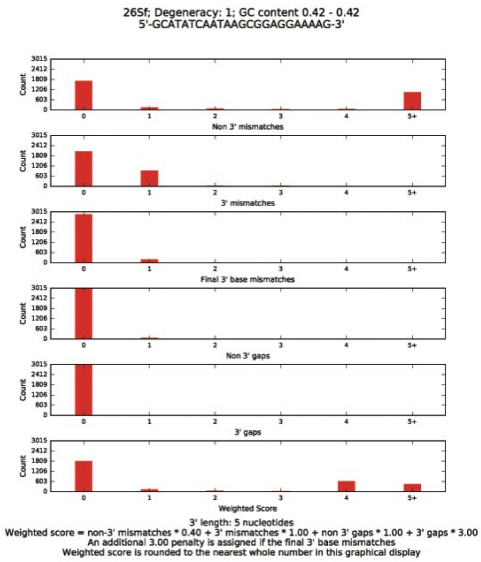
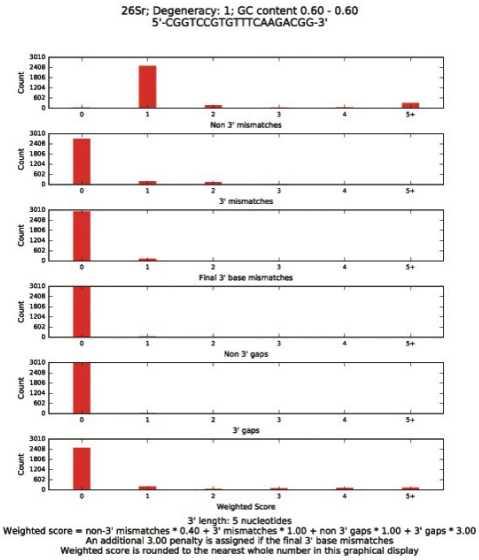
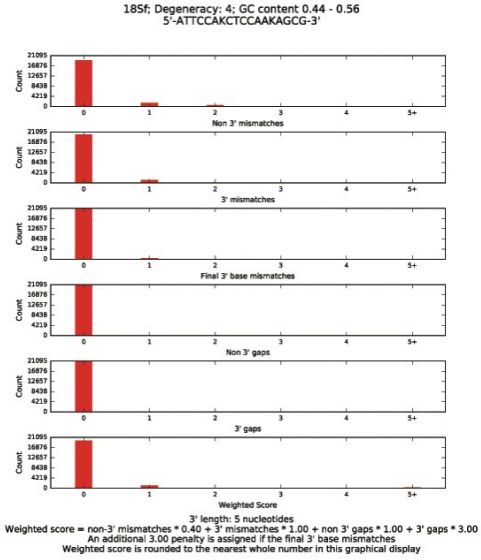
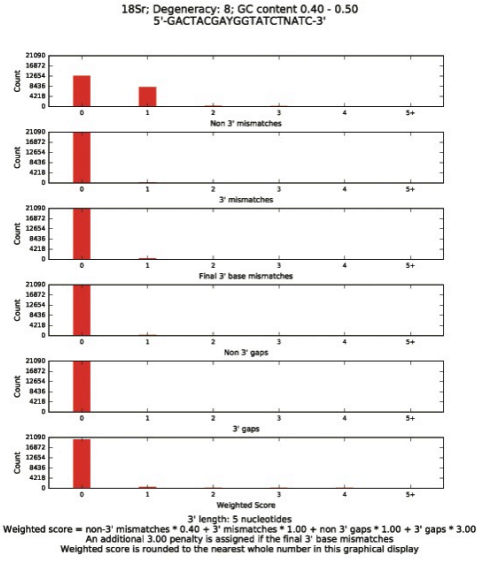
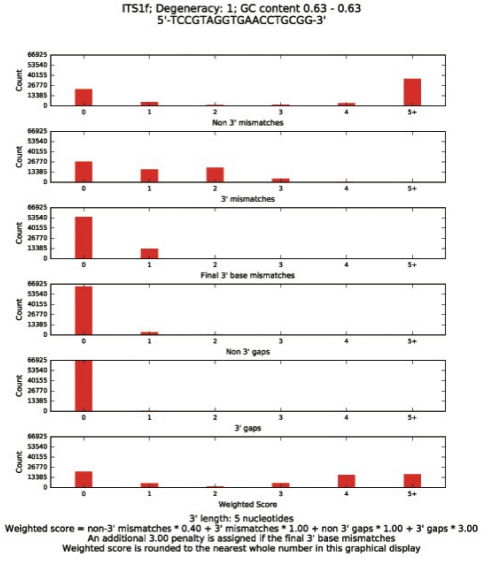
A

