

Materials List for:

CRISPR-Cas9-based Genome Engineering to Generate Jurkat Reporter Models for HIV-1 Infection with Selected Proviral Integration Sites

Julia K. Bialek^{*1,2}, Thomas Walther^{*1}, Joachim Hauber^{1,3}, Ulrike C. Lange^{1,2,3}

¹Heinrich Pette Institute, Leibniz Institute for Experimental Virology

²Department of Anesthesiology, University Medical Center Hamburg-Eppendorf

³German Center for Infection Research (DZIF)

*These authors contributed equally

Correspondence to: Ulrike C. Lange at ulrike.lange@leibniz-hpi.de

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Materials

Name	Company	Catalog Number	Comments
pX330-U6-Chimeric_BB-cBh-hSpCas9	Addgene	42230	vector for expression of SpCas9 and gRNA
pMK	GeneArt		mammalian expression vector for cloning
cDNA3.1	Invitrogen	V79020	mammalian expression vector for cloning
BbsI	New England Biolabs	R0539S	restriction enzyme
NEBuilder HiFi DNA Assembly Cloning Kit	New England Biolabs	E5520S	Assembly cloning kit used for target vector generation
TaqPlus Precision PCR System	Agilent Technologies	600210	DNA polymerase with proofreading activity used for amplification of homology arms (step 1.2.2.2), verification of integration site and reporter sequence (step 3.3.3 and 3.3.5), generation of genomic probe for Southern blot (step 3.4.1.5) and analysis of off-target events (step 3.5.4)
96-well tissue culture plate (round-bottom)	TPP	92097	tissue culture plates for dilution plating
Phusion High-Fidelity DNA polymerase	New England Biolabs	M0530 L	DNA polymerase used for detection of targeting events (step 2.4.2) and generation of reporter-specific probe for Southern blot (step 3.4.1.4)
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D9170	dimethyl sulfoxide as PCR additive
Magnesium Chloride (MgCl ₂) Solution	New England Biolabs	B9021S	MgCl ₂ solution as PCR additive
Deoxynucleotide (dNTP) Solution Mix	New England Biolabs	N0447S	dNTP mixture with 10 mM of each nt for PCR reactions
5PRIME HotMasterMix	5PRIME	2200400	ready-to-use PCR mix used for screening PCR (step 3.2.11)
QIAamp DNA blood mini kit	Qiagen	51106	DNA isolation and purification kit
QIAquick PCR Purification Kit	Qiagen	28106	PCR Purification Kit
RPMI 1640 without glutamine	Lonza	BE12-167F	cell culture medium
Fetal Bovine Serum South Africa Charge	PAN Biotech	P123002	cell culture medium supplement
L-glutamine	Biochrom	K 0282	cell culture medium supplement

Penicillin/Streptomycin 10.000 U/ mL/ 10.000 µg/mL	Biochrom	A 2212	cell culture medium supplement
Gibco Opti-MEM Reduced Serum Media	Thermo Fisher Scientific	31985062	cell culture medium with reduced serum concentration optimized for transfection
TransIT-Jurkat	Mirus Bio	MIR2125	transfection reagent
phorbol 12-myristate 13-acetate	Sigma-Aldrich	P8139-1MG	cell culture reagent
Ionomycin	Sigma-Aldrich	I0634-1MG	cell culture reagent
Syringe-driven filter unit, PES membrane, 0,22 µm	Millex	SLGP033RB	filter unit for sterile filtration
Heracell 150i incubator	Thermo Fisher Scientific	51026280	tissue culture incubator
Amershan Hybond-N+	GE Healthcare	RPN1520B	positively charged nylon membrane for DNA and RNA blotting
Stratalinker 1800	Stratagene	400072	UV crosslinker
High Prime	Roche	11585592001	kit for labeling of DNA with radioactive dCTP using random oligonucleotides as primers
illustra ProbeQuant G-50 Micro Columns	GE Healthcare	28-9034-08	chromatography spin-columns for purification of labeled DNA