

Supplementary file (S1)

Primer information

Target group	Primer name	Primer sequence	Fragment length (bp)	References
AOB	amoA-1F	GGGGTTTCTACTGGTGGT	491	[1]
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC		
AOA	Arch-amoAF	STAATGGTCTGGCTTAGACG	635	[2]
	Arch-amoAR	GCGGCCATCCATCTGTATGT		
NOB	F	TACATGTGGTGGAAACA	485	[3]
	R	CGGTTCTGGTCRATCA		

Note: AOB; ammonia oxidizing bacteria, AOA; ammonia oxidizing archaea, NOB; nitrite oxidizing bacteria

Supplementary file (S2)

Polymerase chain reaction (PCR) reaction system

Component	Volume
2×PCR Master Mix	12.5 µl
Forward primer (10 µM)	0.5 µl
Reverse Primer (10 µM)	0.5 µl
Template (mixed DNA)	1 µl
ddH ₂ O	25 µl

Setting of primer amplification conditions: 94°C for 5 min; 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, 35 cycles; 72°C for 10 min. The products were subjected to agarose gel electrophoresis as follows:

Amplification curve: 94°C for 30 s; 94°C for 10 s, 60°C for 12 s, 72°C 30 s for 40 cycles, 72°C single point detection signal.

Thymine-Adenine (TA) cloning reaction system

Component	Volume
The purified PCR product/or PCR1 ul 1 kb control	1 μ l
PMD-19T Vector	1 μ l
10 \times Enhancer	1 μ l
Sterilized water	10 μ l

Fluorescent quantitative polymerase chain reaction (FQ-PCR) system

Component	Volume	Final concentration
SYBR Premix Ex Taq (2 \times)	10 μ l	1 \times
Forward Primer (10 μ M)	0.8 μ l	0.4 μ M
Reverse Primer (10 μ M)	0.8 μ l	0.4 μ M
Genomic DNA	1 μ l	
ddH ₂ O	20 μ l	

Fluorescence quantitative PCR amplification conditions setting:

Amplification curve: 94°C for 30 s; 94°C for 10 s, 60°C for 12 s, 72°C 30 s for 40 cycles, 72°C single point detection signal.

Dissolution curve: 95°C 0 s, 65°C 15 s, 95°C 0 s, continuous detection signal.

References:

- [1] Rotthauwe JH, Witzel KP, Liesack W (1997) The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl Environ Microbiol 63:4704-4712

- [2] Nicol GW, Leininger S, Schleper C, Prosser JI (2008) The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ Microbiol* 10:2966–2978
- [3] Pester M, Maixner F, Berry D, Rattei T, Koch H, Lückner S, Nowka B, Richter A, Spieck E, Lebedeva E, Loy A, Wagner M, Daims H (2014) NxrB encoding the beta subunit of nitrite oxidoreductase as functional and phylogenetic marker for nitrite-oxidizing Nitrospira. *Environ Microbiol* 16:3055–3071