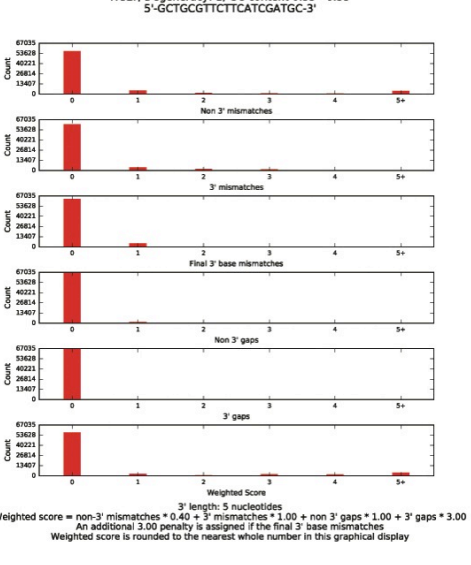

26Sf; Degeneracy: 1; GC content 0.42 - 0.42
5'-GCATATCAATAAGCGGAGGAAAAG-3'
Count
Non 3' mismatches
3' mismatches
Final 3' base mismatches
Non 3' gaps
3' gaps
Weighted Score
3' length: 5 nucleotides
Weighted score = non-3' mismatches * 0.40 + 3' mismatches * 1.00 + non 3' gaps * 1.00 + 3' gaps * 3.00
An additional 3.00 penalty is assigned if the final 3' base mismatches
Weighted score is rounded to the nearest whole number in this graphical display


26Sr; Degeneracy: 1; GC content 0.60 - 0.60
5'-CGGTCCGTGTTTCAAGACGG-3'
Count
Non 3' mismatches
3' mismatches
Final 3' base mismatches
Non 3' gaps
3' gaps
Weighted Score
3' length: 5 nucleotides
Weighted score = non-3' mismatches * 0.40 + 3' mismatches * 1.00 + non 3' gaps * 1.00 + 3' gaps * 3.00
An additional 3.00 penalty is assigned if the final 3' base mismatches
Weighted score is rounded to the nearest whole number in this graphical display


18Sf; Degeneracy: 4; GC content 0.44 - 0.56
5'-ATTCCAKCTCCAAKAGCG-3'
Count
Non 3' mismatches
3' mismatches
Final 3' base mismatches
Non 3' gaps
3' gaps
Weighted Score
3' length: 5 nucleotides
Weighted score = non-3' mismatches * 0.40 + 3' mismatches * 1.00 + non 3' gaps * 1.00 + 3' gaps * 3.00
An additional 3.00 penalty is assigned if the final 3' base mismatches
Weighted score is rounded to the nearest whole number in this graphical display


18Sr; Degeneracy: 8; GC content 0.40 - 0.50
5'-GACTACGAYGGTATCTNATC-3'
Count
Non 3' mismatches
3' mismatches
Final 3' base mismatches
Non 3' gaps
3' gaps
Weighted Score
3' length: 5 nucleotides
Weighted score = non-3' mismatches * 0.40 + 3' mismatches * 1.00 + non 3' gaps * 1.00 + 3' gaps * 3.00
An additional 3.00 penalty is assigned if the final 3' base mismatches
Weighted score is rounded to the nearest whole number in this graphical display


