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qPCR quantification of methanogens using general mcrA primers

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1 Works for me dx.doi.org/10.17504/protocols.io.qcudsww

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ABSTRACT

General *mcrA* primers for quantifying methanogens using a SYBR Green-based assay.

Name	Direction	Sequence	Citation
mlas_mod	Fwd	GGY GGT GTM GGD TTC ACM CAR TA	Angel et al., 2011
mcrA_rev	Rev	CGT TCA TBG CGT AGT TVG GRT AGT	Steinberg and Regan, 2008

Steinberg L M, Regen J M (2008). Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. Applied and Environmental Microbiology. http://10.1128/AEM.00553-08

Angel R, Matthies D, Conrad R (2011). Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen.. PloS one. https://doi.org/10.1371/journal.pone.0020453

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Angel, R., Matthies, D., and Conrad, R. (2011). Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. PLoS ONE 6, e20453. doi:10.1371/journal.pone.0020453.

ATTACHMENTS

Introduction_QPCR_Stratagene.pdf AB_rt-QPCRguide.pdf

QPCR mixture

1	Reagent	Final conc.	1 tube (25 µl)	plate (25 µl x 100)

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PCR H_2O		2.85	285
qPCR master mix	1x	12.5	1250
$MgCl_2$ (25mM)*	3.5 mM	3.5	350
BSA (50 µg $µl^{-1}$)	0.8 µg $µl^{-1}$	0.4	40
mlas-mod (25 µM)	0.25 µM	0.25	25
mrcA-rev (25 µM)	0.25 µM	0.25	25
Template		5	5 x 100

* Different master mixes vary in concentration of $MgCl_2$ and the amount should be adjusted accordingly

Programme 2h

2 1. 94°C – 5'
2. **x 40** {
   a. 95°C – 30"
   b. 57°C – 45"
   c. 72°C – 30"
   d. 84°C - 10" take a snapshot
   }
3. Melt curve: 75°C – 94.8°C –6''. Increase by 0.2°C

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