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_2 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 www.idtdna.com/CRISPR-Cas9
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
Total volume*	**5.4**

* 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
Total volume*	**6.0**

* 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 µL drops of Opti-MEM I in a sterilized Petri dish.

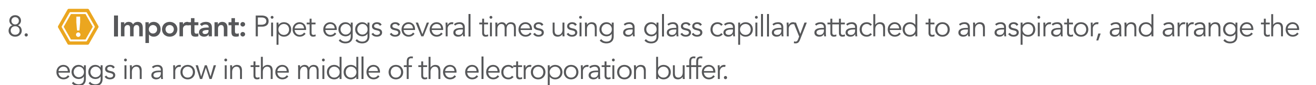
4. Prepare three 50 µL drops of M2 medium in a sterilized Petri dish.

5. Add 5 µL 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 ≤10 washed eggs to 20 µL of KSOM medium.

12. Culture the eggs overnight in a CO_2 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.

For Research Use Only. Not for use in diagnostic procedures.
© 2017 Integrated DNA Technologies, Inc. All rights reserved. Trademarks contained herein are the property of Integrated DNA Technologies, Inc. or their respective owners. For specific trademark and licensing information, see www.idtdna.com/trademarks.
CRS-10071-PR 02/17