

22/04/2016

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Additive: DMSO		0	%		
35 uL	1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)	
Amount	Compound			Amount	Compound
24.85 uL	ddH <sub>2</sub> O	0.71		49.7 uL	ddH <sub>2</sub> O
0 uL	DMSO 100%	0		0 uL	DMSO 100%
3.5 uL	10x PCR buffer	0.1		7 uL	10x PCR buffer
2.8 uL	dNTPs (2.5mM each)	0.08		5.6 uL	dNTPs (2.5mM each)
2.1 uL	MgCl <sub>2</sub>	0.06		4.2 uL	MgCl <sub>2</sub>
0.35 uL	Pf, 5µM	0.01		0.7 uL	Pf, 5µM
0.35 uL	Pr, 5 µM	0.01		0.7 uL	Pr, 5 µM
0.7 uL	DNA template	0.02		1.4 uL	DNA template
0.35 uL	Polymerase	0.01		0.7 uL	Polymerase
35 uL	Total	1		70 uL	Total  mm per25 ul:

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

Reaction mixture with Pfu polymerase per 30ul reaction:

water 22.23ul

Primer: 100 nm: use twice less : 0.6 uL

buffer 3ul

1a light colony pcr from phototrophs 16s

dNTPs 0.2ul of a 25uM solution

1b light colony pcr from phototrophs 18s

forward primer 1.56ul of a 10uM solution

2a dark colony pcr from phototrophs 16s

reverse primer 1.56ul of a 10uM solution

2b dark colony pcr from phototrophs 16s

Pfu polymerase 0.25ul

3a crp2-2 plate 16s

template DNA 1ul

3b crp2-2 plate 18s

4a bl2 16s

PCR conditions:

4b bl2 18s

!!!Primers are 100 uM, therefore used twice less!!!

For template 1:50 diluted DNA from genomic extractions was used

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 5 minutes. 30s, and 72C for 5 minutes.

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