

Materials List for:

Visualizing Single-molecule DNA Replication with Fluorescence Microscopy

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Materials

Name	Company	Catalog Number	Comments
M13mp18 ssDNA	New England Biolabs	N4040	
Biotinylated Tail Oligo	Integrated DNA Technologies		
T7 DNA Polymerase	New England Biolabs	M0274	Use T7 replication buffer for substrate preparation
Phenol/ Isoamyl Alcohol/ Chloroform	Roche Group	03117987001	24:24:1 v/v
3-aminopropyl-triethoxysilane	Sigma-Aldrich	A3648	Other aminosilanes can be used or mixed with non-amine reactive silanes for sparser surfaces
Succinimidyl propionate PEG	Nektar		Similar PEGs can be purchased from Nanocs, CreativePEGWorks, etc.
Biotin-PEG-NHS	Nektar		
Double-sided tape	Grace Bio-Lab Inc.	SA-S-1L	100 µm thickness
Quartz slide	Technical Glass	20 mm (W)x 50 mm (L)x 1mm (H)	Size to fit on coverslips. Drill holes with diamond-tip drill bits (DiamondBurs.net)
Polyethylene tubing	BD Biosciences	427416	0.76 mm ID, 1.22 OD Other size tubing can be substituted.
Streptavidin	Sigma-Aldrich	S4762	Make 1 mg/mL solution, 25 µL aliquots in PBS pH 7.3
Deoxyribonucleotide triphosphate solution mix	New England Biolabs	N0447	
Ribonucleotide triphosphate solutions	Amersham	272056 272066 272076 272086	
SYTOX Orange	Invitrogen	S11368	Other dsDNA stain can be used
Fluorescence Microscope with 60x TIRF objective	Olympus Corporation	IX-71	Microscope, camera, etc. can be substituted for similar equipment
Syringe Pump	Harvard Apparatus	11 Plus	Operate in refill mode to facilitate solution changes
532 nm laser	Coherent Inc.	Compass 215M-75	Select wavelength to correspond to stain of choice
EMCCD Camera	Hamamatsu Corp.	ImagEM	
Emission filter	Chroma Technology Corp.	HQ600/75m	

T7 Replication: 40 mM Tris pH 7.5, 50 mM potassium glutamate, 10 mM magnesium chloride, 100 µg/mL BSA, with 5 mM dithiothreitol, 600 µM dNTPs, 300 µM ATP, 300 µM CTP, and 15 nM SYTOX Orange added immediately before use. Proteins added as: 5 nM gp4 (hexamer), 40 nM polymerase (1:1 gp5: thioredoxin), 360 nM gp2.5^{1,5}.

E. coli Replication: 50 mM HEPES pH 7.9, 12 mM magnesium acetate, 80 mM potassium chloride, 100 µg/mL BSA with 10 mM dithiothreitol, 40 µM dNTPs, 200 µM rNTPs, and 15 nM SYTOX Orange added immediately before use. Proteins added as: 30 nM DnaB (hexamer), 180 nM

DnaC (monomer), 30 nM $\alpha\epsilon\theta$, 15 nM $\tau_2\gamma_1\delta\delta'\chi\psi$ or $\tau_3\delta\delta'\chi\psi$, 30 nM β (dimer), 300 nM DnaG, 250 nM SSB (tetramer), 20 nM PriA, 40 nM PriB, 320 nM PriC, 480 nM DnaT^{1,6}

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