

Universal 16S rRNA Bacteria 338F-516P-805R

qPCR mixture and conditions

Name	Sequence	Target (<i>E.coli</i>)	Reference
BAC338F	ACT CCT ACG GGA GGC AG	338-354	Yu et al. 2005, B&B
BAC516P*	TGC CAG CAG CCG CGG TAA TA	516-536	"
BAC805R	GAC TAC CAG GGT ATC TAA TC	785-805	"

Reagent	Final conc.	1 tube (25 µL)	Plate (25 µL × 100)
PCR H ₂ O		1.75	175
ReadyMix (Sigma)	1X	12.5	1250
MgCl ₂ (25 mM)	4 mM	2.5**	250**
BSA (50 µg/µL) [†]	0.8 µg/µL	0	0
338F (10 µM)	0.5 µM	1.25	125
805R (10 µM)	0.5 µM	1.25	125
516P (10 µM)	0.2 µM	0.5	50
Template		5	5 × 100

*Probe must be dual-labelled with either 5'-6-FAM, 3'-BHQ1 or any other valid combination.

**BSA often contains traces of bacterial DNA and so is avoided here.

[†] ReadyMix already contains 1.5mM of MgCl₂ at final concentration.

Programme:

1. 95 °C – 5'
- X35 {**
2. 95 °C – 30"
3. 62 °C – 60" – take snapshot}