Title: A protocol to amplify *pmoA* with PCR
Date: 4-17-2012
Author: Akihiro Koyama

Overview
This protocol describes how to amplify *pmoA*, a methanotroph functional gene, with PCR following Bourne et al. (2001), Horz et al. (2005), and Lüke et al. (2010). A nested-PCR is employed (Fi.g 1) based on Horz et al. (2005). In the first round of PCR, a touch-down PCR (Lüke et al. 2010) is used with a primer set of A189F/A682R (Bourne et al. 2001, Horz et al. 2005). Amplicons from the first round of PCR are used for the second rounds of PCR with two primer sets, A189F/A650R and A189F/mb661R (Horz et al. 2005). The two sets of primers amplify different groups of methanotrophs (Bourne et al. 2001). Amplicons of the second round of PCR can be used for downstream processes.

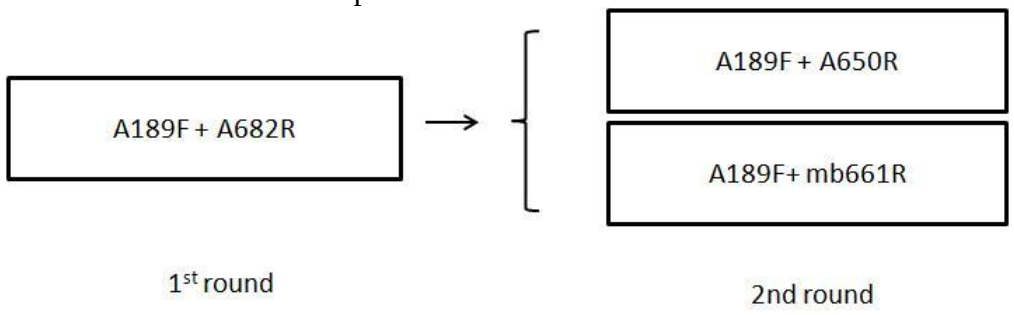

Fig. 1. Nested PCR to amplify *pmoA*.

Procedure
1. Mix reagents and DNA templates with the ratio listed in Table 1.
2. Prepare triplicates for template DNA to obtain total 75µL of amplicons. One 25µL assay is enough for negative control.
3. Run the touch-down PCR. The thermal profile is listed in Table 2.
4. Mix reagents with amplicons from the first round of PCR. Mixing ratio is listed in Table 3. Prepare sextuplicate to obtain total 150 µL of final amplicons.
5. Run the second-round of PCR for each set of primers. Prepare triplicate for each set of primers to obtain total 75 µL of final amplicons. The thermal profile is listed in Table 4.
6. Combine amplicons for each sample and store them at -20 C°.

References
Bourne, D. G., I. R. McDonald, and J. C. Murrell. 2001. Comparison of pmoA PCR Primer Sets as Tools for Investigating Methanotroph Diversity in Three Danish Soils. Applied and Environmental Microbiology **67**:3802-3809.

Horz, H.-P., V. Rich, S. Avrahami, and B. J. M. Bohannan. 2005. Methane-Oxidizing Bacteria in a California Upland Grassland Soil: Diversity and Response to Simulated Global Change. Applied and Environmental Microbiology **71**:2642-2652.

Lüke, C., S. Krause, S. Cavigiolo, D. Greppi, E. Lupotto, and P. Frenzel. 2010. Biogeography of wetland rice methanotrophs. Environmental Microbiology **12**:862-872.

Table 1. Reagents and their quantities in a 25µL assay for the first round of PCR

Reagents	Quantity (µL)	Reagent Conc.	Final Conc.
KAPA2G	12.5	2X	1X
BSA	1.25	10 ng/µL	0.5ng/µL
PCR H2O	7.75	N/A	N/A
F primer (A189F)	1.25	10 µM	0.2 µM
R primer (A682R)	1.25	10 µM	0.2 µM
Template DNA†	1	~ 1 ng/µL	~ 0.05 ng/µL
Total	25	N/A	N/A

†PCR H_2O for negative controls

Table 2. Thermal profile for the first round of PCR

Cycling step	Temperature (C°)	Duration (second)	# of cycles
Initial Denaturation	94	180	1
Denaturation	94	10	
Annealing	62 (touchdown 1 C° per cycle)	10	11
Extension	72	1	
Denaturation	94	10	
Annealing	52	10	24
Extension	72	1	

Table 3. Reagents and their quantities in a 25µL assay for the second round of PCR

Reagents	Quantity (µL)	Reagent Conc.	Final Conc.
KAPA2G	12.5	2X	1X
BSA	1.25	10 ng/µL	0.5ng/µL
PCR H2O	7.75	N/A	N/A
F primer (A189F)	1.25	10 µM	0.2 µM
R primer (A682R)	1.25	10 µM	0.2 µM
Amplicon†	1	-	-
Total	25	N/A	N/A

†PCR H_2O for negative controls

Table 4. Thermal profile for the first round of PCR

Cycling step	Temperature (C°)	Duration (second)	# of cycles
Initial Denaturation	94	180	1
Denaturation	94	10	
Annealing	52.5	10	40
Extension	72	1	