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

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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/6N mice by electroporation of intact zygotes using a common electroporator with synthetic CRISPR/Cas9 components and minimal technical requirement.

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Guidelines

Use only embryo-grade reagents.

M2 and M16 media are prepared 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).

Commercial M2 (Sigma, #M7167) and KSOM/AA (Merck Millipore, #MR-106-D) can be used instead.

Mouse pre-implantation embryos are incubated in at least 4h pre-equilibrated M16 or KSOM/AA in a CO_2 incubator (5% CO_2, 37°C, 95% humidity).

Embryo-grade $T_{10}E_{0.1}$ (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4) buffer is prepared as described (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).

Materials

Cas9 Nuclease 1074181 by Integrated DNA Technologies

tracrRNA 1072532 by Integrated DNA Technologies

crRNA by Integrated DNA Technologies

ssODN (Ultramer DNA Oligonucleotides) by Integrated DNA Technologies

Opti-MEM (Reduced Serum Medium) 31985062 by Thermo Fisher Scientific

✓ M2 Medium by Contributed by users

✓ M16 Medium by Contributed by users

✓ T10E0.1 Buffer by Contributed by users

Electroporation Cuvette 1mm 1652089 by BioRad Sciences

60 mm Center Well Organ Culture Dish 353037 by Corning

Protocol

guide RNA annealing

Step 1.

Resuspend lyophilized crRNA, tracrRNA and ssODN in $T_{10}E_{0.1}$ buffer to 100 µM (e.g. 5 nmol in 50 µl)

(Store at -80 °C until use)

guide RNA annealing

Step 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

guide RNA annealing

Step 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 weeks at -80 °C)

Preparation of the electroporation mix

Step 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

Preparation of the electroporation mix

Step 5.

Incubate mix at room temperature for 10 min

Preparation of the electroporation mix

Step 6.

Place tube on ice, add 2 µl ssODN and vortex

Preparation of the electroporation mix

Step 7.

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

Preparation of the electroporation mix

Step 8.

Summary of the 20 µl electroporation mix:

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	100 µM	10 µM	2.0 µl
Opti-MEM	-	-	15.1 µl

Electroporation of zygotes

Step 9.

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

Electroporation of zygotes

Step 10.

Wash the zygotes in five drops of M2

Electroporation of zygotes

Step 11.

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

Electroporation of zygotes

Step 12.

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

Electroporation of zygotes

Step 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)

Electroporation of zygotes
Step 14.

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

Electroporation of zygotes
Step 15.

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

Electroporation of zygotes
Step 16.

Retrieve the zygotes by flushing the cuvette with 100 µl pre-incubated M16 using a 100 µl pipette into a culture dish (e.g. 60 mm Center Well Organ Culture Dish)

Electroporation of zygotes
Step 17.

Wash the cuvette with 100 µl pre-incubated M16

Electroporation of zygotes
Step 18.

Transfer all zygotes to a new culture dish with 500 µl pre-incubated M16

Electroporation of zygotes
Step 19.

Incubate zygotes in M16 until the two cell stage and transfer the developed embryos into pseudopregnant foster mice

(Embryos may also be transferred at the one cell stage)