

	Bacterial 16S rRNA	Thaumarchaeal 16S rRNA	WCA <i>amoA</i>	WCB <i>amoA</i>	Thaumarchaeal <i>ureC</i>	AOB <i>amoA</i>	<i>Nitrospina</i> 16S rRNA
Forward primer	BACT1369F	GI_334F	WCA-amoA-F	Arch-amoAFB	Thaum-UreC forward	amoA-1F	NitSSU_130F
	CGGTGAATACGTTTCYCGG	AGATGGGTACTGAGACACGGAC	ACACCAGTTTGGCTWCCDTCAGC	CATCCRATGTGGATTCCATCDTG	ATG CAA TYT GTA ATG GAA CWA CW	GGGGTTTCTACTGGTGGT	GGGTGAGTAACACGTGAATAA
Reverse primer	PROK1492R	GI_554R	WCA-amoA-R	WCB-amoA-R	Thaum-UreC reverse	amoAr-NEW	NitSSU_282R
	GGWTACCTTGTTACGACTT	CTGTAGGCCCAATAATCATCCT	TCAGCCACHGTGATCAAATTG	AAYGCAGTTTCTAGYGGATC	AGT TGT YCC CCA ATC TTC ATG TAA	CCCCTCBGSAAAVCCTTCTTC	TCAGGCCGGCTAAMCA
Probe¹	TM1389F	TM519AR	WCA-amoA-P	WCB-amoA-P			
	CTTGTACACACGCCCGTC	TTACCGCGGGCGCTGGCAC	ACTCCGCCGAACAGTATCA	CCAAAGAATATYAGCGARTG			
Reference(s)	Suzuki et al. 2000	Suzuki et al. 2000	Mosier and Francis 2011	Mosier and Francis 2011; Beman et al. 2008	Alonso-Sáez et al., 2012	Rotthauwe et al. 1997; Hornek et al.	Mincer et al. 2007
Cycling conditions	94°C, 2 m	94°C, 2 m	95°C, 10 m	95°C, 10 m	95 °C, 5 m	95°C, 5 m	94°C, 15 m
	35 cycles of:	40 cycles of:	40 cycles of:	40 cycles of:	40 cycles of:	40 cycles of:	50 cycles of:
	94°C, 15 s	94°C, 15 s	95°C, 15 s	95°C, 15 s	94 °C, 30 s	94°C, 30 s	94°C, 15 s
	56°C, 1 m	59°C, 1 m	56°C, 1 m	56°C, 1 m	53 °C, 40 s	61.5°C, 40 s	57.5°C, 15 s
					72 °C, 40 s	72°C, 40 s	72°C, 30 s
Acquisition at 56°C	Acquisition at 59°C	Acquisition at 56°C	Acquisition at 56°C	Acquisition at 78 °C	Acquisition at 82°C	Acquisition at 80°C	
Number of plates run	8	6	6	6	6	4	5
r² of standard curve (mean±SD)	0.999±0.001	0.997±0.005	0.999±0.001	0.997±0.002	0.999±0.001	0.997±0.001	0.999±0.001
Primer Efficiency (mean±SD, %)	100.5±1.9	106.9±14.9	99.1±1.9	93.5±2.3	91.5±2.7	71.4±4.5	91.7±3.5
Limit of Detection (10³ copies L⁻¹)²	5000.0	2.0	1.0	1.0	8.0	8.0	2.0

1) All probes contained a 5' FAM tag and a 3' BHQ1 quencher.

2) Assumes complete extraction of template DNA from a 2 L sample

LITERATURE CITED

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