

Materials List for

# Delivery of the Cas9/sgRNA Ribonucleoprotein Complex in Immortalized and Primary Cells via Virus-like Particles ("Nanoblades")

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## Materials

Name	Company	Catalog Number	Comments
13.2 mL, Thinwall Polypropylene Tubes, 14 x 89 mm - 50Pk	Beckman Coulter Life Sciences	331372	Ultracentrifugation tubes for Nanoblades purification
Amersham Protran Premium Western blotting membranes, nitrocellulose	Merck	GE10600004	Nitrocellulose membrane for quantifying Cas9 levels within purified Nanoblades
BIC-Gag-CAS9	Addgene	119942	Encodes a GAG (F-MLV)-CAS9(sp) fusion. Allows the production of GAG-CAS9 Virus like particles from producer cells in association with over expressed gRNA(s) and appropriate envelopes
BICstim-Gag-dCAS9-VPR	Addgene	120922	Encodes a GAG-dCAS9-VPR fusion for targeted transcriptional activation
BLADE	Addgene	134912	Empty backbone for cloning sgRNA sequence to be used in Nanoblades system
BsmBI-v2	New England Biolabs	R0739S	Restriction enzyme to digest the BLADE and SUPERBLADES vectors for sgRNA cloning
Cas9 (7A9-3A3) Mouse mAb (HRP Conjugate) #97982	Cell Signaling Technology	97982S	Anti-Cas9 antibody for Cas9 quantification by dot-blot
Cas9 Nuclease, <i>S. pyogenes</i>	New England Biolabs	M0386T	Recombinant Cas9 protein to be used as a reference for absolute quantification of the amount of Cas9 loaded within Nanoblades
Ethidium bromide solution (10 mg/mL in H <sub>2</sub> O)	Sigma-Aldrich	E1510-10ML	For staining agarose gels and visualize DNA
Fisherbrand Wave Motion Shakers	Fisher Scientific	88-861-028	Agitation table to resuspend Nanoblades upon centrifugation

gelAnalyzer			<a href="http://www.gelanalyzer.com">http://www.gelanalyzer.com</a> ; quantifying band intensity after digestion
Gesicle Producer 293T	Takara	632617	Nanoblades producer cell line
Gibco DMEM, high glucose, pyruvate	ThermoFisher Scientific	41966052	Cell culture medium for Gesicle Producer 293T cells
GoTaq G2 DNA Polymerase	Promega	M7848	Taq polymerase for amplification of genomic DNA before T7 endonuclease assays
jetPRIME Transfection Reagent kit for DNA and DNA/siRNA	Polyplus	POL114-15	Transfection reagent for Nanoblade production in Gesicle Producer 293T cells
Millex-AA, 0.80 µm, syringe filter	Millipore	SLAA033SS	Syringe filter to remove cellular debris before concentration of Nanoblades
Millex-GS, 0.22 µm, syringe filter	Millipore	SLGS033SS	Syringe filter to sterilise the sucrose cushion solution
Millex-HP, 0.45 µm, polyethersulfone, syringe filter	Millipore	SLHP033RS	Syringe filter to remove cellular debris before Nanoblades concentration
Monarch DNA Gel Extraction Kit	New England Biolabs	T1020L	DNA gel extraction kit for purification of the pBLADES or pSUPERBLADES plasmid fragment upon digestion with BsmBI
NEB Stable Competent E. coli (High Efficiency)	New England Biolabs	C30401	Competent bacteria for plasmid transformation and amplification
NucleoBond Xtra Midi kit for transfection-grade plasmid DNA	Macherey-Nagel	740410.50	Maxipreparation kit for purification of plasmid DNA from cultured bacteria
Nucleospin gDNA extraction kit	Macherey-Nagel	740952.50	Extraction of genomic DNA from transduced cells
NucleoSpin Plasmid, Mini kit for plasmid DNA	Macherey-Nagel	740588.50	Minipreparation kit for purification of plasmid DNA from cultured bacteria
NucleoSpin Tissue, Mini kit for DNA from cells and tissue	Macherey-Nagel	740952.5	Genomic DNA extraction kit
Optima XE-90	Beckman Coulter Life Sciences	A94471	Ultracentrifuge
pBaEVRless	Els Verhoeyen (Inserm U1111)	Personnal requests have to be sent to: <a href="mailto:els.verhoyen@ens-lyon.fr">els.verhoyen@ens-lyon.fr</a>	Baboon Endogenous retrovirus Rless glycoprotein described in Girard-Gagnepain, A. et al. Baboon envelope pseudotyped LVs outperform VSV-G-LVs for gene transfer into early-cytokine-stimulated and resting HSCs. Blood 124, 1221–1231 (2014)
pBS-CMV-gagpol	Addgene	35614	Encodes the Murine Leukemia Virus gag and pol genes
pCMV-VSV-G	Addgene	8454	Envelope protein for producing lentiviral and MuLV retroviral particles
Phosphate-Buffered Saline (PBS)	ThermoFisher Scientific	14200091	10X PBS to dilute in millipore water
Polybrene Transfection Reagent	Millipore Sigma	TR-1003-G	Cationic polymer that enhances the efficiency of retroviral transduction in specific mammalian cells. It can also allow viral-dependent entry of an Oligodeoxynucleotide (ODN) for homology-directed repair
Sucrose, for molecular biology, ≥99.5% (GC)	Sigma-Aldrich	S0389-5KG	Sucrose to prepare a cushion for Nanoblade purification through ultracentrifugation
SUPERBLADE5	Addgene	134913	Empty backbone for cloning sgRNA sequence to be used in nanoblades system (Optimized for increased

			genome editing efficiency via Chen B et al., 2013)
SuperSignal West Dura Extended Duration Substrate	ThermoFisher Scientific	34076	Enhanced chemiluminescence (ECL) HRP substrate for Cas9 dot blots
SW 41 Ti Swinging-Bucket Rotor	Beckman Coulter Life Sciences	331362	Rotor for ultracentrifugation
SYBR Safe DNA Gel Stain	ThermoFisher Scientific	S33102	Alternative to ethidium bromide for staining agarose gels and visualize DNA
T4 DNA Ligase	New England Biolabs	M0202S	DNA ligase to ligate the BLADE or SUPERBLADES vectors with the duplexed DNA oligos corresponding to the variable region of the sgRNA
T7 Endonuclease I	New England Biolabs	M0302S	T7 Endonuclease I recognizes and cleaves non-perfectly matched DNA. Allows to monitor the extent of genome editing at a specific locus
Triton-containing lysis buffer	Promega	E291A	Lysis buffer to disrupt Nanoblades and allow Cas9 quantification
TWEEN 20	Sigma-Aldrich	P9416	For the preparation of TBST