

Sep 01, 2020

qPCR quantification of methanogens using general mcrA primers

DOI

<https://dx.doi.org/10.17504/protocols.io.qcudsww>

SOWA

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Protocol Citation: Roey Angel, Eva Petrova 2020. qPCR quantification of methanogens using general mcrA primers. protocols.io <https://dx.doi.org/10.17504/protocols.io.qcudsww>



Manuscript citation:

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.

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Protocol status: Working

We use this protocol and it's working

Created: May 24, 2018

Last Modified: September 01, 2020

Protocol Integer ID: 12404

Keywords: PCR, qPCR, mcrA, Methanogens, Archaea, qpcr quantification of methanogen, quantifying methanogen, methanogen, qpcr quantification, using general mcra primers general mcra, based assay, general mcra primers general mcra,

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

Citation

Steinberg L M, Regan 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.

[10.1128/AEM.00553-08](https://doi.org/10.1128/AEM.00553-08)

LINK

Citation

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>

LINK

Attachments



[Introduction_QPCR_St...](#)

10.3MB



[AB_rt-QPCRguide.pdf](#)

1,011KB



QPCR mixture

1

Reagent	Final conc.	1 tube (25 μ l)	plate (25 μ l x 100)
PCR H ₂ O		2.85	285
qPCR master mix	1x	12.5	1250
MgCl ₂ (25mM)*	3.5 mM	3.5	350
BSA (50 μ g μ l ⁻¹)	0.8 μ g μ l ⁻¹	0.4	40
mlas-mod (25 μ M)	0.25 μ M	0.25	25
mrcA-rev (25 μ M)	0.25 μ M	0.25	25
Template		5	5 × 100

* Different master mixes vary in concentration of MgCl₂ 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

Citations

Steinberg L M, Regen J M. Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge
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