ITS1f; Degeneracy: 1; GC content 0.63 - 0.63
5'-TCCGTAGGTGAACCTGCGG-3'
Count
Non 3' mismatches
3' mismatches
Final 3' base mismatches
Non 3' gaps
3' gaps
Weighted Score
3' length: 5 nucleotides
Weighted score = non-3' mismatches * 0.40 + 3' mismatches * 1.00 + non 3' gaps * 1.00 + 3' gaps * 3.00
An additional 3.00 penalty is assigned if the final 3' base mismatches
Weighted score is rounded to the nearest whole number in this graphical display


ITS2r; Degeneracy: 1; GC content 0.55 - 0.55
5'-GCTGCGTTCTTCATCGATGC-3'
Count
Non 3' mismatches
3' mismatches
Final 3' base mismatches
Non 3' gaps
3' gaps
Weighted Score
3' length: 5 nucleotides
Weighted score = non-3' mismatches * 0.40 + 3' mismatches * 1.00 + non 3' gaps * 1.00 + 3' gaps * 3.00
An additional 3.00 penalty is assigned if the final 3' base mismatches
Weighted score is rounded to the nearest whole number in this graphical display

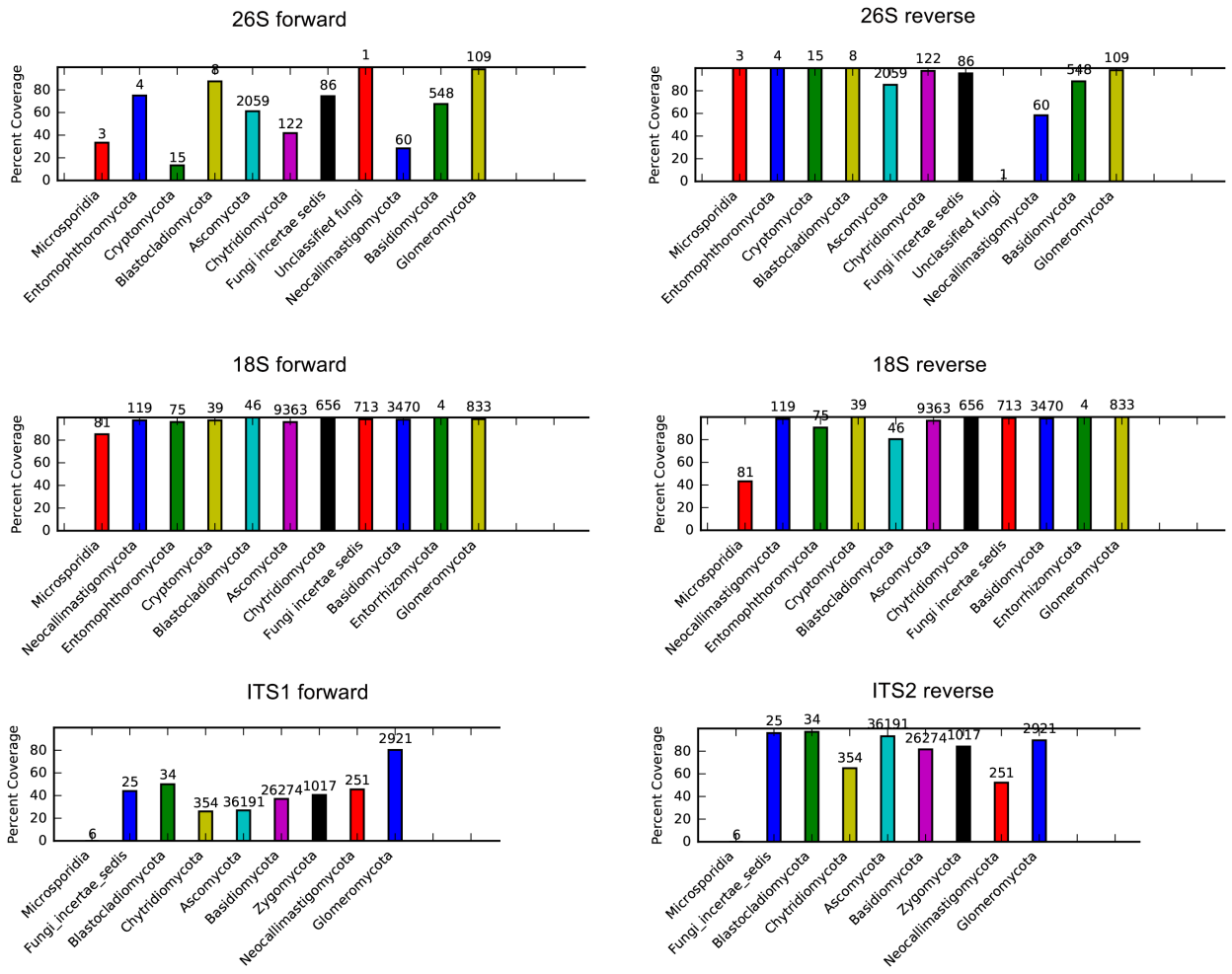


