Table 1. Primer description and thermal profiles for PCR amplification of *amoA*

Primer pair*	Sequence (5'-3')	Round of nested PCR	Thermal profile†	Molecular analysis
A189 (1)	**GGNGACTGGGACTTCTGG**	1	94°C, 30 s 62-52°C,‡ 30 s 72°C, 45 s 30 cycles	T-RFLP
amoA-2R (2)	CCCCTCKGSAAAGCCTTCTTC			
amoA-1F (2)	§**GGGGTTTCTACTGGTGGT**	2	94°C, 60 s 55°C, 90 s 72°C, 90 s 18 cycles	
amoA-2R	CCCCTCKGSAAAGCCTTCTTC			
A189	**GGNGACTGGGACTTCTGG**	1	94°C, 45 s 56°C, 90 s 72°C, 180 s 13 cycles	Real-time PCR‖
amoA-2R	CCCCTCKGSAAAGCCTTCTTC			
amoA-1F	**GGGGTTTCTACTGGTGGT**	2 (Real-time PCR)	94°C, 45 s 55°C, 30 s 72°C, 180 s Plate read at 83°C 30 cycles Melting curve¶	
amoA-2R	CCCCTCKGSAAAGCCTTCTTC			

*Primers in bold represent the 5'-primer.

†All PCR profiles started with an initial denaturation at 94°C for 3 min and ended with a final elongation step at 72°C for 10 min, prior to holding the temperature at 4°C.

‡Annealing temperature was decreased 0.5°C after each cycle until it reached 52°C.

§Primer labeled with 5-carboxyfluorescein.

¶Melting curve analysis: 65-98°C, 0.2°C per read, 1-s hold, final elongation step at 72°C for 10 min, prior to holding the temperature at 10°C.

‖Control reactions with the primers M13F (5'-GTAAAACGACGGCCAG-3') and M13R (5'-CAGGAAACAGCTATGAC-3') were performed with identical thermal profiles.

1. Holmes, A. J., Costello, A., Lidstrom, M. E. & Murrell, J. C. (1995) *FEMS Microbiol. Lett.* **132,** 203-208.

2. Rotthauwe, J., Witzel, K. & Liesack, W. (1997) *Appl. Environ. Microbiol.* **63,** 4704-4712.