

Mock community Full taxonomy	Source	PCR	~Amplicon length (bp)	UNITE Classification	RDP Classification	Rel. %
d:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Pleosporaceae,g:Alternaria,s:Alternaria_alternata	Wild type (LabPLUS)	++	350	d:Fungi_Unclassified	c:Dothideomycetes,s:Alternaria_alternata	2.0%
d:Fungi,p:Ascomycota,c:Dothideomycetes,o:Capnodiales,f:Davidiellaceae,g:Cladosporium,s:Cladosporium_sphaeospermum	Wild type (LabPLUS)	++	380	p:Ascomycota_Unclassified	c:Dothideomycetes,s:Cladosporium_halotolerans	1.2%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_brasiliensis	ATCC® 16404™	++	350	p:Ascomycota_Unclassified*	c:Eurotiomycetes,g:Aspergillus*	9% (incl. A. niger)
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_flavus	Wild type (LabPLUS)	+++	380	c:Eurotiomycetes,g:Aspergillus	c:Eurotiomycetes,s:Aspergillus_flavus	3.6%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_fumigatus	Wild type (LabPLUS)	+++	380	c:Eurotiomycetes,s:Aspergillus_fumigatus	c:Eurotiomycetes,s:Aspergillus_fumigatus	5.8%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_niger	ATCC® 9642™	+++	380	p:Ascomycota_Unclassified*	c:Eurotiomycetes,g:Aspergillus*	9% (incl. A. brasiliensis)
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae,g:Exophiala,s:Exophiala_spinifera	ATCC® 14488™	+++	400	c:Eurotiomycetes,s:Exophiala_exophialae	c:Eurotiomycetes,s:Exophiala_spinifera	1.8%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Penicillium,s:Penicillium_chrysogenum	ATCC® 10002™	+++	400	c:Eurotiomycetes,g:Penicillium	c:Eurotiomycetes,g:Eupenicillium	2.3%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Onygenales,f:Anthroderrmaceae,g:Trichophyton,s:Trichophyton_mentagrophytes	ATCC® 9533™	+++	500	c:Eurotiomycetes,s:Trichophyton_mentagrophytes	c:Eurotiomycetes,g:Arthroderma	4.1%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_albicans	ATCC® 10231™	+++	300	c:Saccharomycetes,g:Candida	c:Saccharomycetes,g:Candida	13.8%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_glabrata	ATCC® 90030™	+++	600	c:Saccharomycetes,o:Saccharomycetales_Unclassified	c:Saccharomycetes,s:Candida_glabrata	1.6%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_parapsilosis	ATCC® 22019™	+++	300	c:Saccharomycetes,s:Candida_parapsilosis	c:Saccharomycetes,s:Candida_parapsilosis	8.5%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_tropicalis	ATCC® 13802™	++	350	c:Saccharomycetes,s:Candida_tropicalis	c:Saccharomycetes,s:Candida_tropicalis	0.8%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Yarrowia,s:Yarrowia_lipolytica	ATCC® 9773™	+	250	c:Saccharomycetes,s:Yarrowia_lipolytica	d:Fungi_unclassified	0.0%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Saccharomyces,s:Saccharomyces_cerevisiae	Wild type (UoA)	+++	520	c:Saccharomycetes,g:Saccharomyces	c:Saccharomycetes,s:Saccharomyces_cerevisiae	3.1%
d:Fungi,p:Ascomycota,c:Sordariomycetes,o:Hypocreales,f:Nectriaceae,g:Fusarium,s:Fusarium_solani	Wild type (LabPLUS)	+++	380	d:Fungi_Unclassified	c:Sordariomycetes,f:Nectriaceae_Unclassified	4.8%
d:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,f:Tremellales_fam_Incertae_sedis,g:Cryptococcus,s:Cryptococcus_neofomans	ATCC® 32045™	+++	350	c:Tremellomycetes,s:Cryptococcus_neofomans	c:Tremellomycetes,g:Filobasidiella	4.6%
d:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Trichosporales,f:Trichosporonaceae,g:Trichosporon,s:Trichosporon_dermatis	ATCC® 204094™	+++	300	c:Tremellomycetes,g:Trichosporon	c:Tremellomycetes,s:Trichosporon_dermatis	33.0%
d:Fungi,p:Basidiomycota,c:Exobasidiomycetes,o:Malasseziales,f:Malasseziaceae,g:Malassezia,s:Malassezia_furfur	NZRM 3493	-	-	c:Exobasidiomycetes,s:Malassezia_furfur	c:Exobasidiomycetes,s:Malassezia_furfur	0.0%
d:Fungi,p:Basidiomycota,c:Exobasidiomycetes,o:Malasseziales,f:Malasseziaceae,g:Malassezia,s:Malassezia_globosa	ATCC® MYA4612™	+	450	c:Exobasidiomycetes,g:Malassezia	c:Exobasidiomycetes,s:Malassezia_globosa	< 0.1%
d:Fungi,p:Basidiomycota,c:Exobasidiomycetes,o:Malasseziales,f:Malasseziaceae,g:Malassezia,s:Malassezia_pachydermatis	Wild type (LabPLUS)	-	-	c:Exobasidiomycetes,g:Malassezia	c:Exobasidiomycetes,s:Malassezia_pachydermatis	0.0%

