Electroporation of human pluripotent or embryonic stem cells with CRISPR reagents

Ribonucleoprotein delivery using the Alt-R® CRISPR-Cas9 System for homology-directed repair or other genome editing applications

Contributed by Kenny Moore and James Martin, Sir William Dunn School of Pathology, Division of Medical Sciences, University of Oxford, UK

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	Supplier information
Neon® Transfection System	Thermo Fisher (cat # MPK5000)
NucleoCounter® NC-3000™ cytometer and consumables (or other live-cell counter solution)	ChemoMetec
Kits and reagents	
Neon Transfection System10 µL Kit	Thermo Fisher (cat # MPK1096)
1X Phosphate buffered saline (PBS) without Mg^{2+} or Ca^{2+}	General laboratory supplier
EDTA	General laboratory supplier
ROCK inhibitor (Y-27632 dihydrochloride)	Abcam (cat # ab120129) or equivalent
mTeSR™ 1 feeder-free maintenance media	STEMCELL Technologies (cat # 85850)

Kits and reagents (continued)	
Basement membrane matrix, for example:	
Matrigel® matrix	Corning (cat # 354277) or equivalent
Geltrex® matrix	Thermo Fisher (cat # A1413301)
Alt-R CRISPR-Cas9 crRNA	IDT predesigned and custom crRNA*: www.idtdna.com/CRISPR-Cas9
Alt-R CRISPR-Cas9 tracrRNA	IDT (cat # 1072532,1072533,1072534)
Alternative:	
Alt-R CRISPR-Cas9 tracrRNA – ATTO™ 550	IDT (cat # 1075927,1075928)
Alt-R *S.p.* Cas9 Nuclease 3NLS†	IDT (cat # 1074181, 1074182)
Alternative:	
Alt-R *S.p.* HiFi Cas9 Nuclease 3NLS	IDT (cat # 1078727, 1078728)
Nuclease-Free Duplex Buffer	IDT (cat # 11-01-03-01)
For homology-directed repair templates:	
Single-stranded oligodeoxynucleotide (ssODN)‡	www.idtdna.com/Ultramer or www.idtdna.com/Megamer
For experiments with no ssODN:	
Alt-R Cas9 Electroporation Enhancer	IDT (cat # 1075915, 1075916)

* We guarantee the performance of our predesigned crRNAs targeting human, mouse, rat, zebrafish, or nematode genes. For other species, you may use our proprietary algorithms to design custom crRNAs. If you have crRNA protospacer designs of your own or from publications, use our design checker tool to assess their on- and off-targeting potential before ordering crRNAs that are synthesized using our Alt-R crRNA modifications. For details about the predesigned crRNA guarantee, see www.idtdna.com/CRISPR-Cas9.

† Alt-R *S.p.* Cas9 Nuclease 3NLS is suitable for most genome editing studies. However, some experiments may benefit from use of Alt-R *S.p.* HiFi Cas9 Nuclease 3NLS, which has been engineered to reduce off-target effects while retaining on-target potency of Alt-R *S.p.* Cas9 Nuclease 3NLS.

‡ We recommend Ultramer® DNA Oligonucleotides for ssODN templates that are up to 200 bases and Megamer® Single-Stranded DNA Fragments for ssODN templates between 200 and 2000 bases. For additional information about using ssODN as HDR templates, see the application note. For additional information about using ssODN as HDR templates, see the application note, Optimizing for CRISPR-Cas9 homology-directed repair (HDR) for efficient, high-fidelity genome editing.

METHODS

A. Prepare cells and plates

1. More than 1 hour before transfection, add ROCK inhibitor (final concentration of 10 µM) to cultured cells.

   Note: Do not use antibiotics (i.e., penicillin-streptomycin) during plate preparation.

2. Coat and prewarm plates.
   a. Coat 24-well plates with your selected basement membrane matrix, then allow matrix to form a gel at 37°C for 1 hr.
   b. For each well, remove the supernatant and replace with 500 µL of mTeSR media supplemented with ROCK inhibitor at a final concentration of 10 µM.
   c. Store prepared plates in a tissue culture incubator to prewarm the media before the transfected cells are added.

