genome editing

protocol

Alt-R® CRISPR-Cas9 system:

Delivery of ribonucleoprotein complexes into Jurkat T cells using the Bio-Rad Gene Pulser® Xcell™ Electroporation System

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Introduction

This protocol describes the delivery of a CRISPR-Cas9 ribonucleoprotein (RNP) complex, containing Alt-R CRISPR-Cas9 crRNA:tracrRNA and a Cas9 endonuclease, into Jurkat T cells using electroporation with the Bio-Rad Gene Pulser Xcell Electroporation System.

Important considerations

1. Use low-passage, healthy Jurkat T cells. A critical factor affecting the success of electroporation is the health of the cells. It is important to do the following:
    - use the lowest passage number cells available
    - subculture cells for at least 2–3 days before the electroporation procedure
    - replace the media the day before electroporation
    - determine the optimal confluency for your cell type
2. Wash the cells. FBS may contain RNase activity that can quickly degrade the critical CRISPR RNA components. Therefore, it is crucial to wash the cells with PBS to remove any FBS-containing media.
3. Assemble RNPs individually. Use separate reactions for each crRNA, if targeting multiple sites per sample.
4. Include Alt-R Cas9 Electroporation Enhancer in the electroporation. To improve electroporation efficiency, we recommend using this non-targeting carrier DNA at a fixed dose of 4.8 µM.

    Note: For more information on the importance of the electroporation enhancer in this protocol, see the DECODED article *Successful CRISPR genome editing in hard-to-transfect cells.*

5. Always include proper controls in your experiment. We recommend using the appropriate Alt-R CRISPR-Cas9 control kit for studies in human, mouse, or rat cells.

    The control kits include an Alt-R CRISPR-Cas9 HPRT Positive Control crRNA targeting the *HPRT* gene and a computationally validated Alt-R CRISPR-Cas9 Negative Control crRNA. The kits also include the Alt-R CRISPR-Cas9 tracrRNA for complexing with the crRNA controls, Nuclease-Free Duplex Buffer, and validated PCR primers for amplifying the targeted *HPRT* region in the selected organism. The inclusion of the PCR assay makes the kits ideal for verification of *HPRT* gene editing using the Alt-R Genome Editing Detection Kit (T7 endonuclease I assay).

Required materials

Kits and reagents	Ordering information
Gene Pulser Xcell Electroporation System	Bio-Rad (multiple options)
Gene Pulser/MicroPulser™ Electroporation Cuvettes, 0.2 cm gap	Bio-Rad (cat # 1652082)
RPMI-1640 Medium (RPMI)	ATCC (cat # 30-2001)
Fetal bovine serum (FBS)	General laboratory supplier
1X Phosphate buffered saline (PBS)	General laboratory supplier
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)
(Optional/Recommended) Alt-R CRISPR-Cas9 Control Kit	IDT (cat # 1072554 [human], 1072555 [mouse], or 1072556 [rat])
Alt-R S.p. Cas9 Nuclease 3NLS† Alternatives: Alt-R S.p. HiFi Cas9 Nuclease 3NLS Alt-R S.p. Cas9 D10A Nickase 3NLS Alt-R S.p. Cas9 H840A Nickase 3NLS	IDT (cat # 1074181, 1074182) IDT (cat # 1078727, 1078728) IDT (cat # 1078729, 1078730) IDT (cat # 1078731, 1078732)
Nuclease-Free IDTE, pH 7.5 (1X TE solution)	IDT (cat # 11-01-02-02)
(Optional/Recommended) Alt-R Cas9 Electroporation Enhancer‡	IDT (cat # 1075915, 1075916) Sequence (100 nt): TTAGCTCTGTTTACGTCCCAGCGGGCATGAGAGTAACA AGAGGGTGTGGTAATATTACGGTACCGAGCACTATCGA TACAATATGTGTCATACGGACACG

* 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 the 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.

‡ The enhancer is designed to avoid homology to human, mouse, or rat genomes, and has been tested as carrier DNA in multiple cell lines, including HEK-293, Jurkat, and K562. Before use in other species, verify that this oligo does not have similarity to your host cell genome to limit participation of the oligo in the repair process of double-stranded DNA breaks.

