

19/02/2016

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Additive: DMSO				5	%				
50 uL		1X_65ul 1.5ul Mg+		1	x	Master Mix (no primers)		10 x	
Amount		Compound				Amount	Compound		
32	uL	ddH ₂ O		0.64		320	uL ddH ₂ O	320	
2.5	uL	DMSO 100%		0.05		25	uL DMSO 100%	25	
5	uL	10x PCR buffer		0.1		50	uL 10x PCR buffer	50	
4	uL	dNTPs (2.5mM each)		0.08		40	uL dNTPs (2.5mM each)	40	
3	uL	MgCl ₂		0.06		30	uL MgCl ₂	30	
1	uL	Pf, 5μM		0.02		10	uL Pf, 5μM	465	
1	uL	Pr, 5 μM		0.02		10	uL Pr, 5 μM		
1	uL	DNA template		0.02		10	uL DNA template		
0.5	uL	Polymerase		0.01		5	uL Polymerase		
50	uL	Total		1		500	uL Total mm per25 ul:	46.5	50

Crp2 mutants + genomic wt colony PCR

2 temperature conditions to get rid of unspecific bands

Notes:

94 C - 5 min

72 C, 10 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	%				
0 uL		1X_65ul 1.5ul Mg+		1ul	x	Master Mix (no primers)		6 x	
Amount		Compound				Amount	Compound		
0	uL	ddH ₂ O		0.69		0	uL ddH ₂ O	0	
0	uL	DMSO 100%		0		0	uL DMSO 100%	0	
0	uL	10x PCR buffer		0.1		0	uL 10x PCR buffer	0	
0	uL	dNTPs (2.5mM each)		0.08		0	uL dNTPs (2.5mM each)	0	
0	uL	MgCl ₂		0.06		0	uL MgCl ₂	0	
0	uL	Pf, 5μM		0.02		0	uL Pf, 5μM	0	
0	uL	Pr, 5 μM		0.02		0	uL Pr, 5 μM		
0	uL	DNA template		0.02		0	uL DNA template		
0	uL	Polymerase		0.01		0	uL Polymerase		
0	uL	Total		1		0	uL Total mm per25 ul:	0	0