PROTOCOL

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Electroporation of Edit-R™ Cas9 Nuclease mRNA and synthetic guide RNA

Successful electroporation of Edit-R Cas9 Nuclease mRNA and synthetic guide RNA (sgRNA, or crRNA complexed with tracrRNA) with subsequent 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 previously.

Materials required

- Edit-R Cas9 Nuclease mRNA (1 µg/µL)
- Synthetic guide RNA (choose one):
  1. Edit-R synthetic sgRNA
  2. Edit-R synthetic crRNA and tracrRNA oligos

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.

- 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)
     or
  2. Edit-R crRNA Non-targeting Control (Cat #U-007501-XX, U-007502-XX, U-007503-XX, U-007504-XX, U-007505-XX)

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
- Phosphate-buffered saline (PBS)
- Assay(s) for detecting gene engineering 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 Nuclease mRNA and synthetic guide RNA

The following example provides a general protocol using electroporation to deliver Edit-R Cas9 Nuclease mRNA and synthetic guide RNAs 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 K-562 cells (2×10^6 cells) 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. Count cells using a hemocytometer or other automated method.
2. Plate cells to achieve 70-80% confluence the next day. For example, plate 8×10^6 K-562 cells in a 150 mm dish.

Cell densities greater than 80% may reduce electroporation efficiency.

3. Incubate cells at 37 °C in 5% CO_2 overnight.

Table 1. Recommended samples for a gene engineering electroporation experiment

Sample	Purpose
Edit-R Cas9 Nuclease mRNA with Non-targeting control guide RNA	Negative control: Cas9 mRNA without targeting guide RNAs
Edit-R Cas9 Nuclease mRNA with gene-specific guide RNA	Gene engineering sample: Cas9 nuclease programmed by guide RNAs for targeted double-stranded break in gene of interest
Untransfected	No treatment control sample: confirmation of cell viability

It is recommended to perform electroporation of guide RNA in triplicate along with the controls listed in Table 1 for high confidence experimental results.

4. Prepare 6-well plates by transferring 2 mL of pre-warmed appropriate culture medium to the number of wells required for each sample in the experiment. Pre-incubate/equilibrate by placing at 37 °C in 5% CO_2 while preparing samples.
5. Prepare guide RNA samples for electroporation.
   For synthetic sgRNA:
   a. 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.

   For crRNA and tracrRNA:
   a. Prepare a 200 µM crRNA stock solution by adding the 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.
   b. 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.
   c. Prepare a 100 µM working solution of crRNA:tracrRNA by combining equal volumes of 200 µM stock solutions (1:1 ratio). Mix gently.
6. Prepare each sample to be electroporated in a 1.7 mL microcentrifuge tube by mixing 5 µg Cas9 mRNA (5 µL) with 5.4 µL of the 100 µM synthetic sgRNA or crRNA:tracrRNA working solution. This will result in 5 µM of guide RNA in the final electroporation mixture.

Cas9 mRNA and synthetic guide RNA volume to be electroporated should not exceed 11 µL (or ~10% of cell resuspension volume).

7. Collect 2 × 10^6 cells for each sample. Centrifuge at ~ 500 × g for 2 minutes at room temperature.
8. Aspirate medium from the cell pellet, wash once with phosphate-buffered saline (PBS) by adding buffer to gently resuspend cells and centrifuging again.
9. Aspirate PBS from the cell pellet and resuspend the cell pellet in 100 µL of Lonza kit V electroporation buffer.

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

10. Transfer resuspended cells to 1.7 mL tube containing Cas9 mRNA and guide RNA. 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.
11. Electroporate sample with program T-016.
12. Use a transfer pipette to gently transfer cells and electroporation mixture from the cuvette to the pre-incubated medium one well of a 6-well plate.
13. Incubate cells at 37 °C in 5% CO_2 for a total of 48 to 72 hours after electroporation; proceed with gene knockout analysis.

When using Fluorescent Cas9 mRNA, we suggest enriching for positive fluorescent cells using FACS 8-24 hours after electroporation. Refer to the protocol for "Using Edit-R Fluorescent Cas9 mRNA for enrichment of transfected cells" for more information.

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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