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Simple electroporation for efficient CRISPR/Cas9 genome editing in murine zygotes V.2

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ABSTRACT

Electroporation of zygotes represents a rapid alternative to the elaborate pronuclear injection procedure for CRISPR/Cas9-mediated genome editing in mice. However, current protocols for electroporation either require the investment in specialized electroporators or corrosive pre-treatment of zygotes which compromises embryo viability. Here, we describe an easily adaptable approach for the introduction of specific mutations in C57BL/6 mice by electroporation of intact zygotes using a common electroporator with synthetic CRISPR/Cas9 components and minimal technical requirement. This protocol works efficiently with zygotes from a variety of genetic backgrounds and is compatible with other CRISPR nucleases like Cas12a.

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GUIDELINES

Use only embryo-grade reagents.

Embryo culture media (M2, M16, KSOM/AA) may be purchased or prepared in-house as published (Behringer, R., et al., (2014) *Manipulating the mouse embryo : a laboratory manual*, Fourth edition. ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Mouse pre-implantation embryos are incubated in at least 4h pre-equilibrated M16 or KSOM/AA in a CO₂ incubator (5% CO₂, 37°C, 95% humidity).

Embryo-grade standard T₁₀E_{0.1} (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4) injection buffer is prepared as described (e.g., Chu, V.T., et al., (2016) Efficient generation of Rosa26 knock-in mice using CRISPR/Cas9 in C57BL/6 zygotes. *BMC Biotechnol* 16, 4.).

Mouse zygotes are collected from the oviducts of superovulated females as described in published protocols (Behringer, R., et al., (2014) *Manipulating the mouse embryo : a laboratory manual*, Fourth edition. ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

This protocol works efficiently with freshly harvested or frozen zygotes from various genetic backgrounds like C57BL/6N, C57BL/6J, FVB/N and SWISS mice. Frozen zygotes may also be purchased from commercial vendors (e.g., Kit Eazygote from Janvier Labs).


Instead of Cas9 other CRISPR nucleases like Cas12a can be used as well. For Cas12a use the same concentration of reagents in the electroporation mix. It is advisable to include the IDT electroporation enhancer at 4 µM (i.e., 0.8 µl of 100 µM in 20 µl of total mix) and use the mix immediately after preparation for the electroporation as Cas12a can have indiscriminate ssDNA nuclease activity. For the same reason the ssODN should be stabilized with with PS (Phosphorothioate) bonds at each end (the last 3 nucleotide bonds are sufficient).

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 [Opti-MEM™ Reduced Serum Medium Thermo Fisher](#)

Scientific Catalog #31985062

 [M2 medium Merck Millipore](#)

Sigma Catalog #M7167

 [KSOM Merck Millipore](#)

Sigma Catalog #MR-106-D

 [M16 Medium Contributed by users](#)

 [T10E0.1 Buffer Contributed by users](#)

 [Electroporation Cuvette 1mm BioRad](#)

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 [60 mm Center Well Organ Culture](#)

Dish Corning Catalog #353037

guide RNA annealing

- 1 Resuspend lyophilized crRNA, tracrRNA and ssODN in T₁₀E_{0.1} buffer to 100 µM (e.g. 5 nmol in 50 µl)
(Store at -80 °C until use)
- 2 Combine 5 µl crRNA (100 µM) and 5 µl tracrRNA (100 µM) in a nuclease-free PCR tube to yield an equimolar crRNA:tracrRNA duplex solution of 50 µM
- 3 Heat to 95 °C for 5 min and cool down at 5 °C/ min in a thermocycler
(crRNA:tracrRNA duplex can be stored for months at -80 °C)

Preparation of the electroporation mix

- 4 Add 1.6 µl crRNA:tracrRNA duplex and 1.3 µl Cas9 nuclease to 15.1 µl Opti-MEM in a nuclease-free tube and vortex
(Instead of Cas9 other CRISPR nucleases like Cas12a can be used as well. See Guidelines & Warnings)
- 5 Incubate mix at room temperature for 10 min

6 Place tube on ice, add 2 µl ssODN and vortex
(The ssODN may be left out if desired but must subsequently be compensated by 2 µl Opti-MEM to reach a total electroporation mix of 20 µl)

7 Quick-spin at 4 °C and keep tube on ice until use

8 Summary of the 20 µl electroporation mix:

A	B	C	D
Reagent	Stock concentration	Final concentration	Volume
crRNA:tracrRNA duplex	50 µM	4 µM	1.6 µl
Cas9 nuclease	61 µM (10 µg/µl)	4 µM	1.3 µl
ssODN (optional)	100 µM	10 µM	2.0 µl
Opti-MEM	-	-	fill up to 20 µl

Electroporation of zygotes

9 Collect zygotes from the oviducts of superovulated females as described in published protocols

10 Wash the zygotes in five drops of M2 medium

11 Wash up to 50 zygotes in one drop of Opti-MEM

12 Transfer zygotes with as little media as possible to the 20 µl electroporation mix

13 Using a 20 µl pipette transfer the entire drop including the zygotes into a pre-warmed (37 °C) 1 mm electroporation cuvette
(Ensure retrieving all zygotes by quickly aspirating the entire drop. Slow aspiration will leave zygotes behind)

14 Insert cuvette into a standard electroporator (e.g. BioRad Gene Pulser Xcell electroporator)

15 Apply two square wave pulses at 30 V and 3 ms duration with a 100 ms interval

16 Retrieve the zygotes by flushing the cuvette with 100 µl M2 medium using a 100 µl pipette into a culture dish (e.g. 60 mm Center Well Organ Culture Dish)

- 100%