

## 16S Crenarchaeote\*

### QPCR mixture and conditions

Name	Sequence	Target	Reference
771F	ACG GTG AGG GAT GAA AGC T	753-771	Ochsenreiter et al. EM 2003
957R	CGG CGT TGA CTC CAA TTG	957-974	"
957R - mod	YGG CGT TGA CTC CAA TTG	957-974	Modified from: Ochsenreiter et al. EM 2003

Reagent	Final conc.	1 tube (25 µL)	Plate (25 µL × 100)
PCR H <sub>2</sub> O		2.85	285
ReadyMix (Sigma)	1X	12.5	1250
MgCl <sub>2</sub> (25 mM)	2 mM	2	200
BSA (50 µg/µL)	0.8 µg/µL	0.4	40
FITC (1:1000)		0.25	25
<b>771F</b> (20 µM)	0.4 µM	1	100
<b>957R – mod</b> (20 µM)	0.4 µM	1	100
Template		5	5 × 100

#### Programme:

1. 95 °C – 5'
2. 95 °C – 30"
3. 62 °C – 30"
4. 72 °C – 30"
5. 83 °C – 10" – take snapshot}
6. **Melt curve:** 72 °C – 94.8 °C – 6". Increase by 0.2 °C

\* **Note:** this assay is not foolproof and it will also amplify Euryarchaeote templates at very high copy numbers (>10<sup>5</sup> in the tube).