

Materials List for:

Determining 3'-Termini and Sequences of Nascent Single-Stranded Viral DNA Molecules during HIV-1 Reverse Transcription in Infected Cells

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Materials

Name	Company	Catalog Number	Comments
293T cells	ATCC	CRL-3216	
Dulbecco's Modified Eagle's Medium	Gibco	31966-021	
Penicillin/Streptomycin	Gibco	15150-122	
Fetal Bovine Serum	Gibco	10270-106	
HeraCell Vios 250i CO2 Incubator	Thermo Scientific	51030966	
Laminar flow hood - CAS BioMAT2	Wolfabs	CAS001-C2R-1800	
10mm TC-treated culture dish	Corning	430167	
TrypLE™ Express (1x), Stable Trypsin Replacement Enzyme	Gibco	12605-010	
OptiMEM® (Minimal Essential Medium)	Gibco	31985-047	
HIV-1 NL4-3 Infectious Molecular Clone (pNL4-3)	NIH Aids reagent program	114	
Polyethylenimine (PEI) - MW:25000	PolySciences Inc	23966-2	dissolved at 1mg/ml and adjusted to pH7
RQ1- Rnase free Dnase	Promega	M6101	
Filter 0.22 µm	Triple Red Limited	FPE404025	
15 mL polypropylene tubes	Corning	CLS430791	
Sucrose	Calbiochem	573113	
Phosphate Buffered Saline (1x)	Gibco	14190-094	
Ultracentrifuge tubes	Beckman Coulter	344060	
Ultracentrifuge	Sorval	WX Ultra Series	Th-641 Rotor
Alliance HIV-1 p24 antigen ELISA kit	Perkin Elmer	NEK050001KT	
CEM-SS cells	NIH Aids reagent program	776	
Roswell Park Memorial Institute Medium	Gibco	31870-025	
CoStar® TC treated multiple well plates	Corning	CLS3513-50EA	
Benchtop centrifuge: Heraeus™ Multifuge™ X3 FR	Thermo Scientific	75004536	
TX-1000 Swinging Bucket Rotor	Thermo Scientific	75003017	
Microcentrifuge: 5424R	Eppendorf	5404000060	
Total DNA extraction kit (DNeasy Blood and Tissue kit)	Qiagen	69504	
Nuclease free H2O	Ambion	AM9937	

Cutsmart buffer	New England Biolabs (part of DpnI enzyme)	R0176S	
DpnI restriction enzyme	New England Biolabs	R0176S	
Oligonucleotides for qPCR	MWG Eurofins	N/A	HPSF purification
TaqMan PCR Universal Mastermix	Thermo	4304437	
LoBind Eppendorf® tubes	Eppendorf	30108078	
Axygen™ aerosol filter pipette tips, 1000 µL	Fisher Scientific	TF-000-R-S	
Axygen™ aerosol filter pipette tips, 200 µL	Fisher Scientific	TF-200-R-S	
Axygen™ aerosol filter pipette tips, 20 µL	Fisher Scientific	TF-20-R-S	
Axygen™ aerosol filter pipette tips, 10 µL	Fisher Scientific	TF-10-R-S	
PCR clean hood	LabCaire	Model PCR-62	
DynaMag™2-magnet	Thermo	12321D	
Streptavidin MagneSphere® paramagnetic particles	Promega	Z5481	
Casein	Thermo Scientific	37582	
End over end rotator, Revolver™ 360°	Labnet	H5600	
Tris-Base	Fisher Scientific	BP152-5	
Hydrochloric Acid	Sigma	H1758-100ML	
EDTA disodium salt dihydrate	Electran (VWR)	443885J	
Sodium Chloride	Sigma	S3014	
Dri-Block® Analog Block Heater	Techne	UY-36620-13	
PCR tubes and domed caps	Thermo Scientific	AB0266	
PCR machine	Eppendorf	Mastercycler® series	
T4 DNA ligase	New England Biolabs	M0202M	
40% Polyethylene glycol solution (PEG) in H ₂ O, MW: 8000	Sigma	P1458-25ML	
Betaine solution, 5M	Sigma	B0300-1VL	
Gel loading buffer II (formamide buffer)	Thermo Scientific	AM8546G	
Precast 6% TBE urea gels	Invitrogen	EC6865BOX	
Mini cell electrophoresis system	Invitrogen, Novex	XCell SureLock™	
Tris/Borate/EDTA solution (10x)	Fisher Scientific	10031223	
Needle 21 G x1 1/2	VWR	613-2022	
SYBR Gold nucleic acid stain (10000x)	Life Technologies	S11494	
Dark Reader DR46B transilluminator	Fisher Scientific	NC9800797	
Ammonium acetate	Merck	101116	
SDS solution 20% (w/v)	Biorad	161-0418	
Centrifuge tube filter	Appleton Woods	BC591	
Filter Glass Fibre Gf/D 10mm	Whatman (VWR)	512-0427	
polyadenylic acid (polyA) RNA	Sigma	10108626001	
Glycogen, molecular biology grade	Thermo Scientific	R0561	
Isopropanol (2-propanol)	Fisher Scientific	15809665	

Ethanol, molecular biology grade	Fisher Scientific	10041814	
Accuprime™ Supermix I (DNA polymerase premix)	Life Technologies	12342-010	
NEBNext® Multiplex Oligo for Illumina (Index Primer Set 1 and 2)	New England Biolabs	E7335S; E7500S	
Tapestation D1000 Screentape High sensitivity	Agilent Technologies	5067- 5584	
Tapestation D1000 Reagents	Agilent Technologies	5067- 5585	
2200 Tapestation - automated gel electrophoresis system	Agilent Technologies	G2965AA	
Agencourt® AMPure® beads XP	Beckman Coulter	A63880	
Qubit™ dsDNA HS Assay Kit	Invitrogen	Q32851	
Qubit™ 2.0 Fluorometer	Invitrogen	Q32866	
Topo™ TA cloning Kit	Invitrogen	450071	
Sequencing platform: MiSeq System	Illumina		
Experiment Manager (Sample sheet software)	Illumina	Note: Use TruSeq LT as a template	
Miseq™ Reagent kit V3 (150 cycle)	Illumina	MS-102-3001	
Sequencing hub: Basespace	Illumina	https://basespace.illumina.com	
<i>Ligase A: Thermostable 5' App DNA/RNA ligase</i>	NEB	M0319S	Not used in this protocol, but tested in optimization process with results described in the discussion.
<i>Ligase B: T4 RNA ligase 1</i>	NEB	M0204	Not used in this protocol, but tested in optimization process with results described in the discussion.
<i>Ligase C: CircLigase</i>	Epicentre	CL4111K	Not used in this protocol, but tested in optimization process with results described in the discussion.