

Isolation of Next-Generation Gene Therapy Vectors through Engineering, Barcoding, and Screening of Adeno-Associated Virus (AAV) Capsid Variants

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Materials

Name	Company	Catalog Number	Comments
Amplification primer	ELLA Biotech (Munich, Germany)	-	Second-strand synthesis of oligonucleotide insert
Agilent DNA 1000 Reagents	Agilent Technologies (Santa Clara, CA, USA)	5067-1504	DNA fragment validation
Agilent 2100 Bioanalyzer System	Agilent Technologies (Santa Clara, CA, USA)	G2938C	DNA fragment validation
AllPrep DNA/RNA Mini Kit	Qiagen (Venlo, Netherlands)	80204	DNA/RNA extraction
Agilent DNA 1000 Reagents	Agilent Technologies (Santa Clara, CA, USA)	5067-1504	NGS Library preparation
Agilent 2100 Bioanalyzer System	Agilent Technologies (Santa Clara, CA, USA)	G2938C	NGS Library preparation
BC-seq fw:	IDT (San Joce, CA, CA, USA)	ATCACTCTCGGCATGGACGAGC	NGS Library preparation
BC-seq rv:	IDT (San Joce, CA, CA, USA)	GGCTGGCAACTAGAAGGCACA	NGS Library preparation
β-Mercaptoethanol	Millipore Sigma (Burlington, MA, USA)	44-420-3250ML	DNA/RNA extraction
BglI	New England Biolabs (Ipswich, MA, USA)	R0143	Digestion of double-stranded insert
C1000 Touch Thermal Cycler	Bio-Rad (Hercules, CA, USA)	1851196	dd-PCR cycler
dNTPS	New England Biolabs (Ipswich, MA, USA)	N0447S	NGS Library preparation
ddPCR Supermix for probes (no dUTP)	Bio-Rad (Hercules, CA, USA)	1863024	dd-PCR supermix
Droplet Generation Oil for Probes	Bio-Rad (Hercules, CA, USA)	1863005	dd-PCR droplet generation oil
DG8 Cartridges for QX100 / QX200 Droplet Generator	Bio-Rad (Hercules, CA, USA)	1864008	dd-PCR droplet generation cartridge
DG8 Cartridge Holder	Bio-Rad (Hercules, CA, USA)	1863051	dd-PCR cartridge holder
Droplet Generator DG8 Gasket	Bio-Rad (Hercules, CA, USA)	1863009	dd-PCR cover for cartridge

