

Materials List for:

A Universal Protocol for Large-scale gRNA Library Production from any DNA Source

Anna Köferle¹, Stefan H. Stricker^{1,2}

¹MCN Junior Research Group, Munich Center for Neurosciences, Ludwig-Maximilian-Universität, BioMedical Center

²Epigenetic Engineering, Institute of Stem Cell Research, Helmholtz Zentrum, German Research Center for Environmental Health

Correspondence to: Stefan H. Stricker at stefan.stricker@helmholtz-muenchen.de

URL: <https://www.jove.com/video/56264>

DOI: [doi:10.3791/56264](https://doi.org/10.3791/56264)

Materials

Name	Company	Catalog Number	Comments
500 mM EGTA	Sigma Aldrich	03777-10G	1.4., Inactivation of Mnase
Novex Hi-Density TBE Sample Buffer	Thermo Fisher Scientific	LC6678	2.1.
Novex® TBE Gels, 20%, 10 well	Thermo Fisher Scientific	EC6315BOX	2.1., pre-made 20 % PAGE gel
O'RangeRuler 5 bp DNA Ladder,	Thermo Fisher Scientific	SM1303	2.1.
Novex® TBE Running Buffer	Thermo Fisher Scientific	LC6675	2.1., PAGE gel running buffer
Disposable scalpel, sterile	VWR	233-5363	2.3., other equivalent reagents may be used
SYBR Green I nucleic acid stain (1000x concentrate in DMSO)	Sigma Aldrich	S9430	2.3. +2.5., also available from Thermo Fisher Scientific (S7563)
UltraPure Phenol:Chloroform:Isoamyl Alcohol (25:24:1)	Thermo Fisher Scientific	15593-031	3.6.1. + 4.3., other equivalent reagents may be used
Glycogen	Sigma	10901393001	3.6.4., other equivalent reagents may be used
3M Sodium acetate , pH5.2	Thermo Fisher Scientific	R1181	3.6.4., other equivalent reagents may be used
Ethanol			3.6.4. + 9.1.8., molecular biology grade
Quick blunting kit	New England Biolabs	E1201	4.1.
ammomium acetate	Sigma	A1542	3.1., other equivalent reagents may be used
magnesium acetate	Sigma	M5661	3.1., other equivalent reagents may be used
0.5 M EDTA (pH 8.0)	VWR	MOLEM37465520 (or Promega V4231)	2.2. + 3.1., other equivalent reagents may be used
Agencourt AMPure XP beads	Beckman coulter	A63881	5.3. + 6.5.
Gel extraction kit	QIAGEN	28704	5.7.+ 7.1. +8.4., other equivalent reagents may be used
concentrated T4 DNA ligase	New England Biolabs	M0202T	6.1.+ 8.1.2.
Long Amp Taq 2X Master Mix	New England Biolabs	M0287S	6.3.
Phusion High-Fidelity PCR Master Mix with HF Buffer	New England Biolabs	M0531S	5.1. + 6.6., other equivalent reagents may be used
HindIII	New England Biolabs	R0104S	5.4.1.
SacII	New England Biolabs	R0157S	5.4.2.
Agel	New England Biolabs	R0552S	8.2.1.
Tris base	Sigma	93362	8.1.1.

2M MgCl	Sigma	93362	8.1.1.
dGTP,dATP, dCTP, dTTP	New England Biolabs	N0446S	8.1.1.
DTT	Sigam	DTT-RO	8.1.1.
PEG-8000	Sigma	P5413	8.1.1.
NAD	Sigma	N6522	8.1.1.
T5 exonuclease	New England Biolabs	M0363S	8.1.2.
Phusion DNA polymerase	New England Biolabs	M0530S	8.1.2.
Taq DNA ligase	New England Biolabs	M0208L	8.1.2.
rSAP	New England Biolabs	M0371S	8.3.1.
TG1 competent cells	Lucigen	60502-1	9.1.
1mm gap electroporation cuvettes	VWR	732-2267	10.2.
Bio-Assay Dish (Polystyrene, 245 mm x 245 mm x 25 mm)	Fisher Scientific	DIS-988-010M	9.4.
NaCl	Sigma	S7653	9.3.
Bacto-tryptone	BD	211705	9.3.
Yeast extract	BD	212750	9.3.
Agar	Sigma	A1296	9.4.
Glycerol	Sigma	G5516	9.17.
MNAse	New England Biolabs	M0247S	1.1.
Nanodrop	Thermo Fisher Scientific	ND-2000	throughout
Micropulser	Biorad	165-2100	10.2.
Electroporation cuvettes	Biorad	732-2267	10.2.
250 ml centrifuge tubes	Corning	430776	9.1-9.9.