18/01/2016 18/01/2016 13:00

Additive: DMSO 5 %

25 uL	1X_65ul 1.5ul Mg+		1 x	Master Mix (no primers)	3 x
Amount	Compound		Amount	Compound	
16 uL	ddH_2O	0.64	48 uL	ddH_2O	48
1.25 uL	DMSO 100%	0.05	3.75 uL	DMSO 100%	3.75
2.5 uL	10x PCR buffer	0.1	7.5 uL	10x PCR buffer	7.5
2 uL	dNTPs (2.5mM each)	0.08	6 uL	dNTPs (2.5mM each)	6
1.5 uL	MgCl2	0.06	4.5 uL	MgCl2	4.5
0.5 uL	Pf, 5µM	0.02	1.5 uL	Pf, 5µM	**69.75**
0.5 uL	Pr, 5 µM	0.02	1.5 uL	Pr, 5 µM	
0.5 uL	DNA template	0.02	1.5 uL	DNA template	
0.25 uL	Polymerase	0.01	0.75 uL	Polymerase	
25 uL	**Total**	1	**75 uL**	**Total |mm per25 ul:**	23.25 25

Gradient PCR with DMSO to optimize p5p8 Reaction

p5 p8 68C, 69C, 70C

Notes: Genomic DNA BL2, and plasmid containing construct, cln5(13.11.15) 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)	3 x
Amount	Compound		Amount	Compound	
17.25 uL	ddH_2O	0.69	51.8 uL	ddH_2O	51.75
0 uL	DMSO 100%	0	0 uL	DMSO 100%	0
2.5 uL	10x PCR buffer	0.1	7.5 uL	10x PCR buffer	7.5
2 uL	dNTPs (2.5mM each)	0.08	6 uL	dNTPs (2.5mM each)	6
1.5 uL	MgCl2	0.06	4.5 uL	MgCl2	4.5
0.5 uL	Pf, 5µM	0.02	1.5 uL	Pf, 5µM	**69.75**
0.5 uL	Pr, 5 µM	0.02	1.5 uL	Pr, 5 µM	
0.5 uL	DNA template	0.02	1.5 uL	DNA template	
0.25 uL	Polymerase	0.01	0.75 uL	Polymerase	
25 uL	**Total**	1	**75 uL**	**Total |mm per25 ul:**	23.25 25