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# pMOA 189F-682R

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Holmes AJ, Costello A, Lidstrom ME, Murrell JC. Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. FEMS Microbiol Lett. 1995;132:203–8.
http://www.sciencedirect.com/science/article/pii/037810979500311R



**Protocol status:** Working
**We use this protocol and it's working**

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**Keywords:** PCR, pmoA, PMMO, Methanotrophs, Methane oxidising bacteria, Particulate methane monooxygenase

## Abstract

A PCR assay targeting the alpha subunit of the particulate methane monooxygenase gene (*pmoA*).
This is a general assay for methane oxidising bacteria using primers 189F–682R.
**Note:** these primers might occasionally also amplify the *amoA* gene of ammonia-oxidising bacteria.

189F GGN GAC TGG GAC TTC TGG (target seq. 189 - 207) Holmes et al. (1995),
682R GAA SGC NGA GAA GAA SGC (target seq. 664 - 682) Holmes et al. (1995).

Fragment size: 508bp.

## PCR mixture

1

| | Reagent | Final. conc. | 1 tube (50 µl) | 1 tube(25 µl) | 96 tubes (25µl x100) | 96 tubes (12.5µl x100) |
|---|---|---|---|---|---|---|
| | PCR $H_2O$ | | 37.9 | 18.95 | 1255 | 627.5 |
| | 10X Buffer (I for RT and II for DNA) | 1x | 5 | 2.5 | 500 (Promega) | 250 (Promega) |
| | dNTP mixture (2.0 mM each | 0.2mM | 5 | 2.5 | 250 | 125 |
| | $MgCl_2$ (50 mM)* | 3mM | 1.5* | 0.75* | 250 | 125 |
| | BSA (20µg/µl) | 0.8 µg/µl | 2 | 1 | 100 | 50 |
| | **189f (25µM)** | 0.4 µM | 0.8 | 0.4 | 40 | 20 |
| | **682r (25 µM)** | 0.4 µM | 0.8 | 0.4 | 40 | 20 |
| | AccuPrime™ Taq | ? | 1 | 0.5 | 15 | 7.5 |
| | Template | | 1 | 0.5 | 0.5 x 100 | 0.25 x 100 |
| | **Final volume** | | **50** | **25** | **2500** | **1250** |

* Buffer contains 1.5 mM $MgCl_2$ at final concentration

## PCR program

2

1. 94˚C – 4'
2. x **10** {
    a. 94˚C – 1'
    b. 62˚C – 1°C – 1’
    c. 68˚C – 1'
   }
3. x **25** {
    a. 94˚C – 1'
    b. 52˚C – 1'
    c. 68˚C – 1'
   }
4. 68˚C – 10'