

Rps10 Locus-specific Illumina Amplicon PCR Protocol

Updated October 2018

The purpose 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).

Rps10 locus-specific PCR mixture

| Reagent | Volume | Final conc. |
|--|--------------|-----------------|
| dH2O, Sterile | 7.0 μ L | NA |
| 2x Qiagen Type-IT PCR Mix ¹ | 17.5 μ L | 1x |
| Rps10 Primer Mix ² | 3.5 μ L | 0.2-0.4 μ M |
| DNA Template (2ng) | 7.0 μ L | 0.4ng/ μ L |
| TOTAL ³ | 35.0 μ L | 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 30 μ L of amplicon, 4 μ L 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 | ∞ |