

02/05/2016

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Additive: DMSO			0	%			
30 uL	1X_65ul 1.5ul Mg+	1ul	x		Master Mix (no primers)	3 x	
Amount	Compound		Amount	Compound			
20.7 uL	ddH <sub>2</sub> O	0.69	62.1 uL	ddH <sub>2</sub> O	62.1		
0 uL	DMSO 100%	0	0 uL	DMSO 100%	0		
3 uL	10x PCR buffer	0.1	9 uL	10x PCR buffer	9		
2.4 uL	dNTPs (2.5mM each)	0.08	7.2 uL	dNTPs (2.5mM each)	7.2		
1.8 uL	MgCl <sub>2</sub>	0.06	5.4 uL	MgCl <sub>2</sub>	5.4		
0.6 uL	Pf, 5μM	0.02	1.8 uL	Pf, 5μM	83.7		
0.6 uL	Pr, 5 μM	0.02	1.8 uL	Pr, 5 μM			
0.6 uL	DNA template	0.02	1.8 uL	DNA template			
0.3 uL	Polymerase	0.01	0.9 uL	Polymerase			
30 uL	Total	1	90 uL	Total  mm per25 ul:	27.9	30	

original protocol with 5x enhancer

Notes:	Genomic DNA BL2 tested in 16S PCR reaction, 2 types with and with no DMSO
	94 C - 5 min 1 phototroph
	94 C, 45 s: 48 C, 1 min: 72 C, 2 min: 33x 2 yeast
	72 C, 5 min 3 BL2
	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

Prepare Mmix Taq			0	%			
500 uL	1X_65ul 1.5ul Mg+	1ul	x		Master Mix (no primers)	1 x	
Amount	Compound		Amount	Compound			
345 uL	ddH <sub>2</sub> O	0.69	345 uL	ddH <sub>2</sub> O	345		
0 uL	DMSO 100%	0	0 uL	DMSO 100%	0		
50 uL	10x PCR buffer	0.1	50 uL	10x PCR buffer	50		
40 uL	dNTPs (2.5mM each)	0.08	40 uL	dNTPs (2.5mM each)	40		
30 uL	MgCl <sub>2</sub>	0.06	30 uL	MgCl <sub>2</sub>	30		
10 uL	Pf, 5μM	0.02	10 uL	Pf, 5μM	465		
10 uL	Pr, 5 μM	0.02	10 uL	Pr, 5 μM			
10 uL	DNA template	0.02	10 uL	DNA template			
5 uL	Polymerase	0.01	5 uL	Polymerase			
500 uL	Total	1	500 uL	Total  mm per25 ul:	465	500	