Mouse zygote electroporation

Ribonucleoprotein delivery of the Alt-R™ CRISPR-Cas9 System

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The method presented in this document has been provided by an IDT customer who has used the Alt-R CRISPR-Cas9 System in their experiments. This document may 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. IDT does not guarantee methods or any performance of such methods. IDT Application Specialists can only provide general technical support and troubleshooting support in relation to the methods outlined in this document.

Equipment and reagents

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 in Opti-MEM I to 1 µg/µL.
2. Resuspend Alt-R CRISPR-Cas9 tracrRNA in Opti-MEM I to 1 µg/µL.
3. Dilute Alt-R S.p. Cas9 nuclease 3NLS with Opti-MEM I to 1 µg/µL.
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
Nuclease-Free Duplex Buffer	4.2
Total volume*	**5.4**

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

B. Transfect mouse zygotes by electroporation

1. Turn the electroporator on, then set pulse settings as follows:
   - Pulse mode: Pd(+)
   - Pulse settings: 30V, 3 ms ON, 97 ms OFF, 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, then chill the electrode on ice to prevent evaporation.
6. Wash fertilized eggs 3 times with Opti-MEM I media by transferring them into Opti-MEM I drops (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.

   Important:

   - Pipette the 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.
   - Ensure that the eggs are submerged in the electroporation buffer to maximize the efficiency of RNP delivery into the zygotes.
8. Start electroporation.

   Note: Air bubbles are generated from the electrode plates if appropriate electric pulses are applied to the electroporation buffer.
9. Wash the eggs 3 times with M2 medium by transferring them into M2 drops (step B4) by glass capillary attached to an aspirator.
10. Transfer the washed eggs to KSOM medium.
11. Culture the eggs overnight in a CO_2 incubator.
12. Clean the electrode.
    a. Wash the used electrode with Milli-Q® water (EMD Millipore), or equivalent.
    b. Wipe the electrode with Kimwipes® tissues (Kimberly-Clark), or equivalent.
    c. Store the electrode in a dry place.
13. Check the embryos, which can be used in either of the following ways:
    - Continue embryo cultures for *in vitro* experiments.
    - Transfer normally developing (2-cell stage) embryos into host mothers for *in vivo* experiments.

Revision history

Version	Release date	Description of changes
2	July 2022	Updated document to internal MAPSS compliance
1	February 2017	Initital release

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