

13/04/2016

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Additive: DMSO				0	%				
60 uL		1X_65ul 1.5ul Mg+		1ul	x	Master Mix (no primers)		8 x	
Amount	Compound					Amount	Compound		
41.4 uL	ddH <sub>2</sub> O			0.69		331 uL	ddH <sub>2</sub> O	331.2	
0 uL	DMSO 100%			0		0 uL	DMSO 100%	0	
6 uL	10x PCR buffer			0.1		48 uL	10x PCR buffer	48	
4.8 uL	dNTPs (2.5mM each)			0.08		38.4 uL	dNTPs (2.5mM each)	38.4	
3.6 uL	MgCl <sub>2</sub>			0.06		28.8 uL	MgCl <sub>2</sub>	28.8	
1.2 uL	Pf, 5µM			0.02		9.6 uL	Pf, 5µM	446.4	
1.2 uL	Pr, 5 µM			0.02		9.6 uL	Pr, 5 µM		
1.2 uL	DNA template			0.02		9.6 uL	DNA template		
0.6 uL	Polymerase			0.01		4.8 uL	Polymerase		
60 uL	Total			1		480 uL	Total  mm per25 ul:	55.8	60

Here are a recipe for master mix and cycling conditions for 18S:

Reaction mixture with Pfu polymerase per 30ul reaction:

water 22.23ul

buffer 3ul

dNTPs 0.2ul of a 25uM solution

forward primer 1.56ul of a 10uM solution

reverse primer 1.56ul of a 10uM solution

Pfu polymerase 0.25ul

template DNA 1ul

PCR conditions:

1a light colony pcr from phototrophs 16s

1b light colony pcr from phototrophs 18s

2a dark colony pcr from phototrophs 16s

2b dark colony pcr from phototrophs 16s

3a crp2-2 plate 16s

3b crp2-2 plate 18s

4a bl2 16s

4b bl2 18s

95C for 5 minutes,10 cycles of touchdown PCR: 95C for 30s, 60C for 30s (decreasing at 0.5C/cycle), and 72C for 30s, followed by 30 cycles: 95C for 30s, 55C for 30s, and 72C for 30s, and 72C for 5 minutes.

Cheers,				0	%				
40 uL		Angela.		1ul	x	Master Mix (no primers)		0 x	
Amount	Compound					Amount	Compound		
27.6 uL	ddH <sub>2</sub> O			0.69		0 uL	ddH <sub>2</sub> O	0	
0 uL	DMSO 100%			0		0 uL	DMSO 100%	0	
4 uL	10x PCR buffer			0.1		0 uL	10x PCR buffer	0	
3.2 uL	dNTPs (2.5mM each)			0.08		0 uL	dNTPs (2.5mM each)	0	
2.4 uL	MgCl <sub>2</sub>			0.06		0 uL	MgCl <sub>2</sub>	0	
0.8 uL	Pf, 5µM			0.02		0 uL	Pf, 5µM	0	
0.8 uL	Pr, 5 µM			0.02		0 uL	Pr, 5 µM		
0.8 uL	DNA template			0.02		0 uL	DNA template		
0.4 uL	Polymerase			0.01		0 uL	Polymerase		
40 uL	Total			1		0 uL	Total  mm per25 ul:	0	2.8