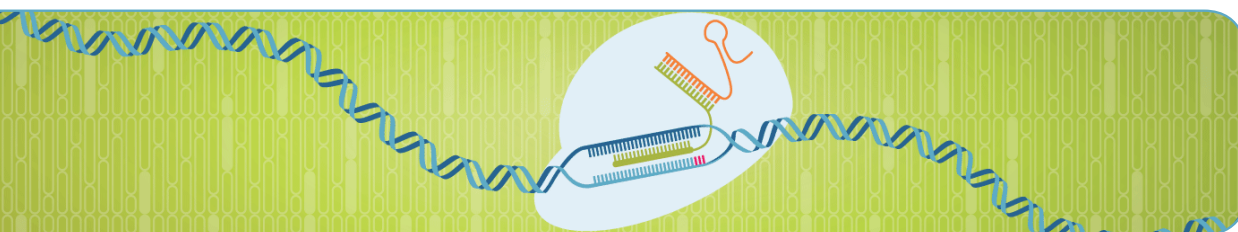


## Alt-R CRISPR-Cas9 system:

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



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## Introduction

This protocol describes the delivery of a CRISPR-Cas9 ribonucleoprotein (RNP) complex, containing Alt-R CRISPR-Cas9 guide RNA (crRNA:tracrRNA duplex or sgRNA) 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 guide RNA (gRNA), 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  $\mu\text{M}$ .

**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. When using crRNA:tracrRNA duplexes, 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).

For assistance with control sgRNAs, contact [applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com).



## 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
<b>Option 1, 2-part guide RNA (crRNA + tracrRNA):</b>	
<ul style="list-style-type: none"> <li>Alt-R CRISPR-Cas9 crRNA or Alt-R CRISPR-Cas9 crRNA XT</li> <li>Alt-R CRISPR-Cas9 tracrRNA or Alt-R CRISPR-Cas9 tracrRNA – ATTO™ 550</li> </ul>	IDT predesigned and custom crRNA*: <a href="http://www.idtdna.com/CRISPR-Cas9">www.idtdna.com/CRISPR-Cas9</a> IDT (cat # 1072532, 1072533, 1072534) IDT (cat # 1075927, 1075928)
<b>Option 2, single guide RNA (sgRNA):</b>	IDT predesigned and custom sgRNA*:
Alt-R CRISPR-Cas9 sgRNA	<a href="http://www.idtdna.com/CRISPR-Cas9">www.idtdna.com/CRISPR-Cas9</a>
(Recommended for option 1, 2-part guide RNAs)	IDT (cat # 1072554 [human], 1072555 [mouse],
Alt-R CRISPR-Cas9 Control Kit	or 1072556 [rat])
Alt-R S.p. Cas9 Nuclease V3†	IDT (cat # 1081058, 1081059)
Alternative:	
Alt-R S.p. HiFi Cas9 Nuclease V3	IDT (cat # 1081060, 1081061)
Nuclease-Free IDTE, pH 7.5 (1X TE solution)	IDT (cat # 11-01-02-02)
(Optional/Recommended)	IDT (cat # 1075915, 1075916)
Alt-R Cas9 Electroporation Enhancer‡	Sequence (100 nt): TTAGCTCTGTTTACGTCCCAGCGGGCATGAGAGTAA CAAGAGGGTGTGGTAATATTACGGTACCGAGCACTA TCGATACAATATGTGTCATACGGACACG

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

† Alt-R S.p. Cas9 Nuclease V3 (wild-type) is suitable for most genome editing studies. However, some experiments may benefit from the 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.

‡ 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<sub>2</sub>.

### B. Prepare RNA

1. Resuspend your RNA oligos in IDTE Buffer.

Guide RNA	Final concentration (µM)
Option 1	
Alt-R CRISPR-Cas9 crRNA	200
Alt-R CRISPR-Cas9 tracrRNA	200
Option 2	
Alt-R CRISPR-Cas9 sgRNA	100

For assistance, use the IDT Resuspension Calculator at [www.idtdna.com/SciTools](http://www.idtdna.com/SciTools).

**Store resuspended RNAs at -20°C.**

2. If using sgRNA, proceed to **step C** (Form the RNP complex).
3. Mix the crRNA and tracrRNA 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
<b>Total volume</b>	<b>20</b>

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

### C. Form the RNP complex

1. Mix the guide RNA 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 range of Cas9 RNP is 1–4  $\mu\text{M}$  in the final 100  $\mu\text{L}$  mixture for electroporation (see Figure 1). The following table demonstrates how to create a 4  $\mu\text{M}$  final concentration to use in **step E14**. Reactions at lower concentrations can be performed by diluting the mixture.

Component	Amount ( $\mu\text{L}$ )
Alt-R guide RNA (100 $\mu\text{M}$ ) (crRNA:tracrRNA duplex from <b>step B5</b> or sgRNA from <b>step B2</b> )	12
Alt-R Cas9 enzyme (62 $\mu\text{M}$ stock)*	16.7
PBS	21.3
<b>Total volume†</b>	<b>50</b>

\* All Alt-R S.p. Cas9 enzymes are provided at a stock concentration of 62  $\mu\text{M}$ .

