

14/01/2016

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Additive: DMSO		5	%			
25 uL		1X_65ul 1.5ul Mg+	1	x	Master Mix (no primers)	6 x
Amount	Compound		Amount	Compound		
16 uL	ddH ₂ O	0.64	96 uL	ddH ₂ O		96
1.25 uL	DMSO 100%	0.05	7.5 uL	DMSO 100%		7.5
2.5 uL	10x PCR buffer	0.1	15 uL	10x PCR buffer		15
2 uL	dNTPs (2.5mM each)	0.08	12 uL	dNTPs (2.5mM each)		12
1.5 uL	MgCl ₂	0.06	9 uL	MgCl ₂		9
0.5 uL	Pf, 5µM	0.02	3 uL	Pf, 5µM	139.5	
0.5 uL	Pr, 5 µM	0.02	3 uL	Pr, 5 µM		
0.5 uL	DNA template	0.02	3 uL	DNA template		
0.25 uL	Polymerase	0.01	1.5 uL	Polymerase		
25 uL	Total	1	150 uL	Total mm per25 ul:	23.25	25

Gradient PCR with DMSO to optimize p5p8 Reaction

p5 68C; p8 70C: 68,0; 68,3; 68,6; 69,0, 69,5; 70

Notes:

Genomic DNA BL2 tested in 16S PCR reaction, 2 types with and with no DMSO

94 C - 5 min

94 C, 45 s: 48 C, 1 min: 72 C, 2 min: 33x

72 C, 5 min

4 C, inf

primers done from stock: 9f 400, 1492r 200: by dilution to 50

	ul	stock: uM	h2o ul	resulting uM	ul resulting
9f	20	400	140	50	160
1492r	40	200	120	50	160

Additive: DMSO			0	%			
0 uL		1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)		0 x
Amount	Compound			Amount	Compound		
0 uL	ddH ₂ O		0.69	0 uL	ddH ₂ O		0
0 uL	DMSO 100%		0	0 uL	DMSO 100%		0
0 uL	10x PCR buffer		0.1	0 uL	10x PCR buffer		0
0 uL	dNTPs (2.5mM each)		0.08	0 uL	dNTPs (2.5mM each)		0
0 uL	MgCl ₂		0.06	0 uL	MgCl ₂		0
0 uL	Pf, 5µM		0.02	0 uL	Pf, 5µM		0
0 uL	Pr, 5 µM		0.02	0 uL	Pr, 5 µM		
0 uL	DNA template		0.02	0 uL	DNA template		
0 uL	Polymerase		0.01	0 uL	Polymerase		
0 uL	Total		1	0 uL	Total mm per25 ul:		0 0