

Transgene Expression in Cultured Cells Using Unpurified Recombinant Adeno-Associated Viral Vectors

 Brian Benyamini¹, Meagan N. Esbin^{1,2}, Oscar Whitney¹, Nike Walther¹, Anna C. Maurer¹
¹Department of Molecular and Cell Biology, University of California, Berkeley ²Biophysics Graduate Group, University of California, Berkeley

Corresponding Author

Anna C. Maurer

amaurer@berkeley.edu

Citation

 Benyamini, B., Esbin, M.N., Whitney, O., Walther, N., Maurer, A.C. Transgene Expression in Cultured Cells Using Unpurified Recombinant Adeno-Associated Viral Vectors. *J. Vis. Exp.* (200), e65572, doi:10.3791/65572 (2023).

Date Published

October 20, 2023

DOI

10.3791/65572

URL

jove.com/video/65572

Materials

Name	Company	Catalog Number	Comments
Snappgene DNA viewing software	Snappgene		
pAAV.CMV.Luc.IRES.EGFP.SV40	AddGene	105533	
pAAV2/2 (Rep/Cap)	AddGene	104963	
pAdΔF6	AddGene	112867	
LB Agar Carbenicillin	Sigma-Aldrich	L0418	
Boekel Scientific Economy Digital Incubator	Boekel Scientific	133000	
LB medium, powder	MP Biomedicals	113002042	
Carbencillin (Disodium)	GoldBio	C-103-5	
New Brunswick I26 Shaker	Eppendorf	M1324-0000	
50 mL Centrifuge Tubes	Corning	430828	
Centrifuge 5810 R	Eppendorf	22627040	
QIAGEN Plasmid Plus Midi Kit	Qiagen	12945	
PCR PULL-APART 8-TUBE STRIPS	USA Scientific	1402-3900	
Mastercycler nexus	Eppendorf	6333000022	
dNTP	Thermo Fisher Scientific	18427013	
5x Phusion Buffer	NEB	B0518S	Provided with purchase of Phusion Polymerase
Phusion High-Fidelity DNA Polymerase	NEB	M0530S	
Gel Loading Dye, Orange (6X)	NEB	B7022S	
DNA Clean & Concentrator-100	Zymo	D4029	
Zymoclean Gel DNA Recovery Kit	Zymo	D4001	
NotI-HF	NEB	R3189S	
EcoRI-HF	NEB	R3101S	
CutSmart Buffer (10x)	NEB	B6004S	Provided with purchase of restriction enzyme
UltraPure Agarose	Thermo Fisher Scientific	16500100	
Ethidium Bromide	Sigma-Aldrich	E1510	

T4 DNA Ligase	NEB	M0202S	
T4 DNA Ligase Reaction Buffer	NEB	B0202S	Provided with purchase of T4 Ligase
Eppendorf Safe-Lock Tubes (1.5mL)	Eppendorf	22363204	
Precision Microprocessor Water Bath	Thermo Scientific	51221046	
Sterile Plastic Culture Tubes	Fisher Scientific	149566B	
2.0 mL Microcentrifuge Tube	Thomas Scientific	1149Y01	
ZR Plasmid Miniprep - Classic	Zymo	D4015	
Xma1	NEB	R0180S	
Sma1	NEB	R0141S	
HEK 293T cells	ATCC	CRL-3216	
Falcon 6-well	Corning	353046	
Gibco DMEM, high glucose, pyruvate	Thermo-Fisher	11995065	
Sanyo MCO-18AIC(UV) CO2 Incubator	Marshall Scientific	MCO-18AIC	
PEI MAX (Polyethylenimine Hydrochloride)	Polysciences	24765-100	
Mixer Vortex Genie 2	Electron Microscopy Sciences	102091-234	
Sanyo Ultra Low Freezer	Sanyo	14656-15267-16219	
INCU-Line IL 10 with transparent window	VWR	390-0384	
Eppendorf Microcentrifuges	Eppendorf	05-400-005	
Falcon 96-well	Corning	353072	
C57BL/6J mice	JAX	strain #000664	
organoid growth medium	STEMCELL Technologies	6005	
L Wnt-3A cells	ATCC	CRL-2647	
nicotinamide	Sigma	N0636-100G	
ROCK inhibitor	STEMCELL Technologies	72302	
CHIR99021	STEMCELL Technologies	72052	
Corning Matrigel Growth Factor Reduced (GFR) Basement Membrane	Fisher	356231	
24-well plate	Fisher	08-772-1	
D-PBS	Thermo Fisher Scientific	14-190-250	
TrypLE Express	Fisher	12604013	
DMEM/F-12 with 15 mM HEPES	STEMCELL Technologies	36254	
polybrene	Millipore Sigma	TR-1003-G	
48-well plates	Fisher	08-772-3D	
Thermo Scientific Nunc Lab-Tek Chambered Coverglass	Fisher	12-565-470	
BeWo cells	ATCC	CCL-98	
F-12K Medium	ATCC	30-2004	
Hepa1-6	ATCC	CRL-1830	
Huh7	UC Berkeley BSD Cell Culture Facility	HUH-7	
C2C12	ATCC	CRL-1772	
HSkMC	ATCC	PCS-950-010	
Skeletal Muscle Cell Growth Medium	Sigma	C-23060	
Skeletal Muscle Differentiation Medium	Sigma	C-23061	

Invitrogen EVOS Digital Color Fluorescence Microscope	Fisher Scientific	12-563-340	
Perkin Elmer Opera Phenix	Perkin Elmer	HH14001000	
PhenoPlate 96-well	Perkin Elmer	6055302	
DMEM, high glucose, no glutamine, no phenol red	Thermo-Fisher	31053028	
GlutaMAX Supplement	Thermo-Fisher	35050079	
Sodium Pyruvate	Thermo-Fisher	11360070	
pAAV2/5 (Rep/Cap)	Addgene	104964	
pAAV2/8 (Rep/Cap)	Addgene	112864	
pAAV-DJ-N589X (Rep/Cap)	Addgene	130878	
pAAV2/9n (Rep/Cap)	Addgene	112865	
pAnc80L65AAP	Addgene	92307	
KP1 (rep/cap)			gifted by Professor Mark Kay (Stanford University)
LK03 (rep/cap)			gifted by Professor Mark Kay (Stanford University)
pAAV4 (rep/cap)			gifted by Professor Luk Vandenberghe (Harvard Medical School)
pAAV.Cas9.sgRNA	Addgene	61591	
pAAV.MCS	Addgene	46954	
gBlock (synthetic DNA fragment)	IDT		