

Jan 21, 2016

Syn33 g20 PCR

DOI

<https://dx.doi.org/10.17504/protocols.io.dai2cd>

Marcia Osburne¹

¹Matthew Sullivan Lab, University of Arizona/Ohio State University

VERVE Net

Sullivan Lab



Verve Team

University of Arizona

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.dai2cd>

Protocol Citation: Marcia Osburne 2016. Syn33 g20 PCR. protocols.io <https://dx.doi.org/10.17504/protocols.io.dai2cd>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: July 06, 2015

Last Modified: November 09, 2017

Protocol Integer ID: 1066

Keywords: syn33 g20 pcr, g20

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	35x
	1µl	100µM 5' primer	2µM	52°C, 1.5 min	
	1µl	100µM 3' primer	2µM	72°C, 1.5 min	
	2.5µl	50mM MgCl ₂	2.5mM		
	35.5µl	H ₂ O	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	35x
	1µl	100µM 5' primer	2µM	52°C, 1.5 min	
	1µl	100µM 3' primer	2µM	72°C, 1.5 min	
	N/A	MgCl ₂	2.5mM		
	35.5µl	H ₂ O	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	35x
	0.5µl	100µM 5' primer	2µM	52°C, 1.5 min	
	0.5µl	100µM 3' primer	2µM	72°C, 1.5 min	
	2.5µl	25mM MgCl ₂	2.5mM		
	16.5µl	H ₂ O	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 reaction 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

