Alt-R™ CRISPR-Cpf1 System:

Delivery of ribonucleoprotein complexes in Jurkat T cells using the Neon® Transfection System

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Introduction

This protocol describes the optimized delivery of a Cpf1 ribonucleoprotein (RNP) complex, containing Alt-R™ CRISPR-Cpf1 crRNA and Alt-R A.s. Cpf1 Nuclease 2NLS, into Jurkat T cells using the Neon® Transfection System (Thermo Fisher) with 10 µL electroporation cuvettes [1]. Application of this protocol can be found in a DECODED newsletter article [2]. Other cell lines will likely require optimization of electroporation parameters and RNP concentration to ensure maximum editing efficiency. To detect on-target mutations and estimate editing efficiency, we recommend using the Alt-R Genome Editing Detection Kit [3].

The CRISPR-Cpf1 system is distinct from the more commonly used CRISPR-Cas9 system. For example, Cpf1 nuclease does not require a tracrRNA; recognizes a T-rich, protospacer-adjacent motif (PAM: TTTV, where V is an A, C, or G base); and creates a staggered double-stranded DNA cut with a 5′ overhang. For additional information, visit www.idtdna.com/CRISPR-Cpf1.

Important considerations

1. Use low-passage, healthy cells. A critical factor affecting the success of electroporation is the health of the cells. It is important to:
   - 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 cells before electroporation.** FBS and trypsin may contain RNase activity that can quickly degrade CRISPR RNA components. Therefore, **it is crucial to wash cells with PBS** to remove FBS-containing media or trypsin. Alternatively, for adherent cells, use "enzyme-free" dissociation media instead of trypsin.
3. **Alt-R Cpf1 Electroporation Enhancer, a non-targeting, single-stranded carrier DNA, is required for successful electroporation.** See the Performance section at www.idtdna.com/CRISPR-Cpf1 for supporting data.
4. **Always include appropriate controls in your experiment.** We recommend using an HPRT-specific, positive control crRNA and a non-targeting negative control. For suggested sequences for studies in human, mouse, or rat cells, see page 4.

   To detect on-target editing of the suggested HPRT control crRNA, use Alt-R HPRT PCR Primer Mix (available for human, mouse, or rat) with the Alt-R Genome Editing Detection Kit (T7 endonuclease I assay).

Required materials

Instruments, kits, and reagents	Ordering information
Neon® Transfection System	Thermo Fisher Scientific (Cat # MPK5000)
Neon® Transfection System 10 µL Kit	Thermo Fisher Scientific (Cat # MPK1096)
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-Cpf1 crRNA	IDT custom crRNA (www.idtdna.com/CRISPR-Cpf1)
Alt-R™ A.s. Cpf1 Nuclease 2NLS	IDT (Cat # 1076158)
Nuclease-Free IDTE, pH 7.5 (1X TE solution)	IDT (Cat # 11-01-02-02)
Alt-R™ Cpf1 Electroporation Enhancer	IDT (Cat # 1076300) **Note:** The electroporation enhancer does not have significant homology to the human, mouse, or rat genomes, and has been tested as carrier DNA in multiple cell lines, including HEK-293, Jurkat, and HeLa.

To order control crRNAs, enter the appropriate sequence into the Cpf1 crRNA ordering tool (accessible at www.idtdna.com/CRISPR-Cpf1). These sequences are available online for copying and pasting into the ordering tool.

Recommended control crRNAs	Sequence
Positive control Cpf1 crRNA, Human *HPRT1*	GGTTAAAGATGGTTAAATGAT
Positive control Cpf1 crRNA, Mouse *Hprt*	GGATGTTAAGAGTCCCTATCT
Positive control Cpf1 crRNA, Rat *Hprt1*	ATGCTTAAGAGGTATTTGTTA
Negative control Cpf1 crRNA #1	CGTTAATCGCGTATAATACGG
Negative control Cpf1 crRNA #2	CATATTGCGCGTATAGTCGCG
Negative control Cpf1 crRNA #3	GGCGCGTATAGTCGCGCGTAT

Protocol

A. Prepare cell cultures for electroporation

1. Do not use freshly thawed cells for electroporation: passage your cells 1X after thawing, and verify that they grow well and look healthy.
2. Use cells with the lowest passage number possible.
3. Change the cell culture media on the cells 1 day before electroporation.
4. Split cells, if necessary, to obtain optimal confluency for electroporation.

> Note: For Jurkat cells, optimal cell density is between 1 x 10^5 and 1 x 10^6 cells/mL at the time of transfection.

B. Prepare the crRNA

1. At first use, resuspend Alt-R CRISPR-Cas9 cRNA in IDTE Buffer to create a 100 µM stock solution. For assistance, use the IDT Resuspension Calculator at www.idtdna.com/scitools.

   Store resuspended RNAs at –20°C.

2. Dilute crRNA (from **Step B1**) to a 75 µM working dilution. You will need 1 µL of working dilution for each electroporation in **Step C1**.

