

21/01/2016

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Additive: DMSO		5	%		
25 uL	1X_65ul 1.5ul Mg+	1	x	Master Mix (no primers)	
Amount	Compound			Amount	Compound
17.5 uL	ddH <sub>2</sub> O	0.7		35 uL	ddH <sub>2</sub> O
1.25 uL	DMSO 100%	0.05		2.5 uL	DMSO 100%
2.5 uL	10x PCR buffer wMg	0.1		5 uL	10x PCR buffer wMg
2 uL	dNTPs (2.5mM each)	0.08		4 uL	dNTPs (2.5mM each)
0 uL	MgCl <sub>2</sub>	0		0 uL	MgCl <sub>2</sub>
0.5 uL	Pf, 5μM	0.02		1 uL	Pf, 5μM
0.5 uL	Pr, 5 μM	0.02		1 uL	Pr, 5 μM
0.5 uL	DNA template	0.02		1 uL	DNA template
0.25 uL	Polymerase	0.01		0.5 uL	Polymerase
25 uL	Total	1		50 uL	Total  mm per25 ul:

23.25 25

Tm=68, the same as the lowest primer

1 Genomic, 2 Plasmid

Genomic DNA BL2, and plasmid containing construct, cln5( 13.11.15) tested in 16S PCR reaction, 2 types with and with no DMSO

Notes:

94 C - 5 min

94 C, 45 s: 48 C, 1 min: 72 C, 2 min: 33: 30x

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	%		
25 uL	1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)	
Amount	Compound			Amount	Compound
18.75 uL	ddH <sub>2</sub> O	0.75		37.5 uL	ddH <sub>2</sub> O
0 uL	DMSO 100%	0		0 uL	DMSO 100%
2.5 uL	10x PCR buffer wMg	0.1		5 uL	10x PCR buffer wMg
2 uL	dNTPs (2.5mM each)	0.08		4 uL	dNTPs (2.5mM each)
0 uL	MgCl <sub>2</sub>	0		0 uL	MgCl <sub>2</sub>
0.5 uL	Pf, 5μM	0.02		1 uL	Pf, 5μM
0.5 uL	Pr, 5 μM	0.02		1 uL	Pr, 5 μM
0.5 uL	DNA template	0.02		1 uL	DNA template
0.25 uL	Polymerase Pfu	0.01		0.5 uL	Polymerase, Pfu
25 uL	Total	1		50 uL	Total  mm per25 ul:

23.25 25