+++ Strong visible band  
 ++ Moderate visible band  
 + Weak visible band  
 - No visible band

Correct species (incl. teleomorph)  
 Correct genus (incl. teleomorph)  
 Correct class, incorrect or unclassified to genus  
 Not classified to class  
 Incorrect class

Over-represented taxa  
 Under-represented taxa

**Image 1. Human mycobiota mock community: ITS1.** Full taxonomy and isolate source for the 21 species that comprised the mock community, together with details of PCR band intensity (visualised via an agarose gel) (**PCR**), approximate amplicon length (bp) including primers and adapters (**Amplicon length (bp)**), taxonomic classifications of sequences based on reference databases (**Reference Classification**), and the relative abundance of sequences for each taxon when sequenced as an equal parts mixed community (**Rel. %**). ATCC = American Type Culture Collection. NZRM = New Zealand Reference Culture Collection. UoA = University of Auckland. \**A. brasiliensis* and *A. niger* shared the same ZOTU for each of ITS1, ITS2, and LSU (i.e. had identical sequences).



Mock_community_Full_taxonomy	Source	PCR	~Amplicon length (bp)	SILVA Classification	Rel. %
d:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pezizales,f:Pezizaceae,g:Alternaria,s:Alternaria_alternata	Wild type (LabPLUS)	+	650	c:Dothideomycetes_Unclassified	
d:Fungi,p:Ascomycota,c:Dothideomycetes,o:Capnodiales,f:Davidiellaceae,g:Cladosporium,s:Cladosporium_sphaeospermum	Wild type (LabPLUS)	+	650	p:Ascomycota_Unclassified	
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_brasiliensis	ATCC® 16404™	+	600	Mixed classification: split across numerous Zotus™	
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_flavus	Wild type (LabPLUS)	+	650	c:Eurotiomycetes,s:Aspergillus_sojae	
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_fumigatus	Wild type (LabPLUS)	+	650	c:Eurotiomycetes_Unclassified	
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_niger	ATCC® 9642™	++	650	c:Eurotiomycetes,g:Aspergillus	
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothiales,f:Herpotrichiellaceae,g:Exophiala,s:Exophiala_spinifera	ATCC® 14488™	+	650	c:Eurotiomycetes_Unclassified	
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Penicillium,s:Penicillium_chrysogenum	ATCC® 10002™	+	650	c:Eurotiomycetes,s:Penicillium_camemberti	
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Onygenales,f:Arthrodermataceae,g:Trichophyton,s:Trichophyton_mentagrophytes	ATCC® 9533™	++	650	c:Eurotiomycetes,g:Trichophyton	
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_albicans	ATCC® 10231™	++	650	c:Saccharomycetes_Unclassified*	
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_glabrata	ATCC® 90030™	+++	650	c:Saccharomycetes_Unclassified	
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_parapsilosis	ATCC® 22019™	+++	650	c:Saccharomycetes_Unclassified*	
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_tropicalis	ATCC® 13802™	+++	650	c:Saccharomycetes_Unclassified	
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Yarrowia,s:Yarrowia_lipolytica	ATCC® 9773™	+++	580	c:Saccharomycetes,s:Yarrowia_lipolytica	
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Yarrowia,s:Yarrowia_lipolytica	ATCC® 9773™	+++	580	c:Saccharomycetes,s:Saccharomyces_cerevisiae	
d:Fungi,p:Ascomycota,c:Sordariomycetes,o:Hypocreales,f:Nectriaceae,g:Fusarium,s:Fusarium_solani	Wild type (LabPLUS)	++	650	d:Eukaryota_Unclassified	
d:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,f:Tremellales_fam_Incertae_sedis,g:Cryptococcus,s:Cryptococcus_neoformans	ATCC® 32045™	+++	650	c:Tremellomycetes,s:Cryptococcus_gattii	
d:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Trichosporonales,f:Trichosporonaceae,g:Trichosporon,s:Trichosporon_dermatis	ATCC® 204094™	+++	600	c:Tremellomycetes,g:Trichosporon	
d:Fungi,p:Basidiomycota,c:Exobasidiomycetes,o:Malasseziales,f:Malasseziaceae,g:Malassezia,s:Malassezia_furfur	NZRM 3493	+	650	c:Exobasidiomycetes_Unclassified	
d:Fungi,p:Basidiomycota,c:Exobasidiomycetes,o:Malasseziales,f:Malasseziaceae,g:Malassezia,s:Malassezia_globosa	ATCC® MYA4612™	++	650	c:Exobasidiomycetes,s:Malassezia_globosa	
d:Fungi,p:Basidiomycota,c:Exobasidiomycetes,o:Malasseziales,f:Malasseziaceae,g:Malassezia,s:Malassezia_pachydermatis	Wild type (LabPLUS)	+	650	c:Exobasidiomycetes,s:Malassezia_pachydermatis	