Protocol

A. Prepare cell cultures before electroporation

Note: Do *not* use freshly thawed cells for electroporation; use cells with the lowest passage number possible.

1. Split cells, if necessary, to obtain optimal confluency for electroporation.
2. Change the cell culture media on the cells 1 day before electroporation.
3. Prepare the culture plate to receive cells following electroporation by filling the applicable wells of a 12–well plate with 2 mL of culture media (RPMI, 10% FBS).
4. Preheat the plate in a tissue culture incubator at 37°C, 5% CO_2.

B. Form the crRNA:tracrRNA duplex

1. Resuspend each RNA oligo (Alt-R CRISPR-Cas9 crRNA and Alt-R CRISPR-Cas9 tracrRNA) in IDTE buffer to the final concentration of 200 µM.

Note: You can use the IDT resuspension calculator at **www.idtdna.com/scitools**. Always store resuspended RNAs at -20°C.

2. Mix the two RNA oligos in equimolar concentrations in a single microcentrifuge tube to a final duplex concentration of 100 µM.

   The following table shows an example of a 20 µL final volume:

Component	Amount (µL)
200 µM Alt-R CRISPR-Cas9 crRNA	10
200 µM Alt-R CRISPR-Cas9 tracrRNA	10
Total volume	**20**

3. Heat at 95°C for 5 min.
4. Remove the duplex from the heat and allow to cool to room temperature (15–25°C).

C. Form the RNP complex

1. Mix the crRNA:tracrRNA duplex and Cas9 enzyme components at a 1:1.2 molar ratio in PBS.

Note: We observe robust editing efficiency in Jurkat T cells when the concentration of Cas9 RNP ranges from 1–4 µM in the final 100 µL mixture for electroporation (see Figure 1). The following table demonstrates how to create a 4 µM final concentration to use in **step E14**. Reactions at lower concentrations can be performed by diluting the mixture.

Component	Amount (µL)
Alt-R crRNA:tracrRNA duplex (100 µM) (from **step B4**)	12
Alt-R Cas9 enzyme (61 µM stock)*	16.7
PBS	21.3
Total volume†	**50**

* All Alt-R S.p. Cas9 nucleases are provided at a stock concentration of 61 µM.

† In the 50 µL mixture, the concentration for Cas9 enzyme is 20 µM, while the concentration for duplexed RNA is 24 µM.

2. Incubate at room temperature for 20 min to allow formation of the RNP complex.
3. After the RNP complex has formed, keep the mixture on ice until electroporation.

D. Prepare the electroporation station

1. Keep cuvettes at 4°C. If any condensation is present, wipe it away before using.
2. Enter these electroporation settings:
    - 250 V
    - 2 ms pulse width
    - 1 pulse
    - unipolar polarity

Note: We have found these settings to be optimal for Jurkat T cells. Other cell lines may require different optimized parameters.

E. Perform electroporation of cells

1. Create a working solution by resuspending the Alt-R Electroporation Enhancer to 96 µM in IDTE.
2. Take the cells subject to electroporation and resuspend them in culture media (RPMI, 10% FBS). Pipette mix to dissociate any cell clumps.
3. Count the cells in the suspension culture.
4. Determine the total number of cells for each experiment. In this example, we will use 1 x 10^7 cells which is enough for 10 electroporations.

> **Note:** For Jurkat cells, we recommend using 1 x 10^6 cells per electroporation.

5. Dilute 1 x 10^7 cells to a final volume of 40 mL using PBS.
6. Centrifuge the required number of cells for all electroporation samples at 200 x *g* for 5 min at room temperature (15–25°C).
7. Remove as much of the supernatant as possible without disturbing the pellet.
8. Wash cells in 5 mL of PBS.
9. Centrifuge at 200 x *g* for 5 min at room temperature.
10. Remove as much of the supernatant as possible without disturbing the pellet.
11. Resuspend the cells by adding 750 µL PBS , which results in a cell density of 1 x 10^6/75 µL.
12. Aliquot 75 µL of the resuspended cells for each electroporation in 1.5 mL microcentrifuge tubes.
13. Keep the resuspended cells on ice for at least 5 min before starting electroporation.
14. For each electroporation, combine the following components into a sterile, microcentrifuge tube:

Component	Amount (µL)
Alt-R RNP complex (from **step C3**)	20
96 µM Alt-R Electroporation Enhancer (from **step E1**)	5
Aliquoted cell resuspension (from **step E12**)	75
Total volume*	**100**

* The final concentration for each electroporation in the 100 µL total volume is 4 µM Cas9 nuclease—4.8 µM crRNA:tracrRNA duplex, and 4.8 µM Electroporation Enhancer.

