

06/01/2016

06/01/2016 16:06

Additive: DMSO		5	%				
40 uL	1X_65ul 1.5ul Mg+	5	x	Master Mix (no primers)		2	x
Amount	Compound	Amount	Compound	Amount	Compound		
26.5 uL	ddH ₂ O	0.64	53 uL	ddH ₂ O		53	
2 uL	DMSO 100%	0.05	4 uL	DMSO 100%		4	
4 uL	10x PCR buffer	0.1	8 uL	10x PCR buffer		8	
3.2 uL	dNTPs (2.5mM each)	0.08	6.4 uL	dNTPs (2.5mM each)		6.4	
2.4 uL	MgCl ₂	0.06	4.8 uL	MgCl ₂		4.8	
0.5 uL	Pf, 5µM	0.02	1 uL	Pf, 5µM		76.2	
0.5 uL	Pr, 5 µM	0.02	1 uL	Pr, 5 µM			
0.5 uL	DNA template	0.02	1 uL	DNA template			
0.4 uL	Polymerase	0.01	0.8 uL	Polymerase			
40 uL	Total	1	80 uL	Total mm per25 ul:		38.1	40

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

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	%				
40 uL	1X_65ul 1.5ul Mg+	5	x	Master Mix (no primers)		2	x
Amount	Compound	Amount	Compound	Amount	Compound		
28.5 uL	ddH ₂ O	0.69	57 uL	ddH ₂ O		57	
0 uL	DMSO 100%	0	0 uL	DMSO 100%		0	
4 uL	10x PCR buffer	0.1	8 uL	10x PCR buffer		8	
3.2 uL	dNTPs (2.5mM each)	0.08	6.4 uL	dNTPs (2.5mM each)		6.4	
2.4 uL	MgCl ₂	0.06	4.8 uL	MgCl ₂		4.8	
0.5 uL	Pf, 5µM	0.02	1 uL	Pf, 5µM		76.2	
0.5 uL	Pr, 5 µM	0.02	1 uL	Pr, 5 µM			
0.5 uL	DNA template	0.02	1 uL	DNA template			
0.4 uL	Polymerase	0.01	0.8 uL	Polymerase			
40 uL	Total	1	80 uL	Total mm per25 ul:		38.1	40