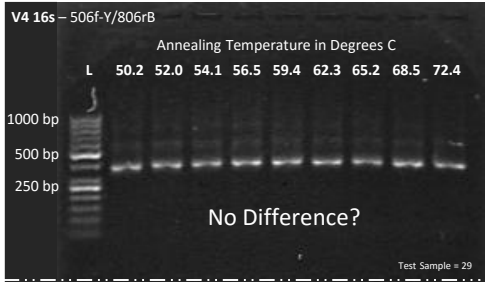

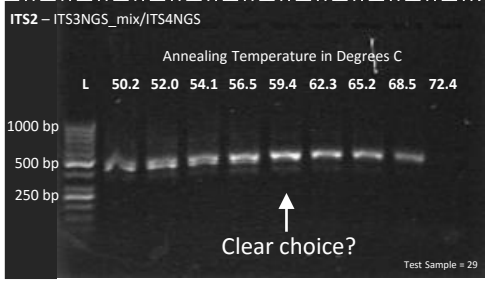

Gradient PCR using degenerate bacterial/archaeal primers (V4 region 16 – 506f-Y/806rB) and fungal primers (ITS2 region – ITS3NGS_mix/ITS4NGS). Primers contain Nextera Adapters.

PCR Recipe:

For one 10 ul reaction mixture:

Ingredient	Stock Conc.	ul per sample	Final Conc.
Invtirogen 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_2O		3	
		Total rxn volume =	10

Thermocycler Program

Cyc1 (1X) 98ºC for 2 min
Cyc2 (35X) 98 ºC for 10 s
Gradient (50-72 ºC) for 10 s
72 ºC for 15 s
Cyc3 (1X) 72 ºC for 5 min
Cyc4 4 ºC HOLD