

25/05/2016

25/05/2016 13:18

Additive: DMSO		0	%			
40 uL	1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)		20 x
Amount	Compound			Amount	Compound	
28.64 uL	ddH ₂ O	0.716		573 uL	ddH ₂ O	572.8
0 uL	DMSO 100%	0		0 uL	DMSO 100%	0
4 uL	10x PCR buffer	0.1		80 uL	10x PCR buffer	80
3.2 uL	dNTPs (2.5mM each)	0.08		64 uL	dNTPs (2.5mM each)	64
2.4 uL	MgCl ₂	0.06		48 uL	MgCl ₂	48
0.4 uL	Pf, 5µM	0.01		8 uL	Pf, 5µM	764.8
0.4 uL	Pr, 5 µM	0.01		8 uL	Pr, 5 µM	
0.8 uL	DNA template	0.02		16 uL	DNA template	
0.16 uL	Polymerase	0.004		3.2 uL	Polymerase	
40 uL	Total	1		800 uL	Total mm per25 ul:	38.24 40

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

1 - 1a that didn't work (60 m)

dNTPs 0.2ul of a 25uM solution

2 - negative control - no template

forward primer 1.56ul of a 10uM solution

(probably less than 60 ml)

reverse primer 1.56ul of a 10uM solution

Pfu polymerase 0.25ul

template DNA 1ul

PCR conditions:

Cheers,		0	%			
40 uL	Angela.	1ul	x	Master Mix (no primers)		0 x
Amount	Compound			Amount	Compound	
27.6 uL	ddH ₂ O	0.69		0 uL	ddH ₂ 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 ₂	0.06		0 uL	MgCl ₂	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