B. Prepare crRNA:tracrRNA duplex

1. Resuspend each RNA oligo (Alt-R CRISPR-Cas9 crRNA and Alt-R CRISPR-Cas9 tracrRNA) in Nuclease-Free Duplex Buffer to final concentrations of 100 µM.

   Note: For assistance, use the Resuspension Calculator at www.idtdna.com/scitools.

2. Mix the two RNA oligos in equimolar concentrations in a sterile microcentrifuge tube. The following table shows an example for a 10 µL final volume, which provides sufficient material for 20 transfections:

Component	Amount (µL)
100 µM Alt-R CRISPR-Cas9 crRNA	4.4
100 µM Alt-R CRISPR-Cas9 tracrRNA	4.4
Nuclease-Free Duplex Buffer	1.2
Total volume	10

3. Heat at 95°C for 5 min.
4. Remove from heat, and allow to cool to room temperature on the bench top (5–10 min).

C. Form the ribonucleoprotein (RNP) complex

1. For each well undergoing electroporation, dilute Alt-R Cas9 nuclease to 36 µM by combining the following:

Component	Amount (µL)
Alt-R Cas9 nuclease (61 µM stock)	0.3
Resuspension Buffer R (from the Neon system kit)	0.2
Total volume	0.5

2. For each well undergoing electroporation, combine the crRNA:tracrRNA duplex and the diluted Cas9 nuclease.

Component	Amount (µL)
Diluted Alt-R Cas9 enzyme (36 µM) (from step C1)	0.5
crRNA:tracrRNA duplex (from step B4)	0.5
Total volume	1

3. Incubate at room temperature for 20 min.

D. Prepare the Alt-R Cas9 Electroporation Enhancer or ssODN

1. At first use, resuspend the Alt-R Cas9 Electroporation Enhancer or ssODN to 100 µM in Nuclease-Free Duplex Buffer to create a stock solution.
2. For each set of experiments, dilute stock (from step D1) to 10.8 µM in Neon Resuspension Buffer R to create a working solution.

   Note: You will need 2 µL of working solution for each electroporation. For assistance, use the Resuspension Calculator at www.idtdna.com/scitools.

E. Prepare cell cultures for electroporation

1. Wash cells with 0.5 mM EDTA in PBS, and then incubate cells in 0.5 mM EDTA in PBS at 37°C for 3 min.
2. Remove the supernatant and incubate the cells at 37°C for another 4 min.
3. Resuspend the cells in 1 mL 1X PBS and transfer to a sterile, microcentrifuge tube.
4. Count the cells.
5. Centrifuge the cells at 120 x *g* for 10 min in a swing-out rotor.

   Note: The cell-containing microcentrifuge tubes can be placed inside a 50 mL centrifuge tube.
6. Remove the supernatant and resuspend the cells in Neon Resuspension Buffer R to a density of 2.2 x 10^7 cells/mL.

   Note: 2 x 10^5 iPSCs or ESCs are used per electroporation with the Neon system.

F. Prepare Neon Transfection System

1. Turn on the Neon system.
2. Enter the following pulse settings:
   - 1400 V
   - 20 ms pulse width
   - 1 pulse
3. Set up the Neon Pipette Station.
   a. Fill the Neon Tube with Electrolytic Buffer (from the Neon Transfection System10 µL Kit).
   b. Insert the tube into the station.

G. Perform electroporation of cells

1. For each electroporation, add the following to a sterile microcentrifuge tube:

Component	Amount (µL)
tracrRNA:Cas9 RNP complex (from step C3)	1
Cell suspension (from step E6)	9
10.8 µM Alt-R Cas9 Electroporation Enhancer or ssODN donor template (from step D2)	2
Total volume	12

2. Pipette 10 µL of the cell:RNP complex mixture (from step G1) into the Neon Tip.
3. Insert the Neon Pipette and Tip into the Pipette Station, and make sure there is Electrolytic Buffer in the Neon Tube.
4. Press Start.
5. After electroporation, transfer cells to a 24-well plate containing prewarmed mTeSR media and ROCK inhibitor (from step A2c).
6. Analyze cells 2–3 days after electroporation.

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