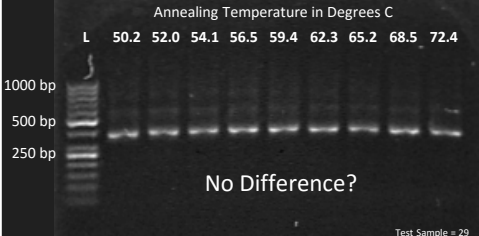
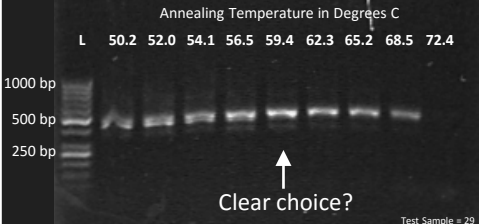


Invitrogen SuperFi Gradient – First Attempt

V4 16s – 506f-Y/806rB



ITS2 – ITS3NGS_mix/ITS4NGS



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	

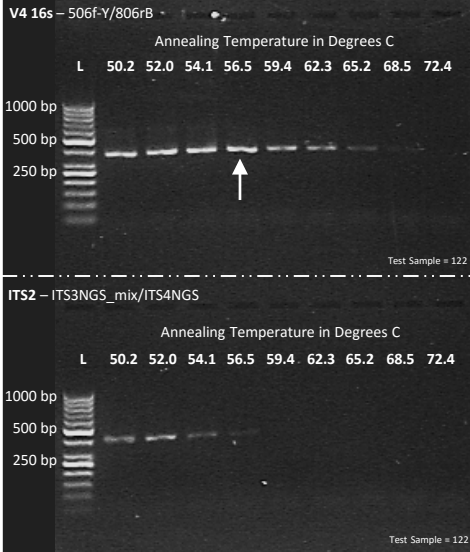
Total rxn volume = 10

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

Invitrogen SuperFi Gradient – Second Attempt

- Reduced the number of cycles from 35 to 25
- Reduced template input from 10 ng to 2.5 ng per 10 uL reaction
- Had to re-order polymerase, so this is from a new lot



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 (122)	2.5 ng/uL	1	0.25 ng/uL
Sterile PCR H ₂ O		3	

Total rxn volume = 10

Thermocycler Program

Cyc1 (1X) 98°C for 2 min
 Cyc2 (25X) 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

According to ThermoFisher Tm Calculator for SuperFi:

506f-Y/806rB Ta = 56.6 C

ITS3NGS_mix/ITS4NGS Ta = 61.1 C