

22/02/2016

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Additive: DMSO		5	%			
50 uL		1X_65ul 1.5ul Mg+	1	x	Master Mix (no primers)	20 x
Amount	Compound		Amount	Compound		
32 uL	ddH ₂ O	0.64	640 uL	ddH ₂ O		640
2.5 uL	DMSO 100%	0.05	50 uL	DMSO 100%		50
5 uL	10x PCR buffer	0.1	100 uL	10x PCR buffer		100
4 uL	dNTPs (2.5mM each)	0.08	80 uL	dNTPs (2.5mM each)		80
3 uL	MgCl ₂	0.06	60 uL	MgCl ₂		60
1 uL	Pf, 5µM	0.02	20 uL	Pf, 5µM		930
1 uL	Pr, 5 µM	0.02	20 uL	Pr, 5 µM		
1 uL	DNA template	0.02	20 uL	DNA template		
0.5 uL	Polymerase	0.01	10 uL	Polymerase		
50 uL	Total	1	1000 uL	Total per tube ul:		46.5 50

Two reactions with A,B, and C,D primers: crp mutants screening

Long extention time

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