† In the 50  $\mu\text{L}$  mixture, the concentration for Cas9 enzyme is 20  $\mu\text{M}$ , while the concentration for duplexed RNA is 24  $\mu\text{M}$ .

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 parameters.

## E. Perform electroporation of cells

1. Create a working solution by resuspending the Alt-R Electroporation Enhancer to 96  $\mu\text{M}$  in IDTE.
2. Resuspend cells for electroporation 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  $1 \times 10^7$  cells which is enough for 10 electroporations.

**Note:** For Jurkat cells, we recommend using  $1 \times 10^6$  cells per electroporation.

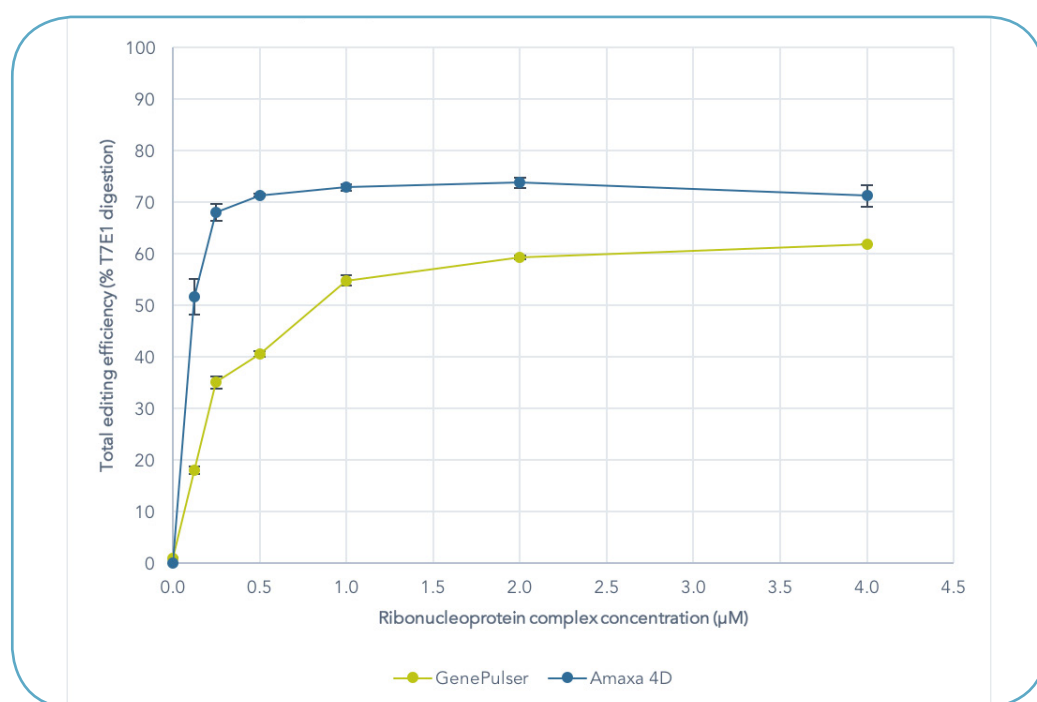
5. Dilute  $1 \times 10^7$  cells to a final volume of 40 mL using PBS.
6. Centrifuge the required number of cells for all electroporation samples at  $200 \times g$  for 5 min at room temperature (15–25°C).
7. Remove as much of the supernatant as possible without disturbing the pellet.
8. Wash cells in 5 mL of PBS.
9. Centrifuge at  $200 \times g$  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  $\mu\text{L}$  PBS, which results in a cell density of  $1 \times 10^6$  per 75  $\mu\text{L}$ .
12. Aliquot 75  $\mu\text{L}$  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 ( $\mu\text{L}$ )
Alt-R RNP complex (from <b>step C3</b> )	20
96 $\mu\text{M}$ Alt-R Electroporation Enhancer (from <b>step E1</b> )	5
Aliquoted cell resuspension (from <b>step E13</b> )	75
<b>Total volume*</b>	<b>100</b>

\* The final concentration for each electroporation in the 100  $\mu\text{L}$  total volume is 4  $\mu\text{M}$  Cas9 nuclease—4.8  $\mu\text{M}$  guide RNA, and 4.8  $\mu\text{M}$  electroporation enhancer.

15. Immediately transfer the mixture to cooled cuvettes (from **step D1**) and start electroporation.
16. After electroporation, transfer cells to the wells of the 12-well tissue culture plate containing 2 mL of preheated culture media (RPMI, 10% FBS) (from **step A4**).
17. Incubate cells in a tissue culture incubator at 37°C, 5% CO<sub>2</sub> for 72 hr.

To detect on-target mutations with the mismatch endonuclease T7E1, use the **Alt-R Genome Editing Detection Kit** (cat # 1075931, 1075932, 1075933) as **instructed**.



**Figure 1. 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).



## Revision history

Version	Date released	Description of changes
2	July 2018	Added instructions for using Alt-R CRISPR-Cas9 sgRNA. Updated names and catalog numbers for Alt-R enzymes (V3).
1.1	May 2018	Added note about use of improved Alt-R enzymes (V3): direct substitution in protocol of V3 enzymes for original enzymes (3NLS).
1	November 2017	Original protocol.

Alt-R CRISPR-Cas9 system:

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

Technical support:

[applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com)

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