

High-Throughput Image-Based Quantification of Mitochondrial DNA Synthesis and Distribution

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Citation

Borowski, L.S., Kasztelan, K., Czerwinska-Kostrzewska, J., Szczesny, R.J. High-Throughput Image-Based Quantification of Mitochondrial DNA Synthesis and Distribution. *J. Vis. Exp.* (195), e65236, doi:10.3791/65236 (2023).

Date Published

May 5, 2023

DOI

10.3791/65236

URL

jove.com/video/65236

Materials

Name	Company	Catalog Number	Comments
2',3'-Dideoxycytidine (ddC)	Sigma-Aldrich	D5782	
384 Well Cell Culture Microplates, black	Greiner Bio-One	#781946	
5-Bromo-2'-deoxyuridine (BrdU)	Sigma-Aldrich	B5002-1G	Dissolve BrdU powder in water to 20 mM stock solution and aliquot. Use 20 µM BrdU solution for labeling.
Adhesive sealing film	Nerbe Plus	04-095-0060	
Alexa Fluor 488 goat anti-mouse IgG1 secondary antibody	Thermo Fisher Scientific	A-21121	
Alexa Fluor 555 goat anti-mouse IgM secondary antibody	Thermo Fisher Scientific	A-21426	
BioTek 405 LS microplate washer	Agilent		
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A4503	
Cell counting chamber Thoma	Heinz Herenz	REF:1080339	
Dulbecco's Modified Eagle Medium (DMEM)	Cytiva	SH30243.01	
Dulbecco's Modified Eagle Medium (DMEM)	Thermo Fisher Scientific	41965-062	
Fetal Bovine Serum (FBS)	Thermo Fisher Scientific	10270-106	
Formaldehyde solution	Sigma-Aldrich	F1635	Formaldehyde is toxic; please read the safety data sheet carefully.
Hoechst 33342	Thermo Fisher Scientific	H3570	
IgG1 mouse monoclonal anti-BrdU (IIB5) primary antibody	Santa Cruz Biotechnology	sc-32323	
IgM mouse monoclonal anti-DNA (AC-30-10) primary antibody	Progen	#61014	
LightCycler 480 System	Roche		
Lipofectamine RNAiMAX Transfection Reagent	Thermo Fisher Scientific	#13778150	
MitoTracker Deep Red FM	Thermo Fisher Scientific	M22426	Mitochondria tracking dye

Multidrop Combi Reagent Dispenser	Thermo Fisher Scientific		
Opti-MEM	Thermo Fisher Scientific	51985-042	
Orca-R2 (C10600) CCD Camera	Hamamatsu		
Penicillin-Streptomycin	Sigma-Aldrich	P0781-100ML	
Phosphate buffered saline (PBS)	Sigma-Aldrich	P4417-100TAB	
PowerUp SYBR Green Master Mix	Thermo Fisher Scientific	A25742	
qPCR primer Fw B2M (reference)			CAGGTACTCCAAAGATTCAGG
qPCR primer Fw GPI (reference gene)			GACCTTTACTACCCAGGAGA
qPCR primer Fw MT-ND1			TAGCAGAGACCAACCGAACC
qPCR primer Fw POLG			TGGAAGGCAGGCATGGTCAAACC
qPCR primer Fw TFAM			GATGAGTTCTGCCTGCTTTAT
qPCR primer Fw TWNK			GCCATGTGACACTGGTCATT
qPCR primer Rev B2M (reference)			GTCAACTTCAATGTCGGATGG
qPCR primer Rev GPI (reference gene)			AGTAGACAGGGCAACAAAGT
qPCR primer Rev MT-ND1			ATGAAGAATAGGGCGAAGGG
qPCR primer Rev POLG			GGAGTCAGAACACCTGGCTTTGG
qPCR primer Rev TFAM			GGACTTCTGCCAGCATAATA
qPCR primer Rev TWNK			AACATTGTCTGCTTCCTGGC
ScanR microscope	Olympus		
siRNA Ctrl	Dharmacon	D-001810-10-5	
siRNA POLG	Invitrogen	POLGHSS108223	
siRNA TFAM	Invitrogen	TFAMHSS144252	
siRNA TWNK	Invitrogen	C10orf2HSS125597	
Suction device	NeoLab	2-9335	Suction device for cell culture
Triton X-100	Sigma-Aldrich	T9284-500ML	
Trypsin	Biowest	L0931-500	
UPlanSApo 20x 0.75 NA objective	Olympus		