

V4 16s – 506f-Y/806rB

Annealing Temperature in Degrees C

L 50.2 52.0 54.1 56.5 59.4 62.3 65.2 68.5 72.4

1000 bp
500 bp
250 bp

No Difference?

Test Sample = 29

ITS2 – ITS3NGS_mix/ITS4NGS

Annealing Temperature in Degrees C

L 50.2 52.0 54.1 56.5 59.4 62.3 65.2 68.5 72.4

1000 bp
500 bp
250 bp

Clear choice?

Test Sample = 29

Gradient PCR using degenerate bacterial/archaeal primers (V4 region 16 – 506f-Y/806rB) and fungal primers (ITS2 region – ITS3NGS_mix/ITS4NGS). Primers contain Nextera Adapters.

PCR Recipe:

For one 10 ul reaction mixture:

Ingredient	Stock Conc.	ul per sample	Final Conc.
Invitrogen Platinum SuperFi Master Mix	2 X	5	1 X
515f-Y (or ITS3NGS_mix)	10 uM	0.5	0.500 uM
806rB (or ITS4NGS)	10 uM	0.5	0.500 uM
Template (FxxB2)	10 ng/uL	1	1 ng/uL
Sterile PCR H ₂ O		3	

Total rxn volume = 10

Thermocycler Program

Cyc1 (1X) 98°C for 2 min

Cyc2 (35X) 98 °C for 10 s

Gradient (50-72 °C) for 10 s

72 °C for 15 s

Cyc3 (1X) 72 °C for 5 min

Cyc4 4 °C HOLD