

Materials List for:

Development of a Quantitative Recombinase Polymerase Amplification Assay with an Internal Positive Control

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Materials

Name	Company	Catalog Number	Comments
qRPA Assay			
HIV-1 forward primer	Integrated DNA Technologies	custom DNA oligos	5'-TGG CAG TAT TCA TTC ACA ATT TTA AAA GAA AAG G-3'
HIV-1 reverse primer	Integrated DNA Technologies	custom DNA oligos	5'-CCC GAA AAT TTT GAA TTT TTG TAA TTT GTT TTT G-3'
HIV-1 probe	BioSearch Technologies	custom DNA oligos	5'- TGC TAT TAT GTC TAC TAT TCT TTC CCC [SIMA/HEX] GC [THF] C [dT-BHQ1] GTA CCC CCC AAT CCC C -3'
IPC probe	BioSearch Technologies	custom DNA oligos	5'-AGG TAG TGA CAA GAA ATA ACA ATA CAG GAC [FAM] T [THF] T [dT-BHQ1] GGT TTT GTA ATT GGA A -3'
RPA exo reagents (pellets, rehydration buffer, magnesium acetate)	TwistDx	TwistAmp exo	
PCR tube strips	BioRad	TLS0801	
PCR flat cap tube strips	BioRad	TCS0803	
Micro-seal adhesive	BioRad	558/MJ	
HIV-1 target (pHIV-IRES-eYFPΔEnvΔVifΔVpr)			custom plasmid, see: Segall, H. I., Yoo, E. & Sutton, R. E. Characterization and detection of artificial replication-competent lentivirus of altered host range. <i>Molecular Therapy</i> 8, 118–129, doi:10.1016/S1525-0016(03)00134-5 (2003).
Human male genomic DNA	Applied Biosystems	360486	
96 well cold-block	Cole Parmer	EW-36700-48	
Thermal cycler	BioRad	CFX96	
MiniFuge	VWR	93000-196	
Vortex	VWR	58816-121	
Tris buffer 1.0 M, pH 8.0	EMD Millipore	648314	
EDTA 0.5 M, pH 8.0	Promega	V4321	
Nuclease free water	Ambion	AM9937	
IPC Development			
Cryptosporidium parvum IPC template	Waterborne Inc	P102C	It is also possible to order a double stranded synthetic target from IDT if the user is unequipped to work with C. parvum (a BSL-2 infectious

			agent). PCR and RPA primers for <i>C. parvum</i> were designed using GenBank accession number AF115377.1
PCR long forward primer	Integrated DNA Technologies	custom DNA oligos	5'-TGG CAG TAT TCA TTC ACA ATT TTA AAA GAA AAG G/ ATC TAA GGA AGG CAG CAG GC-3'
PCR long reverse primer	Integrated DNA Technologies	custom DNA oligos	5'- CCC GAA AAT TTT GAA TTT TTG TAA TTT GTT TTT G/ TGC TGG AGT ATT CAA GGC ATA -3'
Phusion High-Fidelity DNA Polymerase	New England Biolabs	M0530S	
Qiaquick Gel Extraction Kit	Qiagen	28704	
TAE 10X buffer	EMD Millipore	574797	
Agarose	Sigma Aldrich	A9539	
Microscope Experiments			
Upright fluorescence microscope	Zeiss	Zeiss Imager.J1	
Stage heater	Bioscience Tools	TC-GSH	
1-Channel Precision High Stability Temperature Controller	Bioscience Tools	TC-1100S	
FAM/GFP filter cube	Zeiss	filter set 38 (000000-1031-346)	excitation BP 470/40 nm, emission BP 520/50 nm
HEX filter cube	Chroma	49014	excitation BP 530/30 nm, emission BP 575/40 nm
Laser cutter	Engraver's Network	VLS3.60	
1/8" black acrylic	McMaster Carr	8505K113	
1.5 mm clear acrylic	McMaster Carr	PD-72268940	
Super glue	Office Depot	Duro super glue	
PCR grade mineral oil	Sigma Aldrich	M8662-5VL	
Data Analysis			
Microsoft Excel	Microsoft		
MATLAB	MATLAB		
MATLAB script: "JoVE_qRPA_standard_curve.m"	Included in SI		
MATLAB script: "JoVE_qRPA_validation_and_quantification.m"	Included in SI		
MATLAB script: "JoVE_real_time_intensity_to_excel.m"	Included in SI		
Adobe Illustrator	Adobe		
JoVE_qRPA_well.ai	Included in SI		
JoVE_qRPA_base.ai	Included in SI		
AxioVision software	Zeiss		
JoVE_AxioVision_Script.ziscript	Included in SI		