Mouse zygote electroporation

Ribonucleoprotein delivery using the Alt-R CRISPR-Cas9 System and the NEPA21 Electroporator

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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 biological systems but may not be fully optimized for your gene or application. 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
NEPA21 Electroporator	Nepa Gene: www.nepagene.jp
Electrode cable	Nepa Gene (cat # C115CB)
Electrode cable	Nepa Gene (cat # C117)
Electrodes, options: Up to 150 embryos, 5 mm gap, 50 µL Up to 50 embryos, 1 mm gap, 5 µL	 Nepa Gene (cat # CUY505P5) Nepa Gene (cat # CUY501P1-1.5)
Kits and reagents	
Opti-MEM® I Reduced Serum Medium	Thermo Fisher Scientific (cat # 31985062)
CTK solution	ReproCELL (cat # RCHETP002) or equivalent
Alt-R CRISPR-Cas9 crRNA	IDT predesigned and custom crRNA: www.idtdna.com/CRISPR-Cas9
Alt-R CRISPR-Cas9 tracrRNA Alternative: Alt-R CRISPR-Cas9 tracrRNA – ATTO™ 550	IDT (cat # 1072532,1072533,1072534) IDT (cat # 1075927,1075928)
Alt-R S.p. Cas9 Nuclease 3NLS Alternative: Alt-R S.p. HiFi Cas9 Nuclease 3NLS	IDT (cat # 1074181, 1074182) IDT (cat # 1078727, 1078728)
Nuclease-Free Duplex Buffer	IDT (cat # 11-01-03-01)

Methods

Prepare crRNA:tracrRNA duplex

1. Resuspend crRNA and tracrRNA in Nuclease-Free Duplex Buffer to final concentrations of 200 µM.

   Note: Resuspended RNAs can be stored at –20°C.

2. Mix the crRNA and tracrRNA to a final duplex concentration of 100 µM as shown:

Component	Amount (µL)
200 µM crRNA	2
200 µM tracrRNA	2
Total volume	**4**

3. Heat at 95°C for 5 min.
4. Remove from heat and allow to cool to room temperature.

Prepare the ribonucleoprotein (RNP) complex

1. Combine the following:

Component	Final concentration (µM)	Amount (µL)
Opti-MEM medium	—	46
crRNA:tracrRNA duplex (from step A4)	6	3
Alt-R Cas9 Nuclease 3NLS (61 µM stock)	1.2	1
Total volume*	**—**	**50**

2. Incubate at room temperature for 10–20 min.

Measure impedance of RNP complex

If you are using the **CUY505P5** electrode:

1. Pipette 50 µL of RNP complex into the chamber of the electrode.
2. Press the **Ω** button of the NEPA21 electroporator to measure the impedance.

   Note: The impedance should be in the range of 0.5–0.54 kΩ.
3. If needed, adjust the impedance:
   - If the impedance is below 0.5 kΩ, remove some of the cell solution from the chamber to increase the impedance.
   - If the impedance is above 0.54 kΩ, add Opti-MEM to the chamber to decrease the impedance.

If you are using the **CUY501P1-1.5** electrode:

1. Pipette 5 µL of RNP complex into the chamber of the electrode.
2. Press the **Ω** button of the NEPA21 electroporator to measure the impedance.

   Note: The impedance should be in the range of 0.2–0.24 kΩ.
3. If needed, adjust the impedance:
   - If the impedance is below 0.2 kΩ, remove some of the cell solution from the chamber to increase the impedance.
   - If the impedance is above 0.24 kΩ, add Opti-MEM to the chamber to decrease the impedance.

Prepare culture plates and embryos

1. Prepare and warm culture media for use after electroporation.
2. Wash embryos with Opti-MEM.

   Notes:
   - You do not need to weaken the zona pellucida with acidic Tyrode's solution.
   - Use the CUY505P5 electrode to perform electroporation of 20–150 embryos simultaneously.
   - Use the CUY501P1-1.5 electrode to perform electroporation of 5–50 embryos simultaneously.

Perform electroporation

1. Place embryos in the chamber of the electrode.
2. Press the **Ω** button of NEPA21 electroporator, and record the impedance value.

   Note: The final impedance value should be in the following range:

   - For the CUY505P5 electrode, 0.48–0.52 kΩ
   - For the CUY501P1-1.5 electrode, 0.18–0.22 kΩ
3. If needed, adjust the impedance:
   - If the impedance is below the acceptable range, remove some of the cell solution from the chamber to increase the impedance.
   - If the impedance is above the acceptable range, add Opti-MEM to the chamber to decrease the impedance.
4. Set up electroporation program according to the recommendations in the following table:

	Electrode	
Settings	**Nepa Gene CUY505P5**	**Nepa Gene CUY501P1-1.5**
Poring pulse (voltage)		
Voltage	225 V	40 V
Pulse length	1–2 msec	2.5–3.5 msec
Pulse interval	50 msec	50 msec
# of pulses	4	4
Decay rate	10%	10%
Polarity	+	+
Transfer pulse		
Voltage	20 V	7 V
Pulse length	50 msec	50 msec
Pulse interval	50 msec	50 msec
# of pulses	5	5
Decay rate	40%	40%
Polarity	±	±

5. Press the **Start** button to execute the electroporation program, and record the values of current and joules displayed in the Measurements frame.
6. Remove embryos from the chamber.
7. Culture embryos in the prepared culture media (from Prepare culture plates and embryos, step 1).
8. Repeat steps 1–6 (above) for embryos that have not undergone unelectroporation.

   Note: You may reuse the RNP complex solution.

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