

18/04/2016

18/04/2016 15:01

Additive: DMSO		0	%			
60 uL	1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)		2 x
Amount	Compound			Amount	Compound	
41.4 uL	ddH <sub>2</sub> O	0.69		82.8 uL	ddH <sub>2</sub> O	82.8
0 uL	DMSO 100%	0		0 uL	DMSO 100%	0
6 uL	10x PCR buffer	0.1		12 uL	10x PCR buffer	12
4.8 uL	dNTPs (2.5mM each)	0.08		9.6 uL	dNTPs (2.5mM each)	9.6
3.6 uL	MgCl <sub>2</sub>	0.06		7.2 uL	MgCl <sub>2</sub>	7.2
1.2 uL	Pf, 5µM	0.02		2.4 uL	Pf, 5µM	111.6
1.2 uL	Pr, 5 µM	0.02		2.4 uL	Pr, 5 µM	
1.2 uL	DNA template	0.02		2.4 uL	DNA template	
0.6 uL	Polymerase	0.01		1.2 uL	Polymerase	
60 uL	Total	1		120 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	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
	4b bl2 18s

PCR conditions:

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