

## 16S Methanomicrobia 282F-749P-832R

### QPCR mixture and conditions

Name	Sequence	Target	Reference
MMB 282F	ATC GRT ACG GGT TGT GGG	282-299	Yu et al. B&B 2004
MMB 749P	TYC GAC AGT GAG GRA CGA AAG CTG	749-772	"
MMB 832R	CAC CTA ACG CRC ATH GTT TA	812-832	"

Reagent	Final conc.	1 tube (25 µL)	Plate (25 µL × 100)
PCR H <sub>2</sub> O		1.6	160
ReadyMix (Sigma)	1X	12.5	1250
MgCl <sub>2</sub> (25 mM)	4 mM	2.5*	250*
BSA (50 µg/µL)	0.8 µg/µL	0.4	40
<b>282F</b> (10 µM)	0.5 µM	1.25	125
<b>832R</b> (10 µM)	0.5 µM	1.25	125
<b>749P</b> <sup>†</sup> (10 µM)	0.2 µM	0.5	50
Template		5	5 × 100

\*Buffer contains 1.5mM of MgCl<sub>2</sub> at final conc.

<sup>†</sup> Probe must be dual-labelled either with 5'-6-FAM, 3'-BHQ1 or any other valid combination.

#### Programme:

1. 95 °C – 5'  
    **X40 {**
2. 95 °C – 30"
3. 60 °C – 60" – take snapshot}