

# 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.

## Rps10 locus-specific PCR mixture

Reagent	Volume	Final conc.
dH <sub>2</sub> O, Sterile	7.0µL	NA
2x Qiagen Type-IT PCR Mix	17.5µL	1x
Rps10 Primer Mix	3.5µL	0.2-0.4 µM
DNA Template (2ng)	7.0µL	0.4ng/µL
TOTAL	35.0µL	NA

Notes:

- Type-it Microsatellite PCR Kit from Qiagen (Cat No. 206243)
- DNeasy PowerSoil Kit from Qiagen (Cat No. 12888)
- DNeasy Plant mini kit from Qiagen (Cat No. 69106)
- Total volume based on 30µL of amplicon, 4µL to run on a gel, 1ul to account for error
- Rps10 locus-specific amplicon size ≈ 441bp

## 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	∞