Protocol

Dharmacon™ Edit-R™ synthetic guide RNA and Cas9 protein Ribonucleoprotein electroporation protocol

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For research only. Not for use in diagnostic procedures.

Horizon Discovery Biosciences Ltd. (A Revvity Company)

Protocol Description

Successful electroporation of Ribonucleic protein (RNP) composed of Dharmacon™ Edit-R™ synthetic guide RNA [single guide RNA (sgRNA) or crRNA complexed with tracrRNA] and Cas9 protein, with resulting gene knockout, requires careful optimization of delivery conditions with appropriate electroporation reagents and parameters for each cell line of interest. The protocol below assumes that experimental conditions have been optimized.

Materials Required

Materials for gene editing can be ordered at horizondiscovery.com.

- Edit-R Cas9 Protein Hybrid NLS (Cat# CAS135XX)*
  Purified ready-to-use Cas9 protein with optimized nuclear localization (NLS) features enabling efficient nuclear delivery for rapid RNP workflows.
  *Note: This protocol is also recommended for Cas9 nuclease protein NLS (Cat# CAS122XX). Further optimization may be required.

- Synthetic targeting guide RNA (choose one):
  (1) Edit-R synthetic sgRNA
  (2) Edit-R synthetic crRNA and tracrRNA oligos
- Controls and detection primers for editing detection assay – in both sgRNA or crRNA formats
  (1) Edit-R synthetic sgRNA positive controls to optimize CRISPR-Cas9 gene editing
    (a) Detection primers for T7E1 and TIDE editing verification and analysis
  (2) Safe harbor cutting and T-cell receptor target editing controls for specific applications.
- Non-targeting control guide RNA (choose one):
  (1) Edit-R synthetic sgRNA non-targeting control (Cat #U-009501-XX, U-009502-XX, U-009503-XX, U-009504-XX, U-009505-XX)
  (2) Edit-R crRNA non-targeting control (Cat #U-007501-XX, U-007502 XX, U-007503-XX, U-007504-XX, U-007505-XX)

We recommend testing three to five guide RNA designs per gene of interest to identify the guide RNA with highest editing efficiency that also results in complete knockout of functional protein.

- Controls and detection primers for editing detection assay – in both sgRNA or crRNA formats
  (2) Edit-R synthetic sgRNA positive controls to optimize CRISPR-Cas9 gene editing
    a) Detection primers for T7E1 and TIDE editing verification and analysis
  (3) Safe harbor cutting and T-cell receptor target editing controls for specific applications.

Electroporation experiments require standard cell culture reagents and instruments appropriate for maintenance of cells. The following additional materials are required but not supplied:

- Electroporation instrument
- Electroporation reagents (buffer, cuvettes, transfer pipettes)
- Multi-well tissue culture plates or tissue culture dishes
- Antibiotic-free complete medium: Cell culture medium (including serum and/or supplements) without antibiotic; recommended for maintenance and passaging of the cells of interest
- Assay(s) for detecting gene editing events in a cell population

- 10 mM Tris pH 7.4 nuclease-free buffer (Tris buffer) solution (Dharmacon, Cat #B-006000-100)

General protocol for electroporation of Edit-R Cas9 Protein Hybrid NLS and synthetic RNAs

The following example provides a general protocol using electroporation to deliver Cas9 protein and guide RNA into cultured mammalian cells. Exact reagents, amounts and parameters for electroporation should be empirically determined through careful optimization in cells of interest, in accordance with electroporation instrument manufacturer's recommendations. The protocol below describes delivery conditions in U2OS cells (1×10^6) using the Lonza Nucleofector™ 2b instrument and is given for illustrative purposes only. All steps of the protocol should be performed in a laminar flow cell culture hood using sterile technique.

Cell plating

Optimal cell number for plating will vary with growth characteristics of specific cells and should be determined empirically.

1. Trypsinize and count cells.
2. Plate cells to achieve 70–80% confluence the next day. Cell densities greater than 80% may reduce electroporation efficiency. For example, plate $2.5–3 \times 10^6$ U2OS cells in a 150 mm dish.
3. Incubate cells at 37°C in 5% CO_2 overnight.

Electroporation

1. Prepare 6-well plates by transferring 2 mL of pre-warmed culture medium to each well required for the number of samples and replicates in the experiment. Pre-incubate/equilibrate by placing at 37 °C in 5% CO_2 while preparing samples. The following table lists recommended samples for a gene editing electroporation experiment:

Sample	Explanation of sample
Edit-R Cas9 Protein Hybrid NLS with Non-targeting control guide RNA	Negative control: Cas9 protein synthetic guide RNAs designed to result in no functional protein knockout
Edit-R Cas9 Protein Hybrid NLS with Edit-R synthetic positive control guide RNA	Positive control: Cas9 protein with validated control guide synthetic guide RNAs
Edit-R Cas9 Protein Hybrid NLS with gene-specific guide RNA (and tracrRNA if using crRNA for guide format)	Gene editing: Cas9 protein programmed by guide RNAs for targeted double-stranded break in gene of interest
Untransfected	No treatment control: confirmation of cell viability

2. Prepare synthetic sgRNA or crRNA:tracrRNA samples for electroporation according to the following table:

Preparation of synthetic sgRNA

i) Prepare a 100 µM synthetic sgRNA stock solution by adding the appropriate volume of Tris buffer to the sgRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 100 µM.

