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General methanogens: mcrA mlas-mod – mcrA-rev

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Manuscript citation:

Angel R, Matthies D, Conrad R. Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. PLoS ONE. 2011;6:e20453.

<http://dx.doi.org/10.1371/journal.pone.0020453>

Steinberg LM, Regan JM. Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. Appl Environ Microbiol. 2008;74:6663–71.

<http://aem.asm.org/cgi/content/abstract/74/21/6663>

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Protocol status: Working

We use this protocol and it's working

Created: May 24, 2018

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Protocol Integer ID: 12423

Keywords: PCR, Methanogens

Abstract

Amplification of the marker gene methyl coenzyme M reductase alpha subunit (*mcrA*) using the general primers mlas-mod – mcrA-rev

mlas-mod GGY GGT GTM GGD TTC ACM CAR TA Angel et al. (2011), Plos One
mcrA-rev CGT TCA TBG CGT AGT TVG GRT AGT Steinberg and Regan (2008), AEM.

Fragment size: 472bp.

Protocol materials

-  dNTP Set, PCR Grade **Biotechrrabbit Catalog #BR0600601** Step 1
-  DreamTaq Green DNA Polymerase (5 U/μL) **Thermo Fisher Scientific Catalog #EP0711** Step 1
-  Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #B14** Step 1




PCR mixture

1

A	B	C	D	E	F
Reagent	Final. conc.	1 tube (50 μ l)	1 tube(25 μ l)	96 tubes	96 tubes
PCR H ₂ O		25.1	12.55	1255	627.5
	1x	10	5	500	250
dNTP mixture (2.0 mM each)	0.2mM	5	2.5	250	125
	2.5mM	5	2.5	250	125
BSA (20 μ g/ μ l)	0.8 μ g/ μ l	2	1	100	50
mlas-mod	0.4 μ M	0.8	0.4	40	20
mcrA-rev	0.4 μ M	0.8	0.4	40	20
	1.5U/50 μ l	0.3	0.15	15	7.5
Template		1	0.5	0.5 x 100	0.25 x 100
Final volume		50	25	2500	1250

 dNTP Set, PCR Grade **Biotechrabbit Catalog #BR0600601**

 DreamTaq Green DNA Polymerase (5 U/ μ L) **Thermo Fisher Scientific Catalog #EP0711**

 Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #B14**

PCR program

2

1. 94°C – 5'
2. x **5** {
 - a. 60°C – 1°C - 45"
 - b. 72°C – 30"
 - c. 94°C – 30'}
3. x **29** {
 - a. 55°C – 45"
 - b. 72°C – 30"
 - c. 94°C – 30"}
4. 55°C – 45"



5. 72°C - 10'