Rps10 metabarcoding primer ordering and mixing protocol

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The propose of the rps10 locus-specific protocol is to amplify the oomycete rps10 locus for metagenomic research.

Ordering rps10 locus-specific primers

Rps10 oligos were designed by Frank Martin and Val Fieland and are listed here in the 5' -> 3' orientation Both the rps10 forward and reverse primer binding sites have SNPs. To reduce the amount of primer degeneracy in the rps10 locus-specific PCR reaction it is advisable to order multiple specific oligo sequences for the reverse primer sequences rather than ordering oligos with IUPAC ambiguity codes. Using the IUPAC code Y (C,T) for the rps10_F_Conserved oligo is optional but simplifies the primer mixture step.

Rps10 locus-specific forward primers

- before space = Forward 5' Illumina overhang adapter sequence
- after space = Rps10 locus-specific forward primer

Primer name	Forward Primer sequence
rps10_F_Conserved	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTTGGTTAGAGYAAAAGACT
rps10_F2_Conserved	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTTGGTTAGAGTAGAAGACT

Rps10 locus-specific reverse primers

- before space = Reverse 5' Illumina overhang adapter sequence)
- after space = Rps10 locus-specific reverse primer

Primer name	Forward Primer sequence
rps10_R1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCTTAGAAAGATTTGAACT
rps10_R2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATACTTAGAAAGATTTGAACT
rps10_R3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCTTAGAAAGACTTGAACT
rps10_R4	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCTTAGAAAGACTCGAACT
rps10_R5	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCCTAGAAAGACTCGAACT
rps10_R6	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGTTTAGAAAGATTCGAACT
rps10_R7	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCTTAGAAAGATTCGAACT

Rps10 locus-specific primer mixture

Reagent	Volume	Final conc.
rps10_F_Conserved (100μM)	8.0μL	4.0μM
rps10_F2_Conserved (100μM)	4.0μL	2.0μM
rps10_R1 (100μM)	4.0μL	2.0μM
rps10_R2 (100μM)	4.0μL	2.0μM
rps10_R3 (100μM)	4.0μL	2.0μM
rps10_R4 (100μM)	4.0μL	2.0μM
rps10_R5 (100μM)	4.0μL	2.0μM
rps10_R6 (100μM)	4.0μL	2.0μM
rps10_R7 (100μM)	4.0μL	2.0μM
TE buffer	160.0μL	NA
TOTAL	200.0μL	NA

Notes:

- We ordered the Rps10 locus-specific oligos from Life technologies, but other sources will probably work just as well.
- The TE Buffer (Tris-EDTA) is a 1X Solution, pH 8.0, Molecular Biology Grade (Cat. No. BP2473100)
- The two primers represented by rps10_F_Conserved each have a final concentration of 2.0μM