Rps10 Locus-specific Illumina Amplicon PCR Protocol

Updated October 2018

The propose of the rps10 locus-specific protocol is to amplify the oomycete rps10 locus for metagenomic research. This produces an amplicon around about 441bp. We have tested this protocol with DNA from the DNeasy PowerSoil Kit from Qiagen (Cat No. 12888) and the DNeasy Plant mini kit from Qiagen (Cat No. 69106), but other sources of DNA will probably works as well.

Rps10 locus-specific PCR mixture

Reagent	**Volume**	**Final conc.**
dH2O, Sterile	7.0µL	NA
2x Qiagen Type-IT PCR Mix[1]	17.5µL	1x
Rps10 Primer Mix[2]	3.5µL	0.2-0.4 µM
DNA Template (2ng)	7.0µL	0.4ng/µL
TOTAL[3]	35.0µL	NA

[1] Type-it Microsatellite PCR Kit from Qiagen (Cat No. 206243)
[2] Refer to the "Rps10 metabarcoding primer ordering and mixing protocol" for primer information.
[3] Total volume based on 30µL of amplicon, 4µL to run on a gel, 1ul to account for error

Rps10 locus-specific thermocycler conditions

Step	**Temperature**	**Time**
Initial activation	95 °C	5 min
3 Step cycling (35 cycles)		
Denaturation	95 °C	30 s
Annealing	58 °C	3 min
Extension	72 °C	30 s
Final Extension	60 °C	30 min
Hold	10 °C	∞