Rps10 database sequence PCR protocol

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The purpose of this protocol is to amplify the oomycete rps10 locus for use in the rps10 barcode database. If you want to use the Rps10 locus for amplicon metagenomics, use the metabarcoding protocol instead. The rps10 database amplicon is longer than the rps10 locus-specific amplicon and conveniently includes the rps10 locus-specific primer sequences. The amplicon produced is usually around 554bp long.

Ordering rps10 database primers

Both the rps10 forward and reverse database primer binding sites have two SNPs represented with IUPAC codes (Y=C,T and R=A, G). We ordered the Rps10 database oligos from Life technologies.

Direction	Primer name	Primer sequence (5' -> 3')
forward	Prv9r-M	GTTGGTTAGAGYARAAGACT
reverse	Prv9f-M	RTAYACTCTAACCAACTGAGT

Rps10 database PCR mixture

Reagent	Volume	Final conc.
dH2O, Sterile	16.9µL	NA
10X Taq Buffer w/Mg2+[1]	2.5µL	1x; 1.5mM Mg2+
MgCl2 (25mM)[2]	1.5µL	1.5 mM
dNTP (10mM)[3]	0.5µL	0.2 µM
Prv9r-M (FOR) (10mM)	1.3µL	0.5 µM
Prv9f-M (REV) (10mM)	1.3µL	0.5 µM
GenScript Taq (5u/µl)[1]	0.125µL	0.025 U/µl
DNA Template (2ng)	1.0µL	0.08ng/µL
TOTAL	25.0µL	NA

[1] GenScript Taq and 10x buffer from GenScript (Cat. No. E00007)
[2] 25mM MgCl2 from USB (Cat. No 71167)
[3] 100mM dNTP from Invitrogen (Cat. No 10297-018)

Rps10 database thermocycler conditions

Step	Temperature	Time
Initial activation	94 °C	3 min
3 Step cycling (35 cycles)		
Denaturation	94 °C	30 s
Annealing	55 °C	45 s
Extension	72 °C	45 s
Final Extension	72 °C	7 min
Hold	10 °C	∞