

Efficient Genome Editing of Mice by CRISPR Electroporation of Zygotes

 Chantal K. Diallo¹, Andrew J. Modzelewski¹
¹Department of Biomedical Sciences, School of Veterinary Medicine, The University of Pennsylvania

Corresponding Author

 Andrew J. Modzelewski
 amodz@upenn.edu

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Materials

Name	Company	Catalog Number	Comments
0.1-cm-gap electroporation cuvette	Bio-Rad	cat. no. 1652089	Electroporation
26-G, 1/2-inch needle	BD	cat. no. 305111	Superovulation
3–8-month-old male mice and 3- to 5-week-old female mice	JAX	cat. no. 000664	Superovulation
35-mm Tissue culture dish	Greiner Bio-One,	cat. no. 627-160	Embryo Culture
60-mm Tissue culture dish	Greiner Bio-One,	cat. no. 628-160	Embryo Processing
6x loading dye	Thermo Fisher Scientific	cat. no. R0611	sgRNA Synthesis and Genotyping
Acidic Tyrode's (AT) solution, embryo culture grade	Sigma-Aldrich,	cat. no. T1788	Embryo Processing
BSA, embryo culture grade	Sigma-Aldrich	cat. no. A3311	Embryo Processing and Culture
Cas9 protein	Alt-R S.p. Cas9 nuclease 3NLS	cat. no. 1074181	Electroporation
DNase I, RNase-free	New England BioLabs,	cat. no. M0303	sgRNA Synthesis
DPBS(calcium and magnesium free)	Gibco	cat. no. 14190-144	Embryo Processing
EcoRI	NEB	cat. no. R3101S	Genotyping
EDTA, anhydrous	Sigma-Aldrich	cat. no. EDS-100G	RNP Buffer
Ethanol	Koptec	cat. no. V1016	sgRNA Synthesis
Gelatin (powder) type B, laboratory grade	Fisher,	cat. no. G7-500	Lysis Buffer
Glycerol, molecular-biology grade	Fisher	cat. no. BP229	RNP Buffer
Taq Polymerase	Promega	cat. no. M712	Genotyping
HEPES, cell culture grade	Sigma-Aldrich	cat. no. H4034	RNP Buffer
Hinfl (10,000 U/mL)	NEB	cat. no. R0155S	Genotyping
HiScribe T7 High Yield RNA Synthesis Kit	New England BioLabs,	cat. no. E2040	sgRNA Synthesis
Human chorion gonadotropin, lyophilized (hCG)	Millipore	cat. no. 230734	Superovulation
Hyaluronidase/M2	Millipore	cat. no. MR-051-F	Embryo Processing
KSOMaa Evolve medium (potassium-supplemented simplex-optimized medium plus amino acids)	Zenith Biotech	cat. no. ZEKS-050	Embryo Culture

LE agarose, analytical grade	BioExpress	cat. no. E-3120-500	sgRNA Synthesis and Genotyping
M2 medium	Zenith Biotech	cat. no. ZFM2-050	Embryo Processing
Magnesium chloride, anhydrous (MgCl ₂)	Sigma-Aldrich	cat. no. M8266	RNP and Lysis Buffer
Mineral Oil	Millipore	cat. no. ES-005C	Embryo Culture
Nonidet P-40, substitute (NP-40)	Sigma-Aldrich	cat. no. 74385	Lysis Buffer
Nuclease-free water, molecular-biology grade	Ambion	cat. no. AM9937	sgRNA Synthesis and Genotyping
Oligos for sgRNA synthesis, donor oligo and PCR primers for genotyping	Integrated DNA Technologies	custom orders	sgRNA Design
Reduced serum medium	Thermo Fisher Scientific	cat. no. 31985062	Embryo Culture
High-fidelity DNA polymerase	New England BioLabs,	cat. no. M0530	sgRNA Synthesis
Potassium chloridemolecular-biology grade (KCl)	Sigma-Aldrich	cat. no. P9333	RNP and Lysis Buffer
Pregnant mare serum gonadotropin lyophilized ((PMSG)	ProspecBio	cat. no. HOR-272	Superovulation
Proteinase K, molecular-biology grade	Fisher	cat. no. BP1700-100	Lysis Buffer
RNase-free 1.5-mL microcentrifuge tube	VWR	cat. no. 20170-333	sgRNA Synthesis and Genotyping
RNase-free eight-well PCR strip tubes	VWR	cat. no. 82006-606	sgRNA Synthesis and Genotyping
Magnetic purification beads	GE Healthcare	cat. no. 65152105050250	sgRNA Synthesis
Tris (2-carboxyethyl) phosphine hydrochloride (TCEP)	Sigma-Aldrich	cat. no. C4706	RNP Buffer
Tris-HCl solution, pH 8.5 molecular-biology grade	Teknova	cat. no. T1085	Lysis Buffer
Tween 20 molecular-biology grade	Sigma-Aldrich	cat. no. P7949-500	Lysis Buffer