Figure S1. *In silico* primer evaluation results. Panel A shows for each primer the number of mismatches against the database sequences. Panel B shows the proportion of taxa within a given fungal clade that are predicted to be amplified using the specified primer.

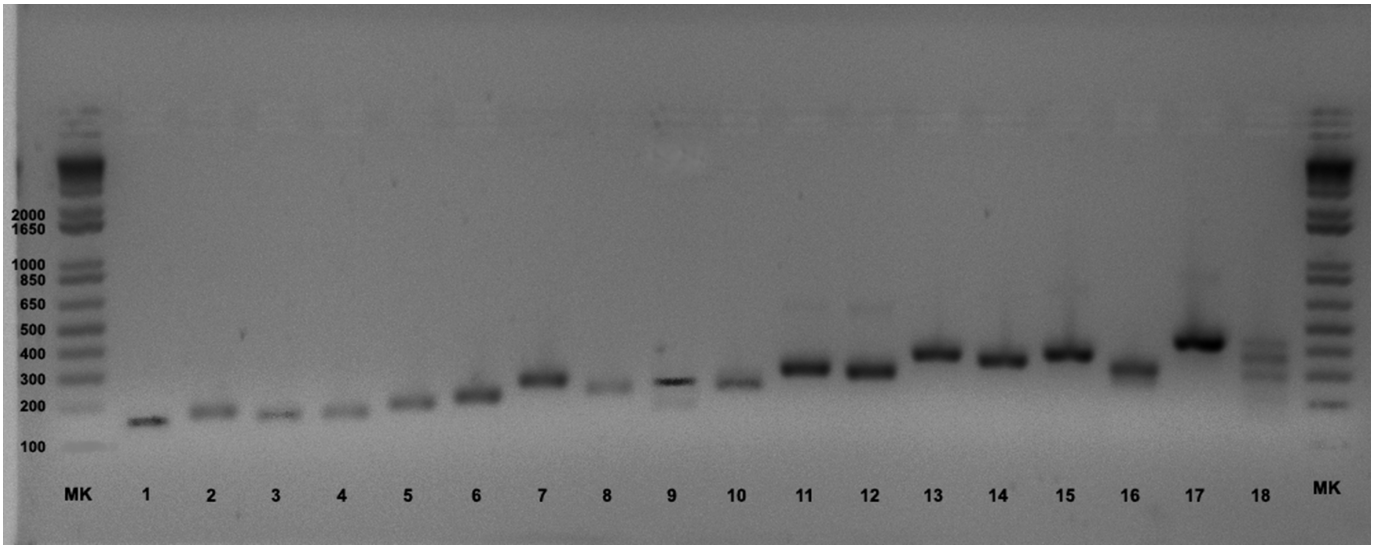


Figure S2. Representative electrophoresis run (1.5% agarose gel) of ITS1-2 amplicons obtained from some of the strains used in the mock community with the primer used in this study (Table S2). MK: 1 Kb Plus DNA Ladder (Invitrogen). 1, *Metschnikowia pulcherrima*; 2, *Issatchenkia orientalis;* 3, *Dekkera bruxellensis*; 4*, Dekkera custersianus*; 5, *Candida zemplinina*; 6, *Candida albicans*; 7, *Candida zeylanoides*; 8, *Botrytis cinerea; 9*, *Sclerotinia sclerotiorum;* 10, *Penicillium brevicompactum*; 11, *Lachancea thermotolerans*; 12, *Thricoderma virens*; 13, *Hanseniaspora guillermondii*; 14, *Zygosaccharomyces bailii*; 15, *Torulaspora delbrueckii*; 16, *Rhizoctonia solani*; 17, *Saccharomyces cerevisiae*; 18, ITS Mock-DNA.

