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16S Arc 109F-934R

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Manuscript citation:

1. Grosskopf R, Janssen PH, Liesack W. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Appl Environ Microbiol. 1998;64:960–9.
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2. Miyashita A, Mochimaru H, Kazama H, Ohashi A, Yamaguchi T, Nunoura T, et al. Development of 16S rRNA gene-targeted primers for detection of archaeal anaerobic methanotrophs (ANMEs). FEMS Microbiol Lett. 2009;297:31–7.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1574-6968.2009.01648.x/full>

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Protocol status: Working

We use this protocol and it's working

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Keywords: PCR, Archaea, 16S rRNA gene, general archaea, marker gene

Abstract

Amplification of the marker gene 16S rRNA for general Archaea using primers 109F-934R.

109F or	ACK GCT CAG TAA CAC GT	(target seq. 109 – 125) Grosskopf et al. (1998),
109F-mod	AHD GCT CAG TAA CAC RT	(target seq. 109 – 125) Miyashita et al. (2009)
934R	GTG CTC CCC CGC CAA TTC CT	(target seq. 915 – 934) Grosskopf et al. (1998)

Expected fragment size: 791bp

Materials

MATERIALS

 GoTaq® Flexi DNA Polymerase **Promega Catalog #M830**



PCR mixture

1		Reagent	Final. conc.	1 tube (50µl)	1 tube(25µl)	96 tubes (25µl x100)
		PCR H ₂ O		27.7	13.85	1385
		GoTaq®Flexi 5x Green Buffer	1x	10	5	500
		dNTP mixture (2.0 mM each	0.2mM	5	2.5	250
		MgCl ₂ (25 mM)	1.5mM	3	1.5	150
		BSA (20 µg/µl)	0.8 µg/ µl	2	1	100
		109f/109f-mod (25 µM)	0.25 µM	0.5	0.25	25
		934r (25 µM)	0.25 µM	0.5	0.25	25
		Go Taq Flexi polymerase	1.5U/5 0µl	0.3	0.15	15
		Template		1	0.5	0.5 × 100
		Final volume		50	25	2500

PCR program

- 2
1. 94°C – 4'
2. x 28 - 32 {
 - a. 52°C – 30"
 - b. 72°C – 45"
 - c. 94°C – 30"}
3. 52°C – 30"
4. 72°C – 10'