

24/02/2016

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Additive: DMSO		5	%		
0 uL	1X_65ul 1.5ul Mg+	1	x	Master Mix (no primers)	0 x
Amount	Compound			Amount	Compound
0 uL	ddH ₂ O	0.64	0 uL	ddH ₂ O	0
0 uL	DMSO 100%	0.05	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:	##### #DIV/0!

PCR products from 18 mutants(220216) + 2 controls used as a template
Due to observed non-specific bands at 67 C, reaction carried out at 69C

Notes:		(Tm primers (Angela) ~ 70, 72)			
94 C - 5 min					
94 C, 45 s: anneal C, 1 min: 69 C, 1 min: 31x					
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	%		
25 uL	1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)	22 x
Amount	Compound			Amount	Compound
17.3 uL	ddH ₂ O	0.69	380 uL	ddH ₂ O	379.5
0 uL	DMSO 100%	0	0 uL	DMSO 100%	0
2.5 uL	10x PCR buffer	0.1	55 uL	10x PCR buffer	55
2 uL	dNTPs (2.5mM each)	0.08	44 uL	dNTPs (2.5mM each)	44
1.5 uL	MgCl ₂	0.06	33 uL	MgCl ₂	33
0.5 uL	Pf, 5µM	0.02	11 uL	Pf, 5µM	511.5
0.5 uL	Pr, 5 µM	0.02	11 uL	Pr, 5 µM	
0.5 uL	DNA template	0.02	11 uL	DNA template	
0.25 uL	Polymerase	0.01	5.5 uL	Polymerase	
25 uL	Total	1	550 uL	Total mm per25 ul:	23.25 25