

16S Methanosarcinales 812F-860P-1159R

QPCR mixture and conditions

Name	Sequence	Target	Reference
MSL 812F	GTA AAC GAT RYT CGC TAG GT	812-831	Yu et al. B&B 2004
MSL 860P	AGG GAA GCC GTG AAG CGA RCC	860-880	"
MSL 1159R	GGT CCC CAC AGW GTA CC	1143-1159	"

Reagent	Final conc.	1 tube (25 µL)	Plate (25 µL × 100)
PCR H ₂ O		1.6	160
ReadyMix (Sigma)	1X	12.5	1250
MgCl ₂ (25 mM)	4 mM	2.5*	250*
BSA (50 µg/µL)	0.8 µg/µL	0.4	40
812F (10 µM)	0.5 µM	1.25	125
1159R (10 µM)	0.5 µM	1.25	125
860P [†] (10 µM)	0.2 µM	0.5	50
Template		5	5 × 100

*Buffer contains 1.5mM of MgCl₂ at final conc.

[†] 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}