Protocol for Non-library 16S PCR
(For Primers 1391R & 27F)

1x Recipe:

10X Taq Buffer:	5ul
"Q" buffer:	10ul
dNTP (10mM):	1.25ul
Primers (P1+P2):	5ul (2.5ul of each)
Taq Polymerase: (red bottle, NOT green hotstart)	0.3ul
H20:	26.45ul
Total:	**48ul Master Mix** **+ (2ul DNA template)** = **50ul per tube**

5x Recipe:

10X Taq Buffer:	25ul
"Q" buffer:	50ul
dNTP (10mM):	6.25ul
Primers (P1+P2):	25ul (12.5ul of each)
Taq Polymerase: (red bottle, NOT green hotstart)	1.5ul
H20:	132.25ul
Total:	**240ul Master Mix** **+ (2ul DNA template)** = **50ul per tube**

8x Recipe:

10X Taq Buffer:	40ul
"Q" buffer:	80ul
dNTP (10mM):	10ul
Primers (P1+P2):	40ul (20ul of each)
Taq Polymerase: (red bottle, NOT green hotstart)	2.4ul
H20:	211.6ul
Total:	**384ul Master Mix** **+ (2ul DNA template)** = **50ul per tube**

10x Recipe:

10X Taq Buffer:	50ul
"Q" buffer:	100ul
dNTP (10mM):	12.5ul
Primers (P1+P2):	50ul (25ul of each)
Taq Polymerase: (red bottle, NOT green hotstart)	3ul
H20:	264.5ul
Total:	**480ul Master Mix** **+ (2ul DNA template)** = **50ul per tube**

1. Keep reagents on ice. Make master mix in separate eppendorf tube according to the appropriate recipe above (be sure to make at least one more sample's worth of master mix than you will need, to account for pipetting errors).
2. Put away reagents in -20 freezer.
3. Put 48 ul of your master mix into each labeled PCR reaction tube. (Don't forget a positive and negative control!)
4. Put 2 ul of template DNA into each tube. (If reactions fail due to low DNA concentration more template may be used in place of water). Cap PCR tubes.

Thermocycler conditions are as follows:
[On most of our lab thermocyclers these can be found labelled "SPACE" or "Space PCR"]
[It's a long story, don't ask]

Lid: 105ºC
Volume: 50ul

1. 95ºC, 3:00
2. 95ºC, 0:15
3. 54ºC, 0:30
4. 72ºC, 1:30
5. Repeat steps 2-4 40x
6. 72ºC, 5:00
7. 4ºC, Infinity