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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. http://aem.asm.org/cgi/content/abstract/64/3/960

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

Protocol status: Working
We use this protocol and it's working

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Keywords: PCR, Archaea, 16S rRNA 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_2O		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/50 µl	0.3	0.15	15
	Template		1	0.5	0.5 x 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'