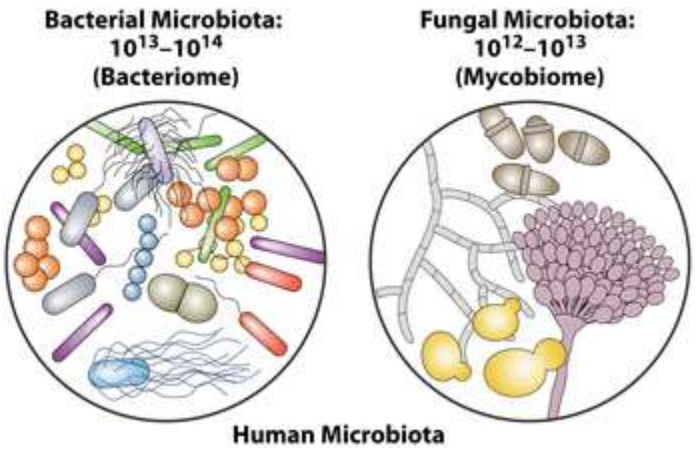


# Summary

- Mycobiome sequencing is a promising tool to provide fungal diagnostics with broad range and speciation.
- The established ITS1 target can significantly under-represent filamentous fungi in mixed communities, leading to poor ID rates
- Novel target can identify filamentous fungi, even when present at 2%, and can ID as few as 2 copies of *A. fumigatus* in a sputum sample.
- Assay validation with clinical samples is ongoing – preliminary data shows it can identify *A. fumigatus* but also reveal additional fungal pathogens - eg. *Scedosporium* – which may inform drug treatments

# Looking to the future

- Combined fungal and bacterial pathogen identification?
- Combined pathogen ID & resistance screening?
- Drug discovery?

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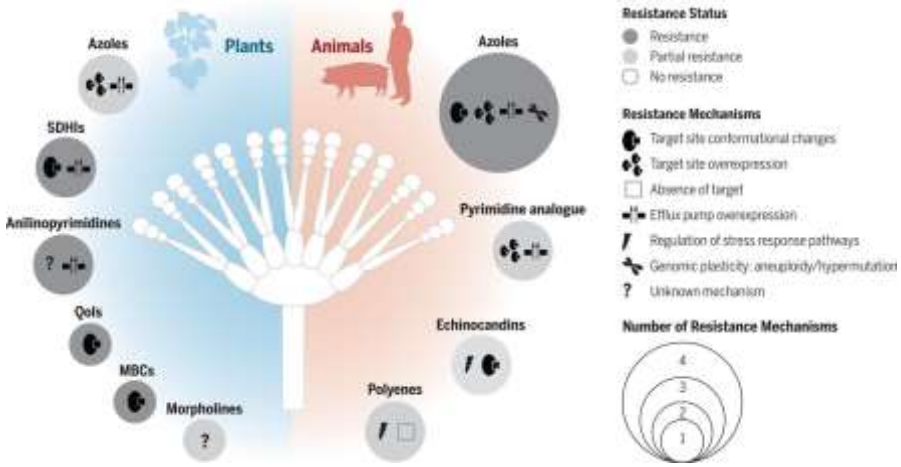


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Thanks for listening!