

Materials List for

Reliably Engineering and Controlling Stable Optogenetic Gene Circuits in Mammalian Cells

Michael Tyler Guinn^{1,2,3}, Damiano Coraci^{*2}, Lesia Guinn^{*2}, Gábor Balázs^{1,2}

¹Biomedical Engineering Department, Stony Brook University ²Lauffer Center for Physical and Quantitative Biology, Stony Brook University ³Stony Brook Medical Scientist Training Program

* These authors contributed equally

Corresponding Authors

Michael Tyler Guinn

Gábor Balázs

michael.guinn@stonybrookmedicine.edu gabor.balazsi@stonybrook.edu

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Materials

Name	Company	Catalog Number	Comments
0.2 mL PCR tubes	Eppendorf	951010006	reagent for carrying out PCR
0.25% Trypsin EDTA 1X	Thermo Fisher Scientific	MT25053CI	reagent for splitting & harvesting mammalian cells
0.5-10 µL Adjustable Volume Pipette	Eppendorf	3123000020	tool used for pipetting reactions
100-1000 µL Adjustable Volume Pipette	Eppendorf	3123000039	tool used for pipetting reactions
20-200 µL Adjustable Volume Pipette	Eppendorf	3123000055	tool used for pipetting reactions
2-20 µL Adjustable Volume Pipette	Eppendorf	3123000039	tool used for pipetting reactions
5 mL Polystyrene Round-Bottom Tube w/ Cell Strainer Cap	Corning	352235	reagent for flow cytometry
5702R Centrifuge, with 4 x 100 Rotor, 15 and 50 mL Adapters, 120 V	Eppendorf	22628113	equipment for mammalian culture work
Agarose	Denville Scientific	GR140-500	reagent for gel electrophoresis
Aluminum Foils for 96-well Plates	VWR®	60941-126	tool used for covering plates in light-induction experiments
Ampicillin	Sigma Aldrich	A9518-5G	reagent for selecting bacteria with correct plasmid
Analog vortex mixer	Thermo Fisher Scientific	02215365PR	tool for carrying PCR, transformation, or gel extraction reactions
Bacto Dehydrated Agar	Fisher Scientific	DF0140010	reagent for growing bacteria
BD LSR Aria	BD	656700	tool for sorting engineered cell lines into monoclonal populations
BD LSRFortessa	BD	649225	tool for characterizing engineered cell lines
BSA, Bovine Serum Albumine	Government Scientific Source	SIGA4919-1G	reagent for IF incubation buffer
Cell Culture Plate 12-well, Clear, flat-bottom w/lid, polystyrene, non-pyrogenic, standard-TC	Corning	353043	plate used for growing monoclonal cells
Centrifuge	VWR	22628113	instrument for mammalian cell culture

Chemical fume hood	N/A	N/A	instrument for carrying out IF reactions
Clear Cell Culture Plate 24 well flat-bottom w/ lid	BD	353047	plate used for growing monoclonal cells
CytoOne T25 filter cap TC flask	USA Scientific	CC7682-4825	container for growing mammalian cells
Dimethyl sulfoxide (DMSO)	Fischer Scientific	BP231-100	reagent used for freezing down engineered mammalian cells
Ethidium Bromide	Thermo Fisher Scientific	15-585-011	reagent for gel electrophoresis
Falcon 96 Well Clear Flat Bottom TC-Treated Culture Microplate, with Lid	Corning	353072	container for growing sorted monoclonal cells
FCS Express	De Novo Software:	N/A	software for characterizing flow cytometry data
Fetal Bovine Serum, Regular, USDA 500 mL	Corning	35-010-CV	reagent for growing mammalian cells
Fisherbrand Petri Dishes with Clear Lid - Raised ridge; 100 x 15 mm	Fisher Scientific	FB0875712	equipment for growing bacteria
Gibco DMEM, High Glucose	Thermo Fisher Scientific	11-965-092	reagent for growing mammalian cells
Hs00932330_m1 KRAS isoform a Taqman Gene Expression Assay	Life Technologies	4331182	qPCR Probe
Hygromycin B (50 mg/mL), 20 mL	Life Technologies	10687-010	reagent for selecting cells with proper gene circuit integration
iScript Reverse Transcription Supermix	Bio-Rad Laboratories	1708890	reagent for converting RNA to cDNA
Laboratory Freezer -20 °C	VWR	76210-392	equipment for storing experimental reagents
Laboratory Freezer -80 °C	Panasonic	MDF-U74VC	equipment for storing experimental reagents
Laboratory Refrigerator +4 °C	VWR	76359-220	equipment for storing experimental reagents
LB Broth (Lennox) , 1 kg	Sigma-Aldrich	L3022-250G	reagent for growing bacteria
LIPOFECTAMINE 3000	Life Technologies	L3000008	reagent for transfecting gene circuits into mammalian cells
MATLAB 2019	MathWorks	N/A	software for analyzing experimental data
Methanol	Acros Organics	413775000	reagent for immunofluorescence reaction
Microcentrifuge Tubes, Polypropylene 1.7 mL	VWR	20170-333	plasticware container
Mr04097229_mr EGFP/YFP Taqman Gene Expression Assay	Life Technologies	4331182	qPCR Probe
MultiTherm Shaker	Benchmark Scientific	H5000-HC	equipment for bacterial transformation
NanoDrop Lite Spectrophotometer	Thermo Fisher Scientific	ND-NDL-US-CAN	equipment for DNA/RNA concentration measurement
NEB Q5 High-Fidelity DNA polymerase 2x Master Mix	NEB	M0492S	reagent for PCR of gene circuit fragments
NEB10-beta Competent E. coli (High Efficiency)	New England Biolabs (NEB)	C3019H	bacterial cells for amplifying gene circuit of interest
NEBuilder HiFi DNA Assembly Master Mix	New England Biolabs (NEB)	E2621L	reagent for combining gene circuit fragments
Nikon Eclipse Ti-E inverted microscope with a DS-Qi2 camera	Nikon Instruments Inc.	N/A	instrument for quantifying gene expression
NIS-Elements	Nikon Instruments Inc.	N/A	software for characterizing fluorescence microscopy data
oligonucleotides	IDT	N/A	reagent used for PCR of gene circuit components