15. Immediately transfer the mixture to cooled cuvettes (from **step D1**) and start electroporation.
16. After electroporation, transfer cells to the preheated wells containing 2 mL of culture media (RPMI, 10% FBS) on the 12-well tissue culture plate (from **step A5**).
17. Incubate cells in a tissue culture incubator at 37°C, 5% CO_2 for 72 hr.

Note: To detect on-target mutations with the mismatch endonuclease T7E1, use the protocol described in *Part 2* of *Alt-R CRISPR-Cas9 System: User guide for cationic lipid delivery of CRISPR-Cas9 ribonucleoprotein into mammalian cells.*

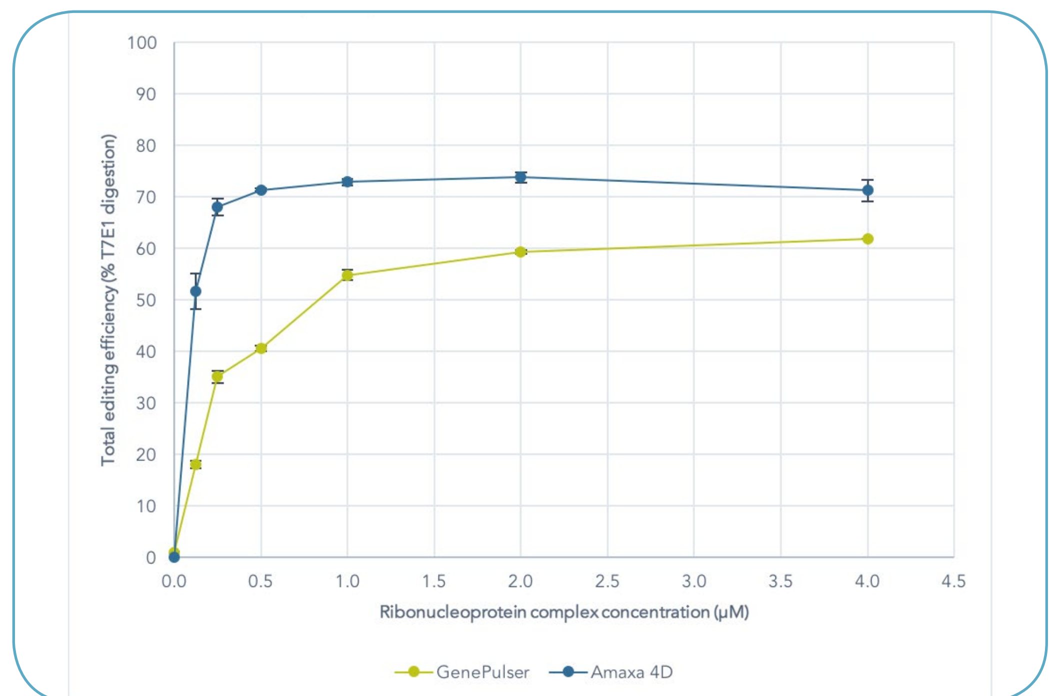

Figure1. Robust genome editing achieved using electroporation with the Bio-Rad Gene Pulser Xcell system. Jurkat T cells were transfected with 0.125–4 µM RNP (Alt-R ***S.p.*** Nuclease 3NLS complexed with Alt-R CRISPR-Cas9 crRNA and tracrRNA) in the presence of Alt-R Cas9 Electroporation Enhancer, on the Amaxa 4D and Bio-Rad Gene Pulser platforms. Genomic DNA was isolated 72 hr after transfection. Total editing efficiency was determined using the Alt-R Genome Editing Detection Kit (T7 endonuclease I assay).

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