+++ Strong visible band  
 ++ Moderate visible band  
 + Weak visible band  
 - No visible band

Correct species (incl. teleomorph)  
 Correct genus (incl. teleomorph)  
 Correct class, incorrect or unclassified to genus  
 Not classified to class  
 Incorrect class

**Supplementary material 1C. Human mycobiota mock community: SSU.** Full taxonomy and isolate source for the 21 species that comprised the mock community, together with details of PCR band intensity (visualised via an agarose gel) (**PCR**), approximate amplicon length (bp) including primers and adapters (**Amplicon length (bp)**), taxonomic classifications of sequences based on reference databases (**Reference Classification**), and the relative abundance of sequences for each taxon when sequenced as an equal parts mixed community (**Rel. %**) (not available for SSU data). ATCC = American Type Culture Collection. NZRM = New Zealand Reference Culture Collection. UoA = University of Auckland. \**C. albicans* and *C. parapsilosis* shared the same ZOTU for SSU. \*\**A. brasiliensis* was split across numerous ZOTUs for SSU sequences and had mixed classification.

Mock_community_Full_taxonomy	Source	PCR	~Amplicon length (bp)	SILVA Classification	RDP Classification	Rel. %
d:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Pleosporaceae,g:Alternaria,s:Alternaria_alternata	Wild type (LabPLUS)	++	700	c:Dothideomycetes_Unclassified	c:Dothideomycetes,g:Alternaria	0.7%
d:Fungi,p:Ascomycota,c:Dothideomycetes,o:Capnodiales,f:Davidiellaceae,g:Cladosporium,s:Cladosporium_sphaeospermum	Wild type (LabPLUS)	++	700	c:Dothideomycetes_Unclassified	c:Dothideomycetes,g:Cladosporium_complex	0.8%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_brasiliensis	ATCC® 16404™	+	700	c:Eurotiomycetes,s:Aspergillus_niger*	c:Agaricomycetes,g:Poria*	7.5% (incl. A. niger)
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_flavus	Wild type (LabPLUS)	+++	700	c:Eurotiomycetes,s:Aspergillus_oryzae	c:Eurotiomycetes,f:Trichocomaceae_Unclassified	2.7%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_fumigatus	Wild type (LabPLUS)	+++	700	c:Eurotiomycetes,s:Aspergillus_fumigatus	c:Eurotiomycetes,g:Aspergillus	4.6%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Aspergillus,s:Aspergillus_niger	ATCC® 9642™	+++	700	c:Eurotiomycetes,s:Aspergillus_niger*	c:Agaricomycetes,g:Poria*	7.5% (incl. A. brasiliensis)
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae,g:Exophiala,s:Exophiala_spinifera	ATCC® 14488™	++	700	p:Ascomycota_Unclassified	c:Eurotiomycetes,f:Herpotrichiellaceae	0.6%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Penicillium,s:Penicillium_chrysogenum	ATCC® 10002™	++	750	c:Eurotiomycetes_Unclassified	c:Eurotiomycetes,g:Penicillium	1.9%
d:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Onygenales,f:Arthrodermataceae,g:Trichophyton,s:Trichophyton_mentagrophytes	ATCC® 9533™	+++	700	c:Eurotiomycetes,s:Trichophyton_tonsurans	c:Eurotiomycetes,g:Arthroderma	2.7%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_albicans	ATCC® 10231™	+++	650	c:Saccharomycetes,s:Candida_albicans	c:Saccharomycetes,f:Saccharomycetales_Unclassified	9.3%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_glabrata	ATCC® 90030™	+++	700	c:Saccharomycetes,s:Candida_glabrata	c:Saccharomycetes,g:Nakaseomyces	4.3%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_parapsilosis	ATCC® 22019™	+++	650	c:Saccharomycetes,s:Candida_parapsilosis	c:Saccharomycetes,g:Vanderwaltozyma	4.1%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Candida,s:Candida_tropicalis	ATCC® 13802™	+	700	c:Saccharomycetes_Unclassified	c:Saccharomycetes,g:Lodderomyces	0.5%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Yarrowia,s:Yarrowia_lipolytica	ATCC® 9773™	+++	650	c:Saccharomycetes,s:Yarrowia_lipolytica	c:Saccharomycetes,g:Yarrowia	16.9%
d:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,f:Saccharomycetales_fam_Incertae_sedis,g:Hexapoda,s:Polistes_fuscatus	Wild type (UoA)	+++	650	c:Hexapoda,s:Polistes_fuscatus	c:Saccharomycetes,g:Saccharomyces	10.3%
d:Fungi,p:Ascomycota,c:Sordariomycetes,o:Hypocreales,f:Nectriaceae,g:Fusarium,s:Fusarium_solani	Wild type (LabPLUS)	+++	700	c:Sordariomycetes_Unclassified	c:Sordariomycetes,g:Nectria	3.7%
d:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,f:Tremellales_fam_Incertae_sedis,g:Cryptococcus,s:Cryptococcus_neoformans	ATCC® 32045™	+++	750	c:Tremellomycetes,s:Cryptococcus_gattii	c:Tremellomycetes,g:Cryptococcus	4.7%
d:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Trichosporales,f:Trichosporonaceae,g:Trichosporon,s:Trichosporon_dermatis	ATCC® 204094™	+++	700	c:Tremellomycetes_Unclassified	c:Tremellomycetes,g:Asterotremella	17.9%
d:Fungi,p:Basidiomycota,c:Exobasidiomycetes,o:Malasseziales,f:Malasseziaceae,g:Malassezia,s:Malassezia_furfur	NZRM 3493	++	750	c:Exobasidiomycetes,s:Malassezia	c:Exobasidiomycetes,g:Malassezia	0.6%
d:Fungi,p:Basidiomycota,c:Exobasidiomycetes,o:Malasseziales,f:Malasseziaceae,g:Malassezia,s:Malassezia_globosa	ATCC® MYA4612™	+++	750	c:Exobasidiomycetes,s:Malassezia_globosa	c:Exobasidiomycetes,g:Malassezia	5.3%
d:Fungi,p:Basidiomycota,c:Exobasidiomycetes,o:Malasseziales,f:Malasseziaceae,g:Malassezia,s:Malassezia_pachydermatis	Wild type (LabPLUS)	++	750	c:Exobasidiomycetes,s:Malassezia	c:Exobasidiomycetes,g:Malassezia	1.2%

+++ Strong visible band

++ Moderate visible band

+ Weak visible band

- No visible band

Correct species (incl. teleomorph)

Correct genus (incl. teleomorph)

Correct class, incorrect or unclassified to genus

Not classified to class

Incorrect class

Over-represented taxa

Under-represented taxa

**Supplementary material 1D. Human mycobiota mock community: LSU.** Full taxonomy and isolate source for the 21 species that comprised the mock community, together with details of PCR band intensity (visualised via an agarose gel) (**PCR**), approximate amplicon length (bp) including primers and adapters (**Amplicon length (bp)**), taxonomic classifications of sequences based on reference databases (**Reference Classification**), and the relative abundance of sequences for each taxon when sequenced as an equal parts mixed community (**Rel. %**). ATCC = American Type Culture Collection. NZRM = New Zealand Reference Culture Collection. UoA = University of Auckland. \**A. brasiliensis* and *A. niger* shared the same ZOTU for each of ITS1, ITS2, and LSU (i.e. had identical sequences).