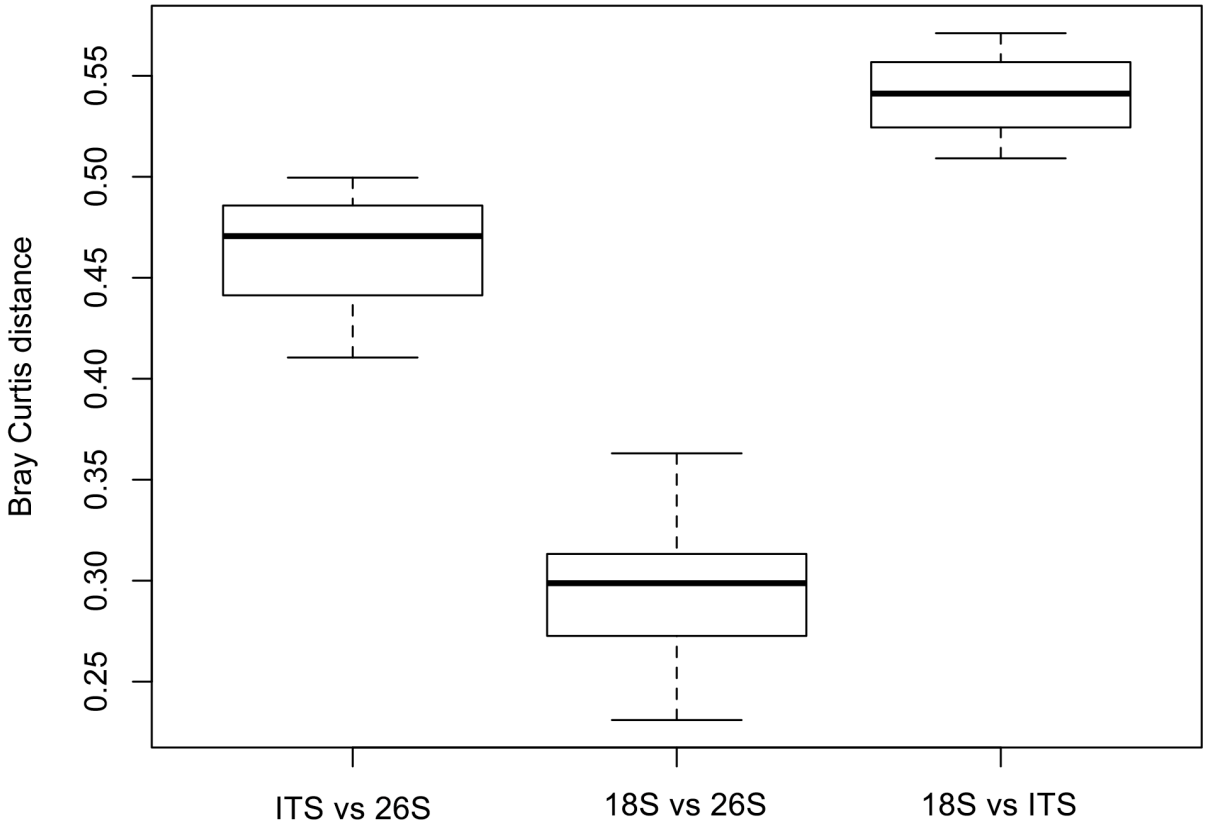


Figure S3. Box plots showing the Bray Curtis distance between mock community samples amplified with different primer sets. Only Mock-DNA samples are shown.