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

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The methods presented in this document has been provided by an IDT customer(s) who have 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.

Consumables and equipment

Table 1. Consumables—IDT

Product name	Catalog number
Alt-R CRISPR-Cas9 tracrRNA	1072532,1072533,1072534
Alternative:	
Alt-R CRISPR-Cas9 tracrRNA – ATTO™ 550	1075927,1075928
Alt-R S.p. Cas9 Nuclease 3NLS†	Discontinued, Alt-R S.p. Cas9 Nuclease V3 should be substituted
Alt-R S.p. Cas9 Nuclease V3	1081058, 1081059, 10000735
Alternative:	
Alt-R S.p. HiFi Cas9 Nuclease V3	1081060, 1081061, 10007803
Nuclease-Free Duplex Buffer	11-01-03-01

Table 1. Consumables—IDT (continued)

Product name	Catalog number
For homology-directed repair templates:	
Single-stranded oligodeoxynucleotide (ssODN)‡	www.idtdna.com/Ultramer or www.idtdna.com/Megamer
Double-stranded donors:	www.idtdna.com/hdrdonoroligos
For experiments with no ssODN:	
Alt-R Cas9 Electroporation Enhancer	1075915, 1075916
Alt-R CRISPR-Cas9 crRNA	IDT predesigned and custom crRNA*: www.idtdna.com/CRISPR-Cas9

† Alt-R S.p. Cas9 Nuclease V3 is suitable for most genome editing studies. However, some experiments may benefit from use of Alt-R S.p. HiFi Cas9 Nuclease V3, which has been engineered to reduce off-target effects, while retaining on-target potency of Alt-R S.p. Cas9 Nuclease V3.

‡ We recommend Alt-R HDR Donor Oligos for ssODN templates that are up to 200 bases and Alt-R HDR Donor Blocks for dsDNA templates between 200–3000 bases. Megamer® Single- Stranded DNA Fragments for ssODN templates of between 200–2000 bases are also available for larger knock-in templates. For additional information about HDR templates, see the application note Improve your CRISPR HDR efficiencies or visit www.idtdna.com/CRISPR-Cas9.

* We guarantee that predesigned Alt-R CRISPR-Cas9 guide RNAs will provide successful editing at the target site, when delivered as a ribonucleoprotein complex as described in the Alt-R User Guides, using Alt-R CRISPR-Cas9 guide RNAs (crRNA:tracrRNA duplex or sgRNA) and either Alt-R S.p. Cas9 nuclease or Alt-R S.p. HiFi Cas9 nuclease. Analysis of editing must be at the DNA level, such as with the Alt-R Genome Editing Detection Kit or DNA sequencing. If successful editing is not observed for a predesigned guide RNA while an appropriate positive control is successful, a one-time "no-cost" replacement of the predesigned Alt-R CRISPR-Cas9 guide RNA will be approved, upon discussion with our Scientific Applications Support team (applicationsupport@idtdna.com). This guarantee does not extend to any replacement product, or to any other incurred or incidental costs or expenses.

Table 2. Consumables—Other suppliers

Item	Ordering information
Neon® Transfection System	Thermo Fisher Scientific (Cat. No. MPK5000)
NucleoCounter® NC-3000™ cytometer and consumables (or another live-cell counter solution)	ChemoMetec
Neon Transfection System 10 µL Kit	Thermo Fisher Scientific (Cat. No. MPK1096)
1X Phosphate buffered saline (PBS) without $Mg2^+$ or $Ca2^+$	General laboratory supplier
EDTA	General laboratory supplier
ROCK inhibitor (Y-27632 dihydrochloride)	Abcam (Cat No. ab120129), or equivalent
mTeSR™1 feeder-free maintenance media	STEMCELL Technologies (Cat. No. 85850)
Basement membrane matrix, for example:	
Matrigel® matrix	Corning (Cat. No. 354277), or equivalent
Geltrex® matrix	Thermo Fisher Scientific (Cat. No. A1413301)

Methods

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 24-well plates with your selected basement membrane matrix, and allow matrix to form a gel for 1 hr.
3. For each well, remove the matrix, and add 500 µL of mTeSR media supplemented with ROCK inhibitor at a final concentration of 10 µM.
4. Store prepared plates in a tissue culture incubator to prewarm the media before the transfected cells are added.

Mix 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.

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).

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 Form the ribonucleoprotein (RNP) complex)	0.5
crRNA:tracrRNA duplex (from Mix crRNA:tracrRNA duplex)	0.5
Total volume	1

3. Incubate at room temperature for 20 min.

Resuspend and dilute 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 this stock 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.

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.

Turn on and set 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. Fill the Neon Tube with Electrolytic Buffer (from the Neon® system kit).
4. Insert the tube into the station.

Electroporate cells

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

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

2. Pipette 10 µL of the cell:RNP complex mixture 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.
6. Analyze cells 2–3 days after electroporation.

Revision history

Version	Release date	Description of changes
2	July 2022	Updated document to internal MAPSS compliance
1	February 2021	Initital release

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