

Visualization of Replisome Encounters with an Antigen Tagged Blocking Lesion

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Citation

Zhang, J., Huang, J., Majumdar, I., James, R.C., Gichimu, J., Paramasivam, M., Pokharel, D., Gali, H., Bellani, M.A., Seidman, M.M. Visualization of Replisome Encounters with an Antigen Tagged Blocking Lesion. *J. Vis. Exp.* (173), e61689, doi:10.3791/61689 (2021).

Date Published

July 27, 2021

DOI

10.3791/61689

URL

jove.com/video/61689

Materials

Name	Company	Catalog Number	Comments
Alexa Fluor 568, Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody	Invitrogen	A-10011	1 in 1000
35 mm plates with glass 1.5 coverslip	MatTek	P35-1.5-14-C	Glass Bottom Microwell Dishes 35mm Petri Dish Microwell
Alexa Fluor 488, Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody	Invitrogen	A-10001	1 in 1000
Bovine serum albumin (BSA)	SeraCare	1900-0012	Blocking solution, reagents need to be stored at 4 °C
CDC45 antibody (rabbit)	Abcam	ab126762	1 in 200
Cell adhesive	Life Science	354240	for cell-TAK solution
Confocal microscope	Nikon	Nikon TE2000 spinning disk microscope	equipped with Velocity software
Digoxigenin (Dig) antibody (mouse)	Abcam	ab420	1 in 200
Dig-TMP	synthesized in the Seidman Lab		
Duolink Amplification reagents (5×)	Sigma-Aldrich	DUO82010	reagents need to be stored at -20 °C
Duolink in situ detection reagents	Sigma-Aldrich	DUO92007	reagents need to be stored at -20 °C
Duolink in situ oligonucleotide PLA probe MINUS	Sigma-Aldrich	DUO92004	anti-mouse MINUS, reagents need to be stored at 4 °C
Duolink in situ oligonucleotide PLA probe PLUS	Sigma-Aldrich	DUO92002	anti-rabbit PLUS, reagents need to be stored at 4 °C
Duolink in situ wash buffer A	Sigma-Aldrich	DUO82046	Duolink Wash Buffers, reagents need to be stored at 4 °C
Duolink in situ wash buffer B	Sigma-Aldrich	DUO82048	Duolink Wash Buffers, reagents need to be stored at 4 °C

epifluorescent microscope	Zeiss	Axiovert 200M microscope	Equipped with the Axio Vision software packages (Zeiss, Germany)
Formaldehyde 16%	Fisher Scientific	PI28906	for fix solution
Goat serum	Thermo	31873	Blocking solution, reagents need to be stored at 4 °C
Image analysis software	open source	Cell profiler	works for analysis of single plane images
Image analysis software-license required	Bitplane	Imaris	Cell Biology module needed. Can quantify PLA dots/nuclei in image stacks (3D) and do 3D reconstructions
Ligase (1 unit/μl)	Sigma-Aldrich	DUO82029	reagents need to be stored at -20 °C
Ligation reagent (5×)	Sigma-Aldrich	DUO82009	reagents need to be stored at -20 °C
MCM2 antibody (rabbit)	Abcam	ab4461	1 in 200
MCM5 antibody (rabbit monoclonal)	Abcam	Ab75975	1 in 1000
Methanol	Lab ALLEY	A2076	pre-cold at -20°C before use
phosphoMCM2S108 antibody (rabbit)	Abcam	ab109271	1 in 200
Polymerase (10 unit/μl)	Sigma-Aldrich	DUO82030	reagents need to be stored at -20 °C
Prolong gold mounting media with DAPI	ThermoFisher Scientific	P36935	
PSF1 antibody (rabbit)	Abcam	ab181112	1 in 200
RNAse A 100 mg/ml	Qiagen	19101	reagents need to be stored at 4 °C
Statistical analysis and data visualization software	open source	R studio	ggplot2 package for generation of dot plot and box plots
Statistical analysis and data visualization software-license required	Systat Software	Sigmaplot V13	
TMP (trioxalen)	Sigma-Aldrich	T6137_1G	
TritonX-100	Sigma-Aldrich	T8787_250ML	
Tween 20	Sigma-Aldrich	P9416_100ML	
UV box	Southern New England Ultraviolet		Discontinued. See Opsytec UV test chamber as a possible replacement
UV test Chamber	Opsytec	UV TEST CHAMBER BS-04	
VE-821	Selleckchem	S8007	final concentrtrion is 1μM