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General methanogens: mcrA mlas-mod – mcrA-rev

DOI

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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

Last Modified: September 26, 2024

Protocol Integer ID: 12423

Keywords: PCR, Methanogens, general methanogen, methanogen, alpha subunit, marker gene, mcra, plos one mcra

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 **Biotechrabbit Catalog #BR0600601**

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

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

PCR mixture

1

	A	B	C	D	E	F
	Reagent	Final. conc.	1 tube (50µl)	1 tube(25 µl)	96 tubes	96 tubes
	PCR H2O		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 (20µg/µl)	0.8 µg/µl	2	1	100	50
	mlas-mod	0.4 µM	0.8	0.4	40	20
	mcrA-rev	0.4 µM	0.8	0.4	40	20
		1.5U/50µl	0.3	0.15	15	7.5
	Template		1	0.5	0.5 × 100	0.25 × 100
	Final volume		50	25	2500	1250

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

⊗ DreamTaq Green DNA Polymerase (5 U/µL) **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'