

## mcrA general

### QPCR mixture and conditions

Name	Sequence	Target	Reference
mlas-mod	GGY GGT GTM GGD TTC ACM CAR TA		Modified from: Steinberg and Regan AEM 2008
mcrA-rev	CGT TCA TBG CGT AGT TVG GRT AGT		Steinberg and Regan AEM 2008

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)	3.5 mM	3.5	350
BSA (50 µg/µL)	0.8 µg/µL	0.4	40
FITC (1:1000)		0.25	25
<b>mlas-mod</b> (25 µM)	0.25 µM	0.25	25
<b>mcrA-rev</b> (25 µM)	0.25 µM	0.25	25
Template		5	5 × 100

#### Programme:

1. 94 °C – 5'  
    **X40 {**
2. 95 °C – 30"
3. 57 °C – 45"
4. 72 °C – 30"
5. 84 °C – 10" – take snapshot}
6. **Melt curve:** 75 °C – 94.8 °C – 6". Increase by 0.2 °C