

PCR (bisulfite primeroptimization)
Temp Gradient → NO Reads

06172014
mg

MgCl ₂	1	2	3	4	5	6	7	8	9	10	11	12
1.5	X			X	✓	B	X		X			X
3.0						B						
4.5						B						
1.5						B						
3.0						B						
4.5						B						
							[X	B	X	X		X]

3.0 MgCl₂
733

Primer Sets: 748, 44098

	15M MgCl ₂	3.0mM MgCl ₂	4.5mM MgCl ₂
10x PCR buffer	2.5 × 8 = 20	= 20	= 20
10mM dNTP	0.5 = 4.0	= 4.0	= 4.0
PF	0.5 = 4.0	= 4.0	= 4.0
PR	0.5 = 4.0	= 4.0	= 4.0
Platinum polymerase	0.2 = 1.6	= 1.6	= 1.6
MgCl ₂	0.75 = 6	(1.5) = 12	(2.25) = 18
H ₂ O	19.05 = 152.4	(18.3) = 146.4	(17.55) = 140.4

primer stocks

Template

5.0 μl

45.0 mL H₂O

50.0 μl @ 10mM

Primer Set 733 @ 3.0 MgCl₂

733

10x PCR buffer	2.5 × 6 = 15.0
10mM dNTP	0.5 = 3.0
PF	0.5 = 3.0
PR	0.5 = 3.0
Plat. pol	0.2 = 1.2
MgCl ₂	1.5 = 9.0
H ₂ O	18.3 = 109.8