

11/01/2016

11/01/2016 15:41

Additive: DMSO		0	%		
25 uL	1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)	10 x
Amount	Compound			Amount	Compound
16 uL	ddH ₂ O	0.64	160	uL	ddH ₂ O
1.25 uL	DMSO 100%	0.05	12.5	uL	DMSO 100%
2.5 uL	10x PCR buffer	0.1	25	uL	10x PCR buffer
2 uL	dNTPs (2.5mM each)	0.08	20	uL	dNTPs (2.5mM each)
1.5 uL	MgCl ₂	0.06	15	uL	MgCl ₂
0.5 uL	Pf, 5μM	0.02	5	uL	Pf, 5μM
0.5 uL	Pr, 5 μM	0.02	5	uL	Pr, 5 μM
0.5 uL	DNA template	0.02	5	uL	DNA template
0.25 uL	Polymerase	0.01	2.5	uL	Polymerase
25 uL	Total	1	250	uL	Total mm per25 ul:
					23.25 25

Primers, template changed from 0.8 -> 0.5 uL

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	%		
25 uL	1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)	10 x
Amount	Compound			Amount	Compound
17.25 uL	ddH ₂ O	0.69	173	uL	ddH ₂ O
0 uL	DMSO 100%	0	0	uL	DMSO 100%
2.5 uL	10x PCR buffer	0.1	25	uL	10x PCR buffer
2 uL	dNTPs (2.5mM each)	0.08	20	uL	dNTPs (2.5mM each)
1.5 uL	MgCl ₂	0.06	15	uL	MgCl ₂
0.5 uL	Pf, 5μM	0.02	5	uL	Pf, 5μM
0.5 uL	Pr, 5 μM	0.02	5	uL	Pr, 5 μM
0.5 uL	DNA template	0.02	5	uL	DNA template
0.25 uL	Polymerase	0.01	2.5	uL	Polymerase
25 uL	Total	1	250	uL	Total mm per25 ul:
					23.25 25