

# Mouse zygote electroporation

## Ribonucleoprotein delivery using the Alt-R™ CRISPR-Cas9 System

Contributed by Masakazu Hashimoto, Osaka University, and Tatsuya Takemoto, Tokushima University

The method presented here is provided by customers who have used the Alt-R CRISPR-Cas9 System. This can serve as a starting point for using the Alt-R CRISPR-Cas9 System in similar model organisms but may not be fully optimized for your gene or application [1,2]. IDT does not guarantee these methods, and application specialists at IDT can only provide general guidance with limited troubleshooting and support.

## Materials

Equipment	Ordering information
Electroporator	BEX Co. Ltd: • CUY21EDIT II (Cat # CUY21EDIT2) or • Genome Editor™ electroporator (Cat # GEB15)
Platinum plate electrode	BEX Co. Ltd (Cat # LF501PT1-10)
Stereo microscope	General laboratory supplier
CO <sub>2</sub> incubator	General laboratory supplier
Glass capillaries for manipulating embryos	General laboratory supplier
Aspirator for glass capillary	Drummond, 15" aspirator tube assembly (Cat # 2-000-000), or equivalent
Reagents	
Alt-R™ CRISPR-Cas9 crRNA	Available at <a href="http://www.idtdna.com/CRISPR-Cas9">www.idtdna.com/CRISPR-Cas9</a>
Alt-R™ CRISPR-Cas9 tracrRNA	IDT (Cat # 1072533)
Alt-R™ S.p. Cas9 Nuclease 3NLS	IDT (Cat # 1074181)
Nuclease-Free Duplex Buffer	IDT (Cat # 11-01-03-01)
Opti-MEM® I Reduced Serum Medium	Thermo Fisher Scientific (Cat # 31985062)
KSOM Medium	General laboratory supplier
M2 Medium	General laboratory supplier

## Methods

### A. Prepare ribonucleoprotein (RNP)

1. Resuspend Alt-R™ CRISPR-Cas9 crRNA to 1 µg/µL in Opti-MEM® I.
2. Resuspend Alt-R CRISPR-Cas9 tracrRNA to 1 µg/µL in Opti-MEM I.
3. Dilute Alt-R S.p. Cas9 Nuclease 3NLS to 1 µg/µL with Opti-MEM I.
4. Add the following reagents to a microcentrifuge tube:

Component	Amount (µL)
Alt-R™ CRISPR-Cas9 crRNA (Step A1)	0.6
Alt-R™ CRISPR-Cas9 tracrRNA (Step A2)	0.6
Nuclease-Free Duplex Buffer	4.2
<b>Total volume*</b>	<b>5.4</b>

\* This table will provide the amount needed for a single electroporation reaction. Scale up as needed for your experiments.

5. Heat the mixed reagents for 3 min at 95°C, and then let the tube cool slowly to room temperature.
6. Add Cas9 nuclease to the microcentrifuge tube:

Component	Amount (µL)
crRNA/tracrRNA duplex solution (Step A5)	5.4
Alt-R™ S.p. Cas9 Nuclease 3NLS (Step A3)	0.6
<b>Total volume*</b>	<b>6.0</b>


\* This table will provide the amount needed for a single electroporation reaction (you will use 5 µL of the 6 µL of RNP solution). Scale up as needed for your experiments.

### B. Transfect mouse zygotes by electroporation

1. Turn on the electroporator and set pulse settings as follows:  
Pulse mode: Pd(+)  
Pulse settings:
  - a. 30 V
  - b. 3 ms ON
  - c. 97 ms OFF
  - d. 7 cycles

2. Connect LF501PT1-10 electrode to the electroporator.
3. Prepare three 50  $\mu$ L drops of Opti-MEM I in a sterilized Petri dish.
4. Prepare three 50  $\mu$ L drops of M2 medium in a sterilized Petri dish.
5. Add 5  $\mu$ L of RNP solution (Step A6) to the gap between the platinum plates of the electrode, and chill the electrode on ice to prevent evaporation.
6. Wash fertilized eggs 3 times with Opti-MEM I media by transferring them into the 3 Opti-MEM I drops from Step B3 using a glass capillary attached to the aspirator.
7. Return the electrode to room temperature and transfer the washed eggs to the electroporation buffer in the electrode.

Note: Minimize the amount of carryover of the washing solution to the electrode to maintain the concentration of RNP in the electroporation buffer.

8.  **Important:** Pipet eggs several times using a glass capillary attached to an aspirator, and arrange the eggs in a row in the middle of the electroporation buffer.

Note: During pipetting, ensure that the eggs are submerged in the electroporation buffer to maximize the efficiency of RNP delivery into the zygotes.

9. Start electroporation.

Note: Air bubbles are generated from the electrode plates if appropriate electric pulses are applied to the electroporation buffer.

10. Wash the eggs 3 times with M2 medium by transferring them into the 3 M2 drops from Step B4 using a glass capillary attached to an aspirator.
11. Transfer  $\leq 10$  washed eggs to 20  $\mu$ L of KSOM medium.
12. Culture the eggs overnight in a CO<sub>2</sub> incubator at 37°C.
13. Clean the electrode by doing the following:
  - e. Wash the used electrode with Milli-Q® water (EMD Millipore), or equivalent.
  - f. Wipe the electrode with Kimwipes® tissues (Kimberly-Clark), or equivalent.
  - g. Store the electrode in a dry place.
14. Check the embryos, which can be used in the following ways:
  - Continue embryo cultures for *in vitro* experiments.
  - Transfer normally developing, 2-cell-stage embryos into host mothers for *in vivo* experiments.

## References

1. Hashimoto M, Takemoto T. (2015) Electroporation enables the efficient mRNA delivery into the mouse zygotes and facilitates CRISPR/Cas9-based genome editing. *Sci Rep*, 5:11315.
2. Hashimoto M, Yamachita Y, Takemoto T. (2016) Electroporation of Cas9 protein/sgRNA into early pronuclear zygotes generates non-mosaic mutants in the mouse. *Dev Biol*, 418:1–9.

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