

Materials List for

# Single-Molecule Dwell-Time Analysis of Restriction Endonuclease-Mediated DNA Cleavage

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## Materials

Name	Company	Catalog Number	Comments
3-aminopropyltriethoxysilane (APTS)	Sigma Aldrich	440140-100ML	Store in dessicator box
5 Minute Epoxy	Devcon	20845	use for sealing the microfluidic device
acetone	Pharmco	329000ACS	use for cleaning coverslips
bath sonicator	Fisher Scientific	CPXH Model 2800	catalog number 15-337-410
Beaker, glass, 100 mL			
Benchtop centrifuge			
Biotin-PEG-Succinimidyl Valerate (MW 5,000)	Laysan Bio	BIO-SVA-5K	Succinimidyl valerate has a longer half-life than succinimidyl carbonate
biotinylated oligonucleotide	Integrated DNA Technologies	custom - see protocol for design considerations	Request 5' Biotin modification and HPLC purification
Bovine Serum Albumin (BSA)	VWR	0903-5G	prepare a 10 mg/mL solution (aq) and heat to 95 °C before using
Centri-Spin-10 Size Exclusion Spin Columns	Princeton Separations	CS-100 or CS-101	used to purify thiolated oligonucleotides after reducing the disulfide bond
Centrifuge tubes 1.5. mL			
Coverslips, 1-inch square glass			
coverslip holders			
diamond point wheel	Dremel	7134	use for drilling holes in quartz flow cell topper
dithiothreitol (DTT)	Thermo Scientific	A39255	No-Weigh Format, 7.7 mg/vial
drill press rotary tool workstation stand	Dremel	220-01	facilitates quartz drilling
EcoRV (REase used to generate example data)	New England Biolabs	R0195T or R0195M	Use 100,000 units/mL stock to avoid adding excess glycerol Check REBASE for suppliers of other REases
ethanol	various	CAS 64-17-5	denatured or 95% are acceptable, use for cleaning coverslips
Ethylenediaminetetraacetic acid (EDTA)	Sigma Aldrich	EDS	BioUltra, anhydrous, store in dessicator box

Flea Micro Spinbar	Fisherbrand	14-513-65	3 mm x 10 mm size to fit beneath coverslip rack
fluorescein	Acros Organics	17324	use to make experimental buffers
gravity convection oven	Binder	9010-0131	
handheld rotary multitool	Dremel	8220	use for drilling holes in quartz flow cell topper
ImagEM X2 EM-CCD Camera	Hamamatsu	C9100-23B	air cooling is adequate for this experiment, use HCLImage software or similar to control
Imaging spacer, double-sided, adhesive			
Jar, glass with screw cap, (approximately 50 mm diameter by 50 mm high)			
magnesium chloride hexahydrate	Fisher Bioreagents	BP214-500	use to make experimental buffer with magnesium
MATLAB software			Data analysis
metal tweezers	Fisher Brand	16-100-110	
methoxy-PEG-Succinimidyl Valerate (MW 5,000)	Laysan Bio	M-SVA-5K	Both PEGs should have the same NHS ester so that the rate of reaction is consistent
microcentrifuge	Eppendorf	5424	
multiposition magnetic stirrer	VWR	12621-022	
N-cyclohexyl-2-aminoethanesulfonic acid (CHES)	Acros Organics	AC20818	CAS 103-47-9, use to make CHES buffer
orbital shaker and heater for microcentrifuge tubes	Q Instruments	1808-0506	with 1808-1061 adaptor for 24 x 2.0 mL or 15 x 0.5 mL tubes
Parafilm			
PE60 Polyethylene tubing (inner diameter 0.76 mm, outer diameter 1.22 mm)	Intramedic	6258917	22 G blunt needles are a good fit for this tubing size
Phosphate-Buffered Saline (PBS) 10x	Sigma Aldrich	P7059	Use at 1x strength
potassium hydroxide	VWR Chemicals BDH	BDH9262	use a 1 M solution to clean coverslips
Qdot 655 ITK Amino (PEG) Quantum Dots	Invitrogen	Q21521MP	
Quartz Slide, 1 inch square, 1 mm thick	Electron Microscopy Sciences	72250-10	holes must be drilled in the corners for inlet and outlet tubing insertion
reinforced plastic tweezers	Rubis	K35a	use for handling coverslips and building microfluidic device
SecureSeal Adhesive Sheets	Grace Biolabs	SA-S-1L	cut to form spacer for microfluidic device
Single channel syringe pump for microfluidics	New Era Pump Systems	NE-1002X-US	fitted with a 50 mL syringe and a 22 G blunt needle
Slide-a-Lyzer MINI Dialysis Devices, 10 kDa MWCO, 0.1 mL	Thermo Scientific	69570 or 69572	used for buffer exchange during quantum dot coupling to DNA
sodium bicarbonate	EMD Millipore	SX0320	use to make buffer for surface functionalization; 100 mM, pH 8
sodium chloride	Macron	7581-12	use to make experimental buffers
Sodium phosphate dibasic solution (BioUltra, 0.5 M in water)	Sigma Aldrich	94046	use to make 100 mM sodium phosphate buffer
Sodium phosphate monobasic solution (BioUltra, 5M in water)	Sigma Aldrich	74092	use to adjust pH of 100 mM sodium phosphate buffer
Streptavidin from <i>Streptomyces avidinii</i>	Sigma Aldrich	S4762	dissolve at 1 mg/mL and store 25 mL aliquots at -20 °C

Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC)	Thermo Scientific	A39268	No-Weigh Format, 2 mg/vial
Syringe fitted with blunt 21 G needle			
Syringe pump			
thiolated oligonucleotide	Integrated DNA Technologies	custom - see protocol for design considerations	Request 5' Thiol Modifier C6 S-S and HPLC purification
TIRF imaging system with 488 nm laser illumination	various		custom built
Tris -HCl	Research Products International	T60050	use to make experimental buffers
Tris base	JT Baker	4101	use to make experimental buffers
Tween-20	Sigma	P7949	use to make blocking buffer
Ultrapure water			
vortex mixer	VWR	10153-842	
Wash-N-Dry Coverslip Rack	Electron Microscopy Sciences	70366-16	used for surface functionalization of coverslips