Electroporation of human induced pluripotent stem (iPS) cells

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**
Cuvette Chamber	Nepa Gene (cat # CU500)
Cuvette Stand Holder	Nepa Gene (cat # CU600)
NEPA Electroporation Cuvettes, 2 mm gap (a pipette* is included with each cuvette)	Nepa Gene (cat # EC-002S)
Kits and reagents	
Opti-MEM® Medium† **NOTE:** Use serum-free and antibiotic-free Opti-MEM medium without supplements.	Thermo Fisher Scientific (cat # 31985062)
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)
For homology-directed repair template: Single-stranded oligodeoxynucleotide (ssODN) For experiments with no ssODN Alt-R Cas9 Electroporation Enhancer	 IDT: **www.idtdna.com** IDT (cat # 1075915, 1075916)
Human iPS cells	ATCC or equivalent
Rock Inhibitor (Y-27632 dihydrochloride)	Abcam (cat # ab120129) or equivalent

Kits and reagents (continued)	
CTK solution	ReproCELL (cat # RCHETP002), or equivalent
Feeder cells	General cell culture laboratory supplier
Recommended for passage before electroporation: • mTeSR™1 feeder-free maintenance medium (without penicillin-streptomycin) • Basement membrane matrix: (Option 1) Matrigel® matrix (Option 2) Geltrex® matrix	 STEMCELL Technologies (cat # 85850) Corning (cat # 354277), or equivalent Thermo Fisher Scientific (cat # A1413301)
Cell detachment solution: (Option 1) TrypLE™ Enzyme (Option 2) Accutase® Solution	 Thermo Fisher Scientific (cat # various) STEMCELL Technologies (cat # 07920)

*After electroporation, use the included pipette, or a gel loading tip, to pipet cells from the cuvette.
†We refer to Opti-MEM medium as "EP buffer" throughout this user method.

Methods

The volumes in this protocol will provide enough reagents and cells for electroporation of 3 samples.

A. 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:

Component	Amount (µL)
200 µM crRNA	3
200 µM tracrRNA	3
Total volume	**6**

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

B. Prepare ribonucleoprotein (RNP) complex

1. Mix the following:

Component	Amount (µL)
crRNA:tracrRNA duplex (from **step A4**)	5
Alt-R Cas9 Nuclease 3NLS (61 µM stock)	4
Total volume	**9**

2. Incubate at room temperature for 10–20 min to allow the RNP complex to form.
3. Suspend ssODN (or Alt-R Cas9 Elctroporation Enhancer) at 1 µg/µL in EP buffer (i.e., Opti-MEM Media).
4. Mix the following:

Component	Amount (µL)
ssODN (or Alt-R Cas9 Electroporation Enhancer) (from **step B3**)	16
RNP complex (from **step B2**)	9
Total volume	**25**

C. Prepare cells

Notes:

- Recommended: To minimize the presence of feeder cells during electroporation, the Nepa Gene labs recommend passaging iPS cells under feeder-free conditions (i.e., use mTeSR media with a basement membrane matrix as directed by manufacturers) once before electroporation.
- Transfection efficiency and cell viability may be severely reduced by residual serum, antibiotic, or supplement.
- Pipette up and down repeatedly to achieve a monodisperse cell suspension without clumps of iPS cells before electroporation.

1. Prepare 6- or 12-well plates for cell culture after electroporation.
   a. Replace the medium on the feeder cells with human iPS cell medium containing a final concentration of 10 µM Rock Inhibitor.
   b. Incubate the feeder cells in a CO_2 incubator until needed in **step D11**.
2. Add Rock Inhibitor to iPS cell cultures at a final concentration of 10 µM, and incubate the cultures in a CO_2 incubator for at least 1 hr.
3. **Important:** Wash the iPS cell cultures with 10 mL of PBS.
4. Remove feeder cells, if needed.
   a. Add 0.5 mL of CTK solution to the iPS cell cultures, and incubate at 37°C for 1–2 min.

      Note: This incubation must not exceed 5 min.

   b. Shake the plates to detach feeder cells, and discard the supernatant containing CTK solution and feeder cells.
   c. **Important:** Add 5 mL of PBS to the iPS cells to wash off as many of the remaining feeder cells as possible. (If feeder cells are present, transfection efficiency of the iPS cells will decrease.)
5. Add 0.5 mL of TrypLE (or Accutase) to the iPS cells, and incubate at 37°C for 3–5 min to detach iPS cell colonies.
6. **Important:** Add 5 mL of the iPS cell medium to the cells, and pipette up and down repeatedly to achieve a monodisperse cell suspension. (Avoid using clumps of cells.)

7. Centrifuge the iPS cells at room temperature.
8. Remove the supernatent, and resuspend the iPS cells in EP buffer (5 or 10 mL).
9. Centrifuge the iPS cells at room temperature.
10. Count the iPS cells, and suspend 3.2 x 10^6 cells in EP buffer (5 or 10 mL).

> **Notes:** If you do not have enough prepared cells, you may decrease the number of cells (for example, suspend 3.2 x 10^5 to 1.6 x 10^6 cells in EP buffer); however, your transfection efficiency may be lower.

11. Centrifuge the iPS cells at room temperature.
12. Remove the supernatent, and resuspend the iPS cells in 295 µL of EP buffer.

D. Perform electroporation

1. Mix the following, being careful to avoid forming bubbles:

Component	Amount (µL)
RNP complex (from **step B4**)	25
iPS cells in EP buffer (from **step C12**)	295
Total volume	**320**

2. Dispense 100 µL of mixture from **step D1** into each cuvette.

> **Note:** Ensure that you use 100 µL to achieve an electric impedance within the optimal range.

Component	Final amount per cuvette
RNP complex	1.6 µM
Cas9 nuclease	0.8 µM
ssODN	5 µg
iPS cells*	1 x 10^6
Total volume	**100 µL**

*As mentioned above, if you do not have enough prepared cells, you can decrease the number of cells per cuvette (for example, use 1 x 10^5 to 5 x 10^5 cells in 100 µL solution per cuvette); however, your transfection efficiency may be lower.

3. Set the electroporation parameters as recommended by Nepa Gene. (For assistance, contact info@nepagene.jp).
4. Mix the cells gently for 2 sec, without forming bubbles, by tapping the bottom of the cuvette with your finger, and place the cuvette into the CU500 Cuvette Chamber.

5. Press the Ω button on the NEPA21 electroporator, and record the impedance value.

   Note: The impedance value should be in the range of 30–55 Ω.

6. If needed, adjust the impedance:
   - If the impedance is below 30 kΩ, remove 2–3 µL of cell solution from the chamber to increase the impedance.
   - If the impedance is above 55 kΩ, add 2–3 µL of Opti-MEM to the chamber to decrease the impedance.

7. Press the **Start** button, and record the values of currents and joules displayed in the Measurements frame.

   Note: To avoid cell damage, plate cells immediately after electroporation (**steps D8–11**).

8. Remove the cuvette from the chamber.

9. Add a sufficient amount (e.g., 300 µL) of human iPS cell medium containing 10 µM Rock Inhibitor into the cuvette, using the included pipette.

10. Mix the cells and medium in the cuvette with the pipette to break up white clumps.

11. Transfer the entire sample in the cuvette to the prepared plate well (from **step C1**).

12. Repeat steps 3–11 for each cuvette.

13. Incubate the cells in a CO_2 incubator overnight.

14. Replace the medium the day after electroporation.

    Note: Adding Rock Inhibitor (final concentration of 10 µM) for a few days can increase cell viability.