Table S1. Taxonomic composition of the fungal ITS and LSU gene training set used for LOOCV comparisons of the ITS and LSU regions. Sequences were modified from Schoch *et al*. (2012) and modified as noted in the Materials & Methods section.

Phylum	Subphylum	Class	Order	No. of Families	No. of Genera	No. of sequences
Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	1	1	3
			Dothideales	2	2	2
			Myriangiales	1	2	15
			Pleosporales	3	5	31
		Eurotiomycetes	Chaetothyriales	1	1	2
			Eurotiales	2	4	103
			Onygenales	1	1	11
		Lecanoromycetes	Lecanorales	3	16	93
			Pertusariales	2	2	4
		Leotiomycetes	Helotiales	3	6	33
		Pezizomycetes	Pezizales	2	2	21
		Sordariomycetes	Coniochaetales	1	1	1
			Diaporthales	1	2	15
			Glomerellales	1	2	52
			Hypocreales	7	17	163
			Magnaporthales	1	2	23
			Microascales	2	2	12
			Ophiostomatales	1	2	28
			Sordariales	2	2	26
			Sordariomycetes incertae sedis	1	1	14
			Xylariales	1	1	6

	Saccharomycotina	Saccharomycetes	Saccharomycetales	7	14	79
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	6	6	60
			Atheliales	1	1	7
			Geastrales	1	1	4
		Tremellomycetes	Tremellales	3	3	88
	Basidiomycota incertae sedis	Wallemiomycetes	Wallemiales	1	1	1
	Pucciniomycotina	Microbotryomycetes	Agaricostilbales	1	1	1
			Microbotryales	1	1	29
			Sporidiobolales	2	2	11
	Ustilaginomycotina	Ustilaginomycetes	Ustilaginales	1	1	6
Blastocladiomycota	Blastocladiomycota incertae sedis	Blastocladiomycetes	Blastocladiales	1	2	19
Chytridiomycota	Chytridiomycota incertae sedis	Chytridiomycetes	Spizellomycetales	1	2	14
Fungi (Early diverging fungal lineages)	Entomophthoromycotina	Entomophthoromycotina incertae sedis	Entomophthorales	1	1	3
	Kickxellomycotina	Kickxellomycotina incertae sedis	Harpellales	1	1	15
			Kickxellales	1	3	7
	Mortierellomycotina	Mortierellomycotina incertae sedis	Mortierellales	1	2	26
	Mucoromycotina	Mucoromycotina incertae sedis	Mucorales	8	13	50
	Zoopagomycotina	Zoopagomycotina incertae sedis	Zoopagales	1	1	1
Neocallimastigomycota	Neocallimastigomycota incertae sedis	Neocallimastigomycetes	Neocallimastigales	1	3	12
6	14	20	40	79	133	1091

Table S2. BLAST and NBC comparison for ITS and LSU using the Matthews correlation coefficient. Four metrics were defined to decompose classification accuracies acquired from each taxonomic assignment classification tool (NBC and BLASTN). For a set of query read fragments, we counted the assignment combinations that could be considered consistent positives (CPs), consistent negatives (CNs), divergent positives (DPs), and divergent negatives (DNs). A set of assignment was considered a consistent positive if the query read fragment was assigned to the same and correct class by each classification tool. A consistent negative was a set of queries those were assigned to the same but incorrect class. A divergent positive denotes that at least one classification tool's assignment was correct. A divergent negative was a query read fragment was assigned to different classes by each classification tool and none of them were correctly assigned. Naïve Bayesian classifier results are above the diagonal line. BLASTN results are below the diagonal line and BLASTN vs naïve Bayesian classifier result at the diagonal. MCC denotes Matthew's correlation coefficient.

Full length

	ITS1					ITS2					LSU				
	CN	CP	DN	DP	M C C	CN	CP	DN	DP	M C C	CN	CP	DN	DP	M C C
ITS1	3.3%	87.2%	4.3%	5.3%	36%	3.2%	89.4%	2.0%	5.5%	44%	2.3%	88.6%	0.8%	8.4%	37%
ITS2	3.4%	86.7%	1.5%	8.4%	40%	2.8%	90.3%	1.1%	5.8%	46%	2.8%	91.3%	0.6%	5.4%	51%
LSU	2.9%	86.5%	1.2%	9.5%	37%	4.2%	91.7%	0.8%	3.5%	66%	2.7%	91.2%	0.3%	6.0%	51%

50-200bp length

	ITS1					ITS2					LSU				
	CN	CP	DN	DP	MCC	CN	CP	DN	DP	MCC	CN	CP	DN	DP	MCC
ITS1	3.2%	89.2%	1.8%	5.8%	44.3%	3.4%	88.2%	1.8%	6.6%	43.5%	2.3%	86.3%	1.7%	9.7%	28.4%
ITS2	2.8%	90.1%	1.1%	6.0%	44.2%	3.5%	89.2%	0.6%	6.7%	51.5%	3.8%	88.5%	0.9%	6.7%	50.5%
LSU	2.2%	89.6%	1.5%	6.7%	34.4%	4.3%	91.0%	1.3%	3.4%	62.7%	2.8%	87.9%	1.0%	8.2%	40.0%

