

26/04/2016

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Additive: DMSO		0	%			
30 uL	1X_65ul 1.5ul Mg+	1ul	x	Master Mix (no primers)		4 x
Amount	Compound			Amount	Compound	
20.18 uL	ddH ₂ O	0.69	80.7 uL	ddH ₂ O	80.72	
0 uL	DMSO 100%	0	0 uL	DMSO 100%	0	
3 uL	10x PCR buffer+Mg	0.1	12 uL	10x PCR buffer	12	
2.4 uL	dNTPs (2.5mM each)	0.08	9.6 uL	dNTPs (2.5mM each)	9.6	
0 uL	MgCl ₂	0	0 uL	MgCl ₂	0	
1.56 uL	Pf, 10µM	0.052	6.24 uL	Pf, 5µM	102.3	
1.56 uL	Pr, 10µM	0.052	6.24 uL	Pr, 5 µM		
1 uL	DNA template	0.0333	4 uL	DNA template		
0.3 uL	Polymerase	0.01	1.2 uL	Polymerase		
30 uL	Total	1.0173	120 uL	Total mm per25 ul:	25.58	30

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. 30s, and 72C for 5 minutes.

No master mix, 1, 2 - concentrated template, phototroph and yeast

3,4 - 50x diluted template phototroph and yeast

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

0.156	30	
0.0052	1	0.0104