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pMOA 189F-682R

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Manuscript citation:
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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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.

Troubleshooting

PCR mixture

1

	Reagent	Final. conc.	1 tube (50µl)	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 × 100	0.25 × 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'