100-200bp length

	ITS1					ITS2					LSU				
	CN	CP	DN	DP	MCC	CN	CP	DN	DP	MCC	CN	CP	DN	DP	MCC
ITS1	2.7%	87.1%	1.2%	9.0%	36.2%	3.6%	88.0%	1.7%	6.6%	45.2%	2.4%	86.4%	1.7%	9.5%	29.6%
ITS2	2.8%	89.9%	1.3%	6.0%	43.1%	3.6%	88.9%	0.7%	6.8%	51.4%	3.8%	88.2%	0.9%	7.1%	50.3%
LSU	2.2%	89.5%	1.6%	6.6%	34.3%	4.3%	90.9%	1.4%	3.4%	62.5%	2.9%	87.8%	0.9%	8.4%	40.8%

150-200bp length

	ITS1					ITS2					LSU				
	CN	CP	DN	DP	MCC	CN	CP	DN	DP	MCC	CN	CP	DN	DP	MCC
ITS1	3.5%	89.3%	1.5%	5.6%	48.4%	4.1%	88.8%	1.3%	5.8%	52.4%	2.9%	86.7%	1.3%	9.1%	36.3%
ITS2	3.1%	89.0%	1.4%	6.5%	43.4%	3.8%	88.0%	0.5%	7.6%	51.3%	4.3%	87.8%	0.7%	7.3%	53.6%
LSU	2.7%	88.5%	1.5%	7.3%	38.2%	5.5%	89.8%	1.1%	3.6%	68.5%	3.5%	86.8%	0.9%	8.8%	44.2%

Table S3. Comparison of ITS1, ITS2 and entire ITS classification accuracy using the ITS-LSU (barcode) and large ITS training sets

	ITS1				ITS2				Entire ITS			
	NBC		BLASTN		NBC		BLASTN		NBC		BLASTN	
	Barcode	Large	Barcode	Large	Barcode	Large	Barcode	Large	Barcode	Large	Barcode	Large
Phylum	97.8%	99.1%	93.0%	98.3%	99.3%	99.4%	99.2%	99.3%	99.5%	99.5%	99.6%	99.4%
Class	97.1%	97.3%	93.0%	97.2%	98.5%	98.2%	98.6%	97.9%	98.7%	98.2%	99.0%	98.2%
Order	96.5%	94.2%	93.0%	94.9%	98.3%	95.4%	98.6%	95.7%	98.4%	95.8%	99.1%	95.9%
Family	95.5%	90.3%	92.6%	91.7%	97.7%	91.4%	98.1%	92.4%	97.7%	92.2%	98.4%	93.0%
Genus	90.8%	80.2%	88.7%	84.2%	93.2%	81.7%	93.1%	84.6%	93.9%	83.1%	94.8%	85.5%

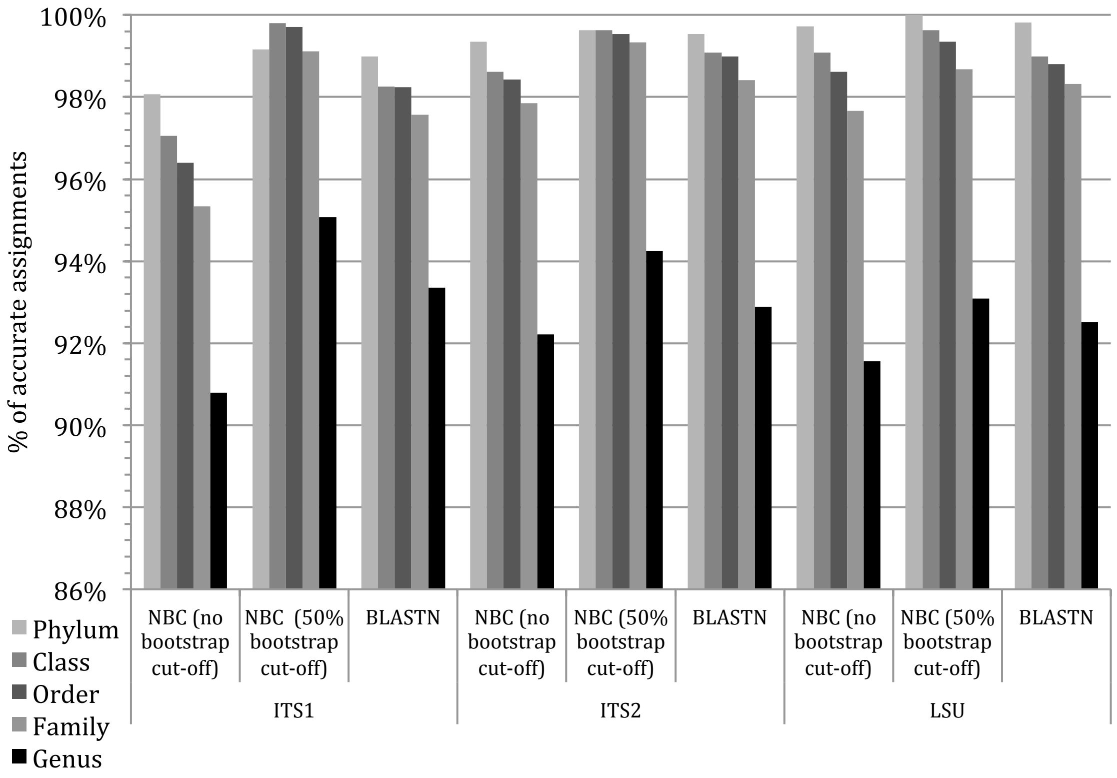

Figure S1. Accuracy comparison among ITS1, ITS2 and LSU regions using the naïve Bayesian classifier and BLASTN at its default setting and 50% bootstrap cutoff. Y axis shows percentages of LOOCV sequences accurately classified. Sequence length shown here is between 50 and 200bp (1054 non-singleton sequences out of 1089 sequences longer than 50bp). Fragments were extracted using the primer anchored method.