Syn33 g20 PCR

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Abstract

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Guidelines

Modified from Marcia Osburne

F1 = 5’-CTTCCTCGTGAAGGTGGAA-3’ (“published”)

R1 = 5’-CAGCGAAGTAATTGTCTGCAATATAATC-3’ (“published”)

F2 = 5’-GTTGCCTAGAAGAGAAGGTGGTCG-3’ (“genomic”)

R2 = 5’-CGGTGAAGTAGTTATCAGCAATGAAGTC-3’ (“genomic”)

Amplicons F1/R1 = 356bp and F2/R2 = 354bp

Marcia Osburne protocol (from 1/12/10 email):

Amt.	Reagent	Final Conc.	Cycling	
2µl	template (i.e., lysate)		94°C, 5min, then add Taq	
5µl	10 x Taq Buffer	1x		
2.5µl	10mM dNTP	0.5mM	94°C, 1 min	
1µl	100µM 5' primer	2µM	52°C, 1.5 min	35x
1µl	100µM 3' primer	2µM	72°C, 1.5 min	
2.5µl	50mM $MgCl_2$	2.5mM		
35.5µl	H_2O	50µl reaction volume	72°C, 10 min	
0.5µl	Taq (add after hot start)	2.5U/reaction	4°C, hold	

Takara LA HS Taq protocol:

Amt.	Reagent	Final Conc.	Cycling
2µl	template (i.e., lysate)		94°C, 5min
5µl	10 x Taq HS LA Buffer	1x	

2.5µl	10mM ea. dNTP	0.5mM ea.	94°C, 30 sec
1µl	100µM 5' primer	2µM	52°C, 1.5 min 35x
1µl	100µM 3' primer	2µM	72°C, 1.5 min
N/A	$MgCl_2$	2.5mM	
35.5µl	H_2O	50µl reaction volume	72°C, 10 min
0.5µl	Takara LA Taq (5U/µl)	2.5U/reaction	15°C, hold

Sigma Taq protocol:

Amt.	Reagent	Final Conc.	Cycling	
1µl	template (i.e., lysate)		94°C, 5min, then add taq	
2.5µl	10 x Buffer w/o $MgCl_2$	1x		
1.25µl	10mM ea. dNTP	0.5mM ea.	94°C, 30 sec	
0.5µl	100µM 5' primer	2µM	52°C, 1.5 min	35x
0.5µl	100µM 3' primer	2µM	72°C, 1.5 min	
2.5µl	25mM $MgCl_2$	2.5mM		
16.5µl	H_2O	25µl reaction volume	72°C, 10 min	
0.25µl	Sigma Taq (5U/µl)	2.5U/reaction	15°C, hold	

Note: most "10mM dNTP" are 2.5mM per dNTP; try decreasing Sigma 10mM ea. dNTP (at. D7295) to 1µl/50µl reation which will be 0.2mM ea. in final reaction.

Conclusions from 1/18/2010 Exp. 108:

* Primers F1/R1 amplify JW Syn 26 lysate
* Primers F2/R2 amplify MBS Syn33a lysate
* JW Syn33 lysate not amplified by either primer set

Protocol