C. Form the RNP complex

1. For each well undergoing electroporation, combine the crRNA and A.s. Cpf1 Nuclease components, gently swirling the pipet tip while pipetting:

Component	Amount
Alt-R™ CRISPR-Cpf1 crRNA (from **Step B2**)	1.0 µL (75 pmol)
Alt-R™ A.s. Cpf1 Nuclease 2NLS	1.0 µL (63 pmol)
Total volume	**2.0 µL***

* This 2 µL volume is for 1 electroporation reaction; scale up as necessary for your experiment—we recommend making 1.2X volume needed to correct for pipetting errors.

2. Incubate the mixture at room temperature for 10–20 min.

> Note: To save time, the RNP can be prepared during the 2 x 10 min centrifugation in Steps E6 and E8 below.

D. Prepare Neon® Transfection System

1. Turn on the Neon system.
2. Enter electroporation settings, or choose a setting from the optimization protocol.

   Note: In our experiments, the optimum settings for Jurkat cells was found to be 1600 V, 10 ms pulse width, 3 pulses [2].

3. Set up the Neon Pipette Station by filling the Neon Tube with Electrolytic Buffer (included in the Neon Transfection System Kit) and insert into the station.

E. Perform electroporation of cells

1. Prepare the Alt-R Cpf1 Electroporation Enhancer. For assistance, use the Resuspension Calculator at www.idtdna.com/scitools.
   a. At first use, resuspend the Alt-R Cpf1 Electroporation Enhancer to 100 µM in IDTE to create a stock solution.
   b. For each set of experiments, dilute stock to 10.8 µM (working solution). You will need 2 µL of working solution for each electroporation in **Step E11**.
2. Prepare culture plates to receive cells following electroporation.
   a. Set 1: for each electroporation sample, fill a well with 190 µL of culture media (RPMI, 10% FBS) to resuspend cells in **Step E16**.
   b. Set 2: for each electroporation sample, fill 3 wells with 150 µL of culture media (RPMI, 10% FBS) for growth in **Step E17**.
   c. Store all plates in a tissue culture incubator (37°C, 5% CO_2).
3. Pipette cells up and down to dissociate cell clumps.
4. Count the cells in the suspension culture.
5. Determine the total number of cells necessary for your experiment.

   Note: For Jurkat cells, we use 5 x 10^5 cells per electroporation.

6. Centrifuge the required number of cells for all electroporation samples at 600 rpm (Beckman GH-3.8 rotor: 58 x *g*) for 10 min at room temperature.
7. Remove as much supernatant as possible without disturbing the pellet.
8. Wash cells in 5 mL of 1X PBS, and then centrifuge at 600 rpm (Beckman GH-3.8 rotor: 58 x *g*) for 10 min at room temperature.

   Note: FBS in the growth media may contain RNase activity. Therefore, it is crucial to wash the cells with PBS after spinning down.

9. Remove as much supernatant as possible without disturbing the pellet.
10. Resuspend cells by adding 8 µL of Resuspension Buffer R per 5 x 10^5 cells.
11. For each electroporation, add the following to a 200 µL PCR tube:

Component	Amount (µL)*
crRNA:Cpf1 RNP complex (from **Step C2**)	2
Cell suspension (from **Step E10**)	8
10.8 µM Alt-R™ Cpf1 Electroporation Enhancer (from **Step E1b**)	2
Total volume	**12**

* The final concentration for each electroporation is 6.25 µM crRNA, 5.25 µM Cpf1 nuclease, and 1.8 µM Cpf1 electroporation enhancer.

12. Insert a Neon Tip into the Neon Pipette.
13. Pipette 10 µL of cell/RNP complex mixture (from **Step E11**) into the Neon Tip, avoiding air bubbles.
14. Insert the Neon Pipette and Tip into the Pipette Station. Verify the presence of Electrolytic Buffer in the Neon Tube.
15. Press **Start**.
16. After electroporation, transfer cells to wells containing 190 µL of pre-warmed culture media (RPMI, 10% FBS) (from **Step E2a**) and slowly resuspend.
17. Transfer 50 µL of resuspended cells in triplicate to the wells containing 150 µL of pre-warmed culture media (RPMI, 10% FBS) (from **Step E2b**).
18. Incubate cells in a tissue culture incubator (37°C, 5% CO_2) for 72 hr.

To detect on-target mutations with the Alt-R Genome Editing Detection Kit (T7EI mismatch assay), follow the protocol [3].

References

1. (2014) Neon® Transfection System for transfecting mammalian cells, including primary and stem cells, with high transfection efficiency. Thermo Fisher. Available at https://tools.thermofisher.com/content/sfs/manuals/neon_device_man.pdf. (Accessed May 19, 2017)
2. Turk R and Prediger E. (2016) Successful CRISPR genome editing in hard-to-transfect cells. Available at www.idtdna.com/pages/decoded/decoded-articles/genome-editing/decoded/2016/06/20/successful-crispr-genome-editing-in-hard-to-transfect-cells-(i.e.-jurkat-cells). (Accessed May 19, 2017)
3. (2017) Alt-R™ Genome Editing Detection Kit protocol. Integrated DNA Technologies. Available at www.idtdna.com/pages/docs/default-source/user-guides-and-protocols/altr-genome-editing-detection-kit-protocol.pdf. (Accessed May 19, 2017)

Revision history

Version	Date released	Description of changes
1	May 2017	Original protocol