

Optical Tweezers to Study RNA-Protein Interactions in Translation Regulation

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Materials

Name	Company	Catalog Number	Comments
Bacterial Strains			
E. coli HB101	lab collection	N/A	cloning of the vectors
Chemicals and enzymes			
Sodium chloride	Sigma-Aldrich	31424	Buffers
Biotin-16-dUTP	Roche	11093070910	Biotinylation
BSA	Sigma-Aldrich	A4737	Buffers
Catalase	Lumicks	N/A	Oxygen scavenger system
Dithiothreitol (DTT)	Melford Labs	D11000	Buffers
DNase I from bovine pancreas	Sigma-Aldrich	D4527	in vitro transcription
dNTPs	Th.Geyer	11786181	PCR
EDTA	Sigma-Aldrich	E9884	Buffers
Formamide	Sigma-Aldrich	11814320001	Buffers
Glucose	Sigma-Aldrich	G8270-1KG	Oxygen scavenger system
Glucose-oxidase	Lumicks	N/A	Oxygen scavenger system
HEPES	Carl Roth	HN78.3	Buffers
Magnesium chloride	Carl Roth	2189.1	Buffers
Phusion DNA polymerase	NEB	M0530L	Gibson assembly, cloning
Potassium chloride	Merck	529552-1KG	Buffers
PrimeSTAR GXL DNA Polymerase	Takara Bio Clontech	R050A	PCR
Pyrophosphatase, thermostabile, inorganic	NEB	M0296L	in vitro transcription
RNase Inhibitor	Molox	1000379515	Buffers
rNTPS	life technologies	R0481	in vitro transcription
Sodium thiosulphate	Sigma-Aldrich	S6672-500G	Bleach deactivation
Sytox Green	Lumicks	N/A	confocal measurements
T4 DNA Polymerase	NEB	M0203S	Biotinylation
T5 exonuclease	NEB	M0363S	Gibson assembly, cloning

T7 RNA polymerase	Produced in-house	N/A	in vitro transcription
Taq DNA polymerase	NEB	M0267S	PCR
Taq ligase	Biozym	L6060L	Gibson assembly, cloning
TWEEN 20 BioXtra	Sigma-Aldrich	P7949	Buffers
Kits			
Monolith Protein Labeling Kit RED-NHS 2nd Generation (Amine Reactive)	Nanotemper	MO-L011	Used for ribosome labeling
Purefrex 2.0	GeneFrontier	PF201-0.25-EX	Ribosomes used for the labeling
Oligonucleotides			
5' handle T7 forward	Microsynth	custom order	5' - CTTAATACGACTCACTATAGGTC CTTTCTGTGGACGCC - 3', used to generate OT in vitro transcription template in PCR 1
3' handle reverse	Microsynth	custom order	5' - GTCAAAGTGCGCCCCGTTATCC - 3', used to generate OT in vitro transcription template in PCR 1
5' handle forward	Microsynth	custom order	5' - TCCTTTCTGTGGACGCCGC - 3', used to generate 5' handle in PCR 2
5' handle reverse	Microsynth	custom order	5' - CATAAATACCTCTTTACTAATATA TATACCTTCGTAAGCTAGCGT - 3', used to generate 5' handle in PCR 2
3' handle forward	Microsynth	custom order	5' - ATCCTGCAACCTGCTCTTCGCC AG - 3', used to generate 3' handle in PCR 3
3' handle reverse 5' labeled with digoxigenin	Microsynth	custom order	5' -[Dig]- GTCAAAGTGCGCCCCGTTATCC - 3', used to generate 3' handle in PCR 3
DNA vectors			
pMZ_OT	produced in-house	N/A	further description in "Structural studies of Cardiovirus 2A protein reveal the molecular basis for RNA recognition and translational control" Chris H. Hill, Sawsan Naphine, Lukas Pekarek, Anuja Kibe, Andrew E. Firth, Stephen C. Graham, Neva Caliskan, Ian Brierley bioRxiv 2020.08.11.245035; doi: https://doi.org/10.1101/2020.08.11.245035
Software and Algorithms			
Atom	https://atom.io/packages/ide-python	N/A	
Bluelake	Lumicks	N/A	
Graphpad	https://www.graphpad.com/	N/A	
InkScape 0.92.3	https://inkscape.org/	N/A	
Matlab	https://www.mathworks.com/products/matlab.html	N/A	
POTATO	https://github.com/lpekarek/POTATO.git	N/A	
RNAstructure	https://rna.urmc.rochester.edu/RNAstructure.html	N/A	
Spyder	https://www.spyder-ide.org/	N/A	
Other			

Streptavidin Coated Polystyrene Particles, 1.5-1.9 μm , 5 ml, 1.0% w/v	Spherotech	SVP-15-5	
Anti-digoxigenin Coated Polystyrene Particles, 2.0-2.4 μm , 2 ml, 0.1% w/v	Spherotech	DIGP-20-2	
Syringes	VWR	TERUMO SS+03L1	
Devices			
C-trap	Lumicks	N/A	optical tweezers coupled with confocal microscopy