PROTOCOL

horizon™
INSPIRED CELL SOLUTIONS

Electroporation of Dharmacon™ Edit-R™ Cas9 Nuclease Expression Plasmid, synthetic crRNA and tracrRNA for genome engineering

Successful electroporation of the three Edit-R components (Cas9 Nuclease Expression Plasmid, gene-specific synthetic crRNA and synthetic tracrRNA), for target 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 CRISPR-Cas9 materials for gene editing can be ordered at dharmacon.horizondiscovery.com.

- Edit-R Cas9 Nuclease Expression Plasmid with cell line-optimized promoter
  - Edit-R hCMV_mKate2-Cas9 Expression Plasmid DNA, 120 µg (Cat #U-004100-120)
  - Edit-R mCMV_mKate2-Cas9 Expression Plasmid DNA, 120 µg (Cat #U-004200-120)
  - Edit-R hEf1α_mKate2-Cas9 Expression Plasmid DNA, 120 µg (Cat #U-004300-120)
  - Edit-R mEf1α_mKate2-Cas9 Expression Plasmid DNA, 120 µg (Cat #U-004400-120)
  - Edit-R PGK_mKate2-Cas9 Expression Plasmid DNA, 120 µg (Cat #U-004500-120)
  - Edit-R CAG_mKate2-Cas9 Expression Plasmid DNA, 120 µg (Cat #U-004600-120)
- Edit-R tracrRNA, 5, 20, 50 nmol (Cat #U-002005-XX)
- Edit-R crRNA Non-targeting Control (Cat #U-007501-xx, U-007502-xx, U-007503-xx, U-007504-xx, U-007505-xx)
- Edit-R crRNA, predesigned for your gene of interest in a variety of sizes, or designed and ordered using the Dharmacon CRISPR Design Tool

It is recommended to test three to five crRNA designs per gene of interest to identify the crRNA with highest editing efficiency that also results in complete knockout of functional protein.

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 solution for resuspension of crRNA and tracrRNA (Horizon Discovery, Cat #B-006000-100)

General protocol for electroporation of Edit-R Cas9 Expression Plasmid and synthetic RNAs

The following example provides a general protocol using electroporation to deliver Cas9 plasmid and crRNA:tracrRNA into cultured mammalian cells. Electroporation of Cas9 plasmid and then sequential electroporation of the crRNA:tracrRNA has demonstrated the best results. 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 K562 cells (2×10^6) 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. For example, plate 4×10^5 K562 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.

Electroporation

Table 1. Recommended samples for a gene editing electroporation experiment.

Sample	Explanation of sample
Edit-R Cas9 Nuclease Expression Plasmid with tracrRNA and Non-targeting control crRNA	**Negative control:** Expressed Cas9 without targeting RNAs
Edit-R Cas9 Nuclease Expression Plasmid with tracrRNA and gene-specific crRNA or positive control crRNA	**Gene editing:** Expressed Cas9 programmed by RNAs for targeted double-stranded break in gene of interest or control gene
Untransfected	**No treatment control:** confirmation of cell viability

It is recommended to perform electroporation of gene-specific 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 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.
5. Prepare samples to be electroporated by adding 10 µg Edit-R Cas9 Nuclease Expression Plasmid to a 1.7 mL tube for each sample. The Cas9 plasmid volume to be electroporated should not exceed 10 µL (or 10% of cell resuspension volume).
6. Collect 1×10^6 cells for each sample. Centrifuge at ~ 500 × g for 5 minutes at room temperature.
7. Aspirate medium from the cell pellet and resuspend 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 viability.

8. Transfer resuspended cells to the 1.7 mL tube containing Cas9 plasmid. 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.
9. Electroporate sample with program T-016.

It is recommended to optimize the electroporation program for your specific cells of interest.

10. 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 back to the appropriate well.
11. Repeat steps 6-10 for remaining Cas9 plasmid samples.
12. Incubate cells at 37 °C in 5% CO_2 for 30–48 hours after electroporation.
13. Prepare new 6-well plates by transferring 2 mL of pre-warmed appropriate 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.
14. Prepare crRNA and tracrRNA samples for electroporation
    a. Prepare a 100 µM tracrRNA stock solution by adding the appropriate volume of nuclease-free, 10 mM Tris pH 7.4 buffer to tracrRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 100 µM.
    b. Prepare a 100 µM crRNA stock solution by adding the appropriate volume of nuclease-free, 10 mM Tris pH 7.4 buffer to crRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 100 µM.
    c. Prepare a 50 µM working solution of crRNA:tracrRNA by combining equal volumes of 100 µM stock solutions (1:1 ratio). Mix gently.
15. In 1.7 mL tubes prepare samples to be electroporated by adding 4 µL of 50 µM crRNA:tracrRNA working solution. This will result in 2 µM of crRNA:tracrRNA in the final electroporation mixture.
16. Collect cells from one well of Cas9 plasmid electroporation from step 12. Centrifuge at ~ 500 × g for 5 minutes at room temperature.
17. Aspirate medium from the cell pellet and resuspend in 100 µL of Lonza kit V buffer.

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

18. Transfer resuspended cells to the 1.7 mL tube containing crRNA:tracrRNA complex sample. 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.
19. Electroporate sample with program T-016.
20. Use a transfer pipette to gently layer pre-incubated medium from one well of a 6-well plate prepared in step 13, on top of electroporated cells. Gently aspirate cells from the bottom of the cuvette and transfer to the well.
21. Repeat steps 16-20 for remaining crRNA:tracrRNA samples.
22. Incubate cells at 37 °C in 5% CO2 for a total of 48 to 72 hours after crRNA:tracrRNA electroporation, before proceeding with gene knockout analysis.

If you have any questions, contact

t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

horizon™
INSPIRED CELL SOLUTIONS

V61019