Panasonic MCO-170 AICUVHL-PA cellIQ Series CO ₂ Incubator with UV and H ₂ O ₂ Control	Panasonic	MCO-170AICUVHL-PA	instrument for growing mammalian cells
Paraformaldehyde, 16% Electron Microscopy Grade	Electron Microscopy Sciences	15710-S	reagent
PBS, Dulbecco's Phosphate-Buffered Saline (D-PBS) (1x)	Invitrogen	14190144	reagent for mammalian cell culture, reagent for IF incubation buffer
Penicillin-Streptomycin (10,000 U/mL), 100x	Fisher Scientific	15140-122	reagent for growing mammalian cells
primary ERK antibody	Cell Signaling Technology	4370S	primary ERK antibody for immunofluorescence
primary KRAS antibody	Sigma-Aldrich	WH0003845M1	primary KRAS antibody for immunofluorescence
QIAprep Spin Miniprep Kit (250)	Qiagen	27106	reagent kit for purifying gene circuit plasmids
QIAquick Gel Extraction Kit (50)	Qiagen	28704	reagent kit for purifying gene circuit fragments
QuantStudio 3 Real-Time PCR System	Eppendorf	A28137	equipment for qRT-PCR
Relative Quantification App	Thermo Fisher Scientific	N/A	software for quantifying RNA/cDNA amplification
RNeasy Plus Mini Kit	Qiagen	74134	kit for extracting RNA of engineered mammalian cells
Secondary ERK antibody	Cell Signaling Technology	8889S	secondary ERK antibody for immunofluorescence
secondary KRAS antibody	Invitrogen	A11005	secondary KRAS antibody for immunofluorescence
Serological Pipets 5.0 mL	Olympus Plastics	12-102	reagents used for setting up a variety of chemical reactions
SmartView Pro Imager System	Major Science	UVCI-1200	tool for imaging correct PCR bands
SnapGene Viewer (free) or SnapGene	SnapGene	N/A	software DNA sequence design and analysis
Stage top incubator	Tokai Hit	INU-TIZ	tool for carrying PCR, transformation, or gel extraction reactions
TaqMan Fast Advanced Master Mix	Thermo Fisher Scientific	4444557	reagent for PCR of gene circuit fragments
TaqMan Human GAPD (GAPDH) Endogenous Control (VIC/MGB probe), primer limited, 2500 rxn	Life Technologies	4326317E	qPCR Probe
Thermocycler	Bio-Rad	1851148	tool for carrying PCR, transformation, or gel extraction reactions
VisiPlate-24 Black, Black 24-well Microplate with Clear Bottom, Sterile and Tissue Culture Treated	PerkinElmer	1450-605	plate used for light-induction experiments
VWR Disposable Pasteur Pipets, Glass, Borosilicate Glass Pipet, Short Tip, Capacity=2 mL, Overall Length=14.6 cm	VWR	14673-010	reagent for mammalian cell culture
VWR Mini Horizontal Electrophoresis Systems, Mini10 Gel System	VWR	89032-290	equipment for DNA gel electrophoresis
Flp-In 293	Thermo Fisher Scientific	R75007	Engineered cell line with FRT site