ddPCR Plates 96-Well, Semi-Skirted	Bio-Rad (Hercules, CA, USA)	12001925	dd-PCR 96-well plate
E.cloni 10G SUPREME Electrocompetent Cells	Lucigen (Middleton, WI, USA)	60081-1	Electrocompetent cells
Electroporation cuvettes, 1mm	Biozym Scientific (Oldendorf, Germany)	748050	Electroporation
GAPDH primer/probe mix	Thermo Fischer Scientific (Waltham, MA, USA)	Mm00186825_cn	Taqman qPCR primer
Genepulser Xcell	Bio-Rad (Hercules, CA, USA)	1652660	Electroporation
High-Capacity cDNA Reverse Transcription Kit	Applied Biosystems (Waltham, MA, USA)	4368814	cDNA reverse transcription
ITR_fw	IDT (San Joce, CA, USA)	GGAACCCCTAGTGATGGAGTT (https://signagen.com/blog/2019/10/25/qpcr-primer-and-probe-sequences-for-raav-titration/)	dd-PCR primer
ITR_rv	IDT (San Joce, CA, USA)	CGGCCTCAGTGAGCGA (https://signagen.com/blog/2019/10/25/qpcr-primer-and-probe-sequences-for-raav-titration/)	dd-PCR primer
ITR_probe	IDT (San Joce, CA, USA)	HEX-CACTCCCTCTCTGCGCGCTCG-BHQ1 (https://signagen.com/blog/2019/10/25/qpcr-primer-and-probe-sequences-for-raav-titration/)	dd-PCR probe
Illumina NextSeq 500 system	Illumina Inc (San Diego, CA, USA)	SY-415-1001	NGS Library sequencing
KAPA HiFi HotStart ReadyMix (2X)*	Roche AG (Basel, Switzerland)	KK2600 07958919001	NGS sample preparation
MagnaBot 96 Magnetic Separation Device	Promega GmbH (Madison, WI, USA)	V8151	Sample preparation for NGS library
NanoDrop 2000 spectrophotometer	Thermo Fischer Scientific (Waltham, MA, USA)	ND-2000	Digestion of double-stranded insert
NGS_fw	Sigma-Aldrich (Burlinght, MA, USA)	GTT CTG TAT CTA CCA ACC TC	NGS primer
NGS_rev	Sigma-Aldrich (Burlinght, MA, USA)	CGC CTT GTG TGT TGA CAT C	NGS primer
NextSeq 500/550 High Output Kit (75 cycles)	Illumina Inc (San Diego, CA, USA)	FC-404-2005	NGS Library sequencing
Ovation Library System for Low Complexity Samples Kit	NuGEN Technologies, Inc. (San Carlos, CA, USA)	9092-256	NGS Library preparation
PX1 Plate Sealer	Bio-Rad (Hercules, CA, USA)	1814000	dd-PCR plate sealer
Pierceable Foil Heat Seal	Bio-Rad (Hercules, CA, USA)	1814040	dd-PCR sealing foil
Phusion High-Fidelity DNA-Polymerase	Thermo Fischer Scientific (Waltham, MA, USA)	F530S	Second-strand synthesis of oligonucleotide insert
PEI MAX - Transfection Grade Linear Polyethylenimine Hydrochloride (MW 40,000)	Polysciences, Inc. (Warrington, PA, USA)	24765-1G	AAV library preparation
ProNex Size-Selective Purification System	Promega GmbH (Madison, WI, USA)	NG2002	Sample preparation for NGS library
Phusion Hot Start II Polymerase	Thermo Fischer Scientific (Waltham, MA, USA)	F549L	NGS Library preparation
Proteinase K	Roche AG (Basel, Switzerland)	5963117103	DNA/RNA extraction
pRep2Cap2_PIS			ITR-Rep2Cap2-ITR vector. Peptide insertion site within the Cap2 ORF, manufactured/prepared in the lab
QX200 Droplet Generator	Bio-Rad (Hercules, CA, USA)	1864002	dd-PCR droplet generator
QX200 Droplet Reader	Bio-Rad (Hercules, CA, USA)	1864003	dd-PCR droplet analysis
QIAquick Nucleotide Removal Kit	Qiagen (Venlo, Netherlands)	28306	Second-strand synthesis of oligonucleotide insert purification
QIAquick Gel Extraction Kit	Qiagen (Venlo, Netherlands)	28704	Plasmid vector purification
QIAGEN Plasmid Maxi Kit	Qiagen (Venlo, Netherlands)	12162	Plasmid library DNA preparation

Qiaquick PCR Purification kit	Qiagen (Venlo, Netherlands)	28104	Sample preparation for NGS library
Qubit fluorometer	Invitrogen (Waltham, MA, USA)	Q32857	NGS Library preparation
Qubit dsDNA HS	Thermo Fischer Scientific (Waltham, MA, USA)	Q32851	NGS Library preparation
QuantiFast PCR Master Mix	Qiagen (Venlo, Netherlands)	1044234	Taqman qPCR
rep_fw	IDT (San Joce, CA, USA)	AAGTCCTCGGCCAGATAGAC	dd-PCR primer
rep_rv	IDT (San Joce, CA, USA)	CAATCACGGCGCACATGT	dd-PCR primer
rep_probe	IDT (San Joce, CA, USA)	FAM-TGATCGTCACCTCCAACA-BHQ1	dd-PCR probe
RNase-free DNase	Qiagen (Venlo, Netherlands)	79254	DNA/RNA extraction
Sfil	New England Biolabs (Ipswich, MA, USA)	R0123	Digestion of vector
5 mm, steel Beads	Qiagen (Venlo, Netherlands)	69989	DNA/RNA extraction
TRIMER-oligonucleotides	ELLA Biotech (Munich, Germany)	-	Degenerate oligonucleotide
T4 Ligase	New England Biolabs (Ipswich, MA, USA)	M0202L	Plasmid library ligation
TissueLyserLT	Qiagen (Venlo, Netherlands)	85600	DNA/RNA extraction
YFP_fw	IDT (San Joce, CA, USA)	GAGCGCACCATCTTCTTCAAG	dd-PCR primer
YFP_rv	IDT (San Joce, CA, USA)	TGTCGCCCTCGAACTTAC	dd-PCR primer
YFP_probe	IDT (San Joce, CA, USA)	FAM-ACGACGGCAACTACA-BHQ1	dd-PCR probe
Zymo DNA Clean & Concentrator-5 (Capped)	Zymo research (Irvine, CA, USA)	D4013	Vector and Ligation purification