

Materials List for:

Pairwise Growth Competition Assay for Determining the Replication Fitness of Human Immunodeficiency Viruses

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URL: <https://www.jove.com/video/52610>

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Materials

Name	Company	Catalog Number	Comments
Construction of recombinant clones			
Chimeric primer/Mutagenic/Sequencing primers	IDT		Custom DNA oligos
pNL4-3VifA and pNL4-3VifB plasmid			Please contact Dr. James I Mullins (jmullins@uw.edu)
High-Fidelity DNA polymerase	Thermo Scientific	F-549S	
High-fidelity buffer	Thermo Scientific	F-549S	
dNTP	Bioline	Bio-39026	
Thermal cycler	Life Technologies		Applied Biosystems® GeneAmp® PCR System 9700
DNA loading dye	Thermo Scientific	R0631	
1 kb Plus DNA ladder	Invitrogen	10787-018	
QIAquick PCR Purification Kit	Qiagen	28104	
QIAquick gel extraction kit	Qiagen	28704	
DpnI enzyme	New England Biolabs	R0176S	
TOP10 chemically competent <i>Escherichia coli</i>	Invitrogen	C4040-10	
Luria Broth base powder	Invitrogen	12795-084	
Carbenicillin	Research Products International	C46000-25.0	
QIAprep Spin Miniprep kit	Qiagen	27104	
EndoFree Plasmid Maxi Kit	Qiagen	12362	
Transfection			
X-tremeGENE 9 DNA transfection reagent	Roche	6365787001	
HEK 293T-17	ATCC	CRL-11268	http://www.atcc.org/
0.22 µm filter top tube	VWR International	89220-716	
Cell culture			
Dulbecco's Modified Eagle Medium (DMEM)	Life Technologies	10566016	
Iscove's Modified Dulbecco's Medium (IMDM)	Life Technologies	31980-030	
RPML-1640 media	Life Technologies	61870-036	
Phytohemagglutinin (PHA)	Thermo Scientific	R30852801	

Human Interleukin-2	Roche	11147528001	
Fetal bovine serum	JR Scientific	43640	
Penicillin and Streptomycin	Corning Cellgro	30-001-CI	
6 well plate	VWR Scientific	73520-906	
48 well plate	VWR Scientific	62407-338	
96 well flat-bottomed plate	ISC Bioexpress	T-3015-4	
96 well round-bottomed plate	BD Falcon	353077	
1.5 ml Microcentrifuge Tube	Mt. Baker Bio	MBD-1500	
1.5 ml tubes w/ O-ring	VWR Scientific	89004-290	
50 ml conical tube	ISC Bioexpress	C-3317-6	
ELISA			
Triton-X 100	Sigma-Aldrich	X100	
Phosphate buffer saline (PBS)	Invitrogen	14190-250	
Fetal bovine serum (FBS)	Sigma-Aldrich	F0392	
glycerol	Sigma-Aldrich	G5516	
Tween 20	Sigma-Aldrich	P1379	
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A2153	
Rabbit anti-HIV-1 SF2 p24 antiserum	NIH AIDS Reagent Program	4250	
Goat anti-rabbit HRP	KPL	474-1516	
TMB Microwell Peroxidase Substrate	KPL	52-00-01	
H ₂ SO ₄	Fisher Scientific	A300-500	Causes severe burns by all exposure routes. Use personal protective equipment. Use only under a chemical fume hood. Wash off immediately with plenty of water for at least 15 min.
HIV p24 standard	AIDS and Cancer Virus program (NCI-Frederick)		For order details please contact Julian Bess, Jr. (bessjw@mail.nih.gov); http://ncifrederick.cancer.gov/Programs/Science/Acyp/bio/Bess.aspx
Microplate reader	Molecular Devices		VMax Kinetic ELISA Microplate Reader
RNA isolation cDNA synthesis			
QIAxtractor	Qiagen		http://www.qiagen.com/products/catalog/automated-solutions/sample-prep/qiaxtractor
QIAamp Viral RNA Mini Kit	Qiagen	52906	
dNTP	Bioline	Bio-39026	
SuperscriptIII	Invitrogen	18080-085	
First-strand buffer	Invitrogen	18080-085	
Dithiothreitol (DTT)	Invitrogen	18080-085	
Rnase inhibitor	Roche	3335402001	
RnaseH	Invitrogen	18021-071	
qPCR			
Real-time qPCR machine	Life Technologies		Applied Biosystems 7300 Real-Time PCR
TaqMan® Gene Expression Master Mix	Life Technologies	4369016	

Forward, reverse primer	IDT		Custom order
Probe	Life Technologies		Custom order
optical 96 well reaction plate	Life Technologies	I19N3Q216	
PCR+sequencing			
Taq DNA polymerase (Biolase)	Bioline	Bio-21043	
NH ₄ buffer	Bioline	Bio-21043	
MgCl ₂	Bioline	Bio-21043	
Sequencing primer	IDT		Custom order
QIAxcel	Qiagen		http://www.qiagen.com/products/catalog/automated-solutions/detection-and-analysis/qiaxcel-advanced-system
DNA sequencing service provider	http://www.htseq.org/		
GRC web tool	http://indra.mullins.microbiol.washington.edu/grc/		
ChromatQuan web tool	http://indra.mullins.microbiol.washington.edu/cgi-bin/chromatquant.cgi		