

Laser Micro-Irradiation to Study DNA Recruitment During S Phase

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Materials

| Name | Company | Catalog Number | Comments |
|--|------------------------------|----------------|---|
| Ammonium chloride | Sigma-Aldrich | A9434-500G | For quenching formaldehyde |
| Anti-EXO1 Rabbit Polyclonal Antibody | Proteintech | 16253-1-AP | primary antibody |
| Anti-phospho-Histone H2A.X (Ser139) Antibody, clone JBW301 | Millipore | 05-636 | primary antibody |
| Bovine Serum Albumin | Sigma-Aldrich | 3117332001 | BSA for blocking |
| BrdU (5-Bromo-2'-deoxyuridine) | Merck | 19-160 | pre-sensitizing agent |
| Citifluor™ Mountant Solution AFR3 | Electron Microscopy Sciences | 17973-10 | antifade containing PBS solution for imaging |
| DAPI | Sigma-Aldrich | D9542-1MG | nucleic acid stain |
| DMEM Medium | Thermo Fisher Scientific | 10569010 | Cell culture medium for HEK293T cells |
| DMSO | Sigma-Aldrich | D2650-100ML | Vehicle control and dissolution solvent |
| EGFP-FBXL10 | Addgene | #126542 | viral expression vector for EGFP-FBXL10 |
| EXO1b-AcGFP (in pRetroQ) | custom cloning | na | EXO1b cDNA was cloned in the NheI, BamHI sites of pRetroQ-AcGFP1-N1 vector. |
| Fetal Bovine Serum | Gibco | 16140071 | Media supplement |
| FluoroBrite DMEM | Thermo Fisher Scientific | A1896701 | Phenol red free medium for microscopy |
| Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 | Thermo Fisher Scientific | A32723 | secondary antibody |
| HEK293T cells | ATCC | ATCC CRL-3216 | Cell line for viral packaging |
| HEPES | Sigma-Aldrich | H0887-100ML | Buffering agent to supplement live cell imaging medium |
| Hoehchst 33342 | Thermo Fisher Scientific | H3570 | pre-sensitizing agent |
| Lipofectamine 3000 | Thermo Fisher Scientific | L3000015 | Transfection reagent |
| McCoy's 5A (Modified) Medium | Life Technologies | 16600-108 | Cell culture medium for U-2 OS cells |

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|---|--------------------------|--------------|---|
| mCherry-PCNA | Addgene | #55117 | non-viral PCNA construct suitable for cell cycle marker |
| mPlum-PCNA | Addgene | #55994 | non-viral PCNA construct suitable for cell cycle marker |
| mPlum-PCNA (in pBABE) | custom cloning | na | mPlum-PCNA cDNA was cloned from Addgene #55994 in the BamHI, Sall sites of pBABE (puro) |
| Nikon A1R-HD25 Confocal Scanhead and Controller | Nikon | na | confocal imaging system |
| Nikon LUN4 laser unit | Nikon | na | excitation system |
| Nikon LUN-F 50 mW 405 nm FRAP laser unit | Nikon | na | FRAP laser unit |
| Nikon NIS Elements Confocal Controller Software | Nikon | na | Confocal controlling software |
| Nikon Ti2-E Inverted Microscope | Nikon | na | inverted epifluorescent microscope base |
| Nikon Ti2-LAPP Modular Illumination System | Nikon | na | illumination system |
| NTHL1-mCherry (in pRetroQ) | custom cloning | na | NTHL1 cDNA was cloned in the NheI, Sall sites of pRetroQ-mCherry-N1 vector. |
| Nunc Lab-Tek II Chambered Coverglass (4 well) | Thermo Fisher Scientific | 155382PK | Live cell microscopy cell culture chamber |
| Olaparib | Selleck Chemicals | S1060 | PARP inhibitor |
| Opti-MEM reduced serum media | Thermo Fisher Scientific | 31985062 | Dilution medium for transient transfection |
| Paraformaldehyde aqueous solution (32%) | Thermo Fisher Scientific | 50-980-494 | Fixative |
| pBABE (hygro) | Addgene | #1765 | retroviral expression vector (for low expression levels) |
| pBABE (neo) | Addgene | #1767 | retroviral expression vector (for low expression levels) |
| pBABE (puro) | Addgene | #1764 | retroviral expression vector (for low expression levels) |
| pBABE (zeo) | Addgene | #1766 | retroviral expression vector (for low expression levels) |
| PCNA Antibody (PC10) | Santa Cruz | sc-56 | primary antibody |
| Penicillin-Streptomycin-Glutamine (100x) | Gibco | 10378016 | Media supplement |
| polybrene | Sigma-Aldrich | TR-1003 | Increase viral infection efficiency |
| pRetroQ-AcGFP-C1 | Takara | 632506 | retroviral expression vector |
| pRetroQ-AcGFP-N1 | Takara | 632505 | retroviral expression vector |
| pRetroQ-mCherry-C1 | Takara | 632567 | retroviral expression vector |
| pRetroQ-mCherry-N1 | Takara | 632568 | retroviral expression vector |
| pUMVC | Addgene | #8449 | Viral packaging vector |
| Sodium-pyruvate | Thermo Fisher Scientific | 11360070 | Supplement for live cell imaging medium |
| Triton X-100 aqueous solution (10%) | Sigma-Aldrich | 11332481001 | Dilute in PBS for cell permeabilization buffer |
| Trypsin-EDTA Solution 10X | Sigma-Aldrich | 59418C-100ML | Dilute in PBS to split cells |
| U-2 OS Cells | ATCC | HTB-96 | Optimal cell line for microscopy experiments |
| Universal Mycoplasma Detection Kit | ATCC | 30-1012K | PCR based Mycoplasma detection kit |
| VSV-G | Addgene | #8454 | Viral protein envelope vector |