Preparation of crRNA and trRNA

i) Prepare a 200 µM crRNA stock solution by adding the appropriate volume of Tris buffer to crRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 200 µM.

ii) Prepare a 200 µM tracrRNA stock solution by adding the appropriate volume of Tris buffer to tracrRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 200 µM.

iii) Prepare a 100 µM working solution of crRNA:tracrRNA by combining equal volumes of 200 µM stock solutions (1:1 ratio). Mix gently.

3. In 1.7 mL tubes, prepare samples to be electroporated. Combine 150 pmol of Cas9 protein with 3 µL (300 pmol) of crRNA:tracrRNA or synthetic sgRNA working solution (according to the following table) for a final concentration of 1.5 µM and 3 µM, respectively. This creates the Cas9 RNP complex.

Cas9 Catalog Number	Cas9 protein concentration	150 pmol Cas9	300 pmol guide RNA	Total volume
Cat# CAS135XX	60.7 µM	2.47 µL	3.00 µL	5.47 µL
Cat# CAS122XX	61.8 µM	2.43 µL	3.00 µL	5.43 µL

Lower amounts of Cas9 protein with higher amounts of guide RNA can be electroporated. We found that using 100 pmol of Cas9 protein with 400-600 pmol guide RNA produced comparable gene editing as 150 pmol Cas9 protein with 300 pmol guide RNA; this ratio should be empirically determined for your cell line.

4. Incubate at room temperature for 10-15 minutes.
5. Collect 1 x 10^6 cells for each sample. Centrifuge at ~500 x g for 1 minute at room temperature.
6. Aspirate medium from the cell pellet and resuspend in 100 µL of appropriate electroporation buffer.

Do not leave cells resuspended in electroporation buffer for more than 15 minutes as this can negatively affect cell viability.

7. Transfer resuspended cells to the 1.7 mL tube containing Cas9 RNP complex. Gently mix components and transfer the entire volume to an electroporation cuvette. Sample should cover the bottom of the cuvette; tap to remove any air bubbles.
8. Electroporate sample with program X-001.
9. Use a transfer pipette to gently layer pre-incubated medium on top of electroporated cells from one well of a 6-well plate. Gently aspirate cells from the bottom of the cuvette and transfer to the well.
10. Repeat steps 8–12 for remaining Cas9 protein gene editing samples.
11. Incubate cells at 37 °C in 5% CO_2 for a total of 48 to 72 hours after electroporation before proceeding with gene knockout analysis.

For more information
To find the contact information in your country for your technology of interest, please visit us at horizondiscovery.com/contact-us
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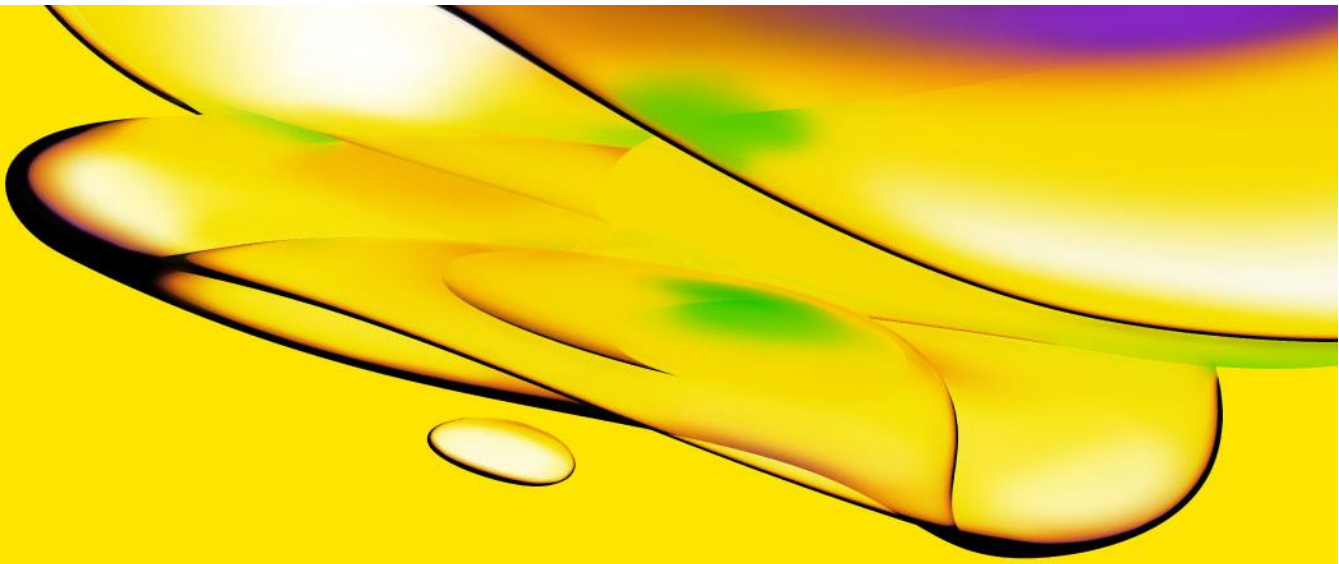

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