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Additive: DMSO 0 %

25 uL	1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)	2 x
Amount	Compound		Amount	Compound	
17.25 uL	ddH_2O	0.69	34.5 uL	ddH_2O	34.5
0 uL	DMSO 100%	0	0 uL	DMSO 100%	0
2.5 uL	10x PCR buffer	0.1	5 uL	10x PCR buffer	5
2 uL	dNTPs (2.5mM each)	0.08	4 uL	dNTPs (2.5mM each)	4
1.5 uL	MgCl2	0.06	3 uL	MgCl2	3
0.5 uL	Pf, 5µM	0.02	1 uL	Pf, 5µM	**46.5**
0.5 uL	Pr, 5 µM	0.02	1 uL	Pr, 5 µM	
0.5 uL	DNA template	0.02	1 uL	DNA template	
0.25 uL	Polymerase	0.01	0.5 uL	Polymerase	
25 uL	**Total**	1	**50 uL**	**Total |mm per25 ul:**	23.25 25

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

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

2 - negative control - no template (probably less than 60 ml)

PCR conditions:

Cheers, 0 %

40 uL	Angela.	1ul	x	Master Mix (no primers)	0 x
Amount	Compound		Amount	Compound	
27.6 uL	ddH_2O	0.69	0 uL	ddH_2O	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	MgCl2	0.06	0 uL	MgCl2	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

8.25