Rps10 metabarcoding primer ordering and mixing protocol

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This protocol is used to prepare PCR primers for the rps10 locus for metabarcoding.

Ordering rps10 locus-specific primers

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

Primer name	Forward Primer sequence (5' -> 3')
rps10_F_Conserved	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG[1] GTTGGTTAGAGYAAAAGACT[2]
rps10_F2_Conserved	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTTGGTTAGAGTAGAAGACT

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

Rps10 locus-specific reverse primers

Primer name	Reverse Primer sequence (5' -> 3')
rps10_R1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG[1]ATGCTTAGAAAGATTTGAACT[2]
rps10_R2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATACTTAGAAAGATTTGAACT
rps10_R3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCTTAGAAAGACTTGAACT
rps10_R4	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCTTAGAAAGACTCGAACT
rps10_R5	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCCTAGAAAGACTCGAACT
rps10_R6	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGTTTAGAAAGATTCGAACT
rps10_R7	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCTTAGAAAGATTCGAACT

[1] before space = Reverse 5' Illumina overhang adapter sequence
[2] after space = Rps10 locus-specific reverse primer

Rps10 locus-specific primer mixture

Reagent	Volume	Final conc.
rps10_F_Conserved (100µM)	8.0µL	4.0µM[2]
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[3]	160.0µL	NA
TOTAL	200.0µL	NA

[1] We ordered the Rps10 locus-specific oligos from Life technologies
[2] The two primers represented by rps10_F_Conserved each have a final concentration of 2.0µM
[3] The TE Buffer (Tris-EDTA) is a 1X Solution, pH 8.0, Molecular Biology Grade (Cat. No. BP2473100)