PROTOCOL

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# Electroporation of CRISPRmod dCas9-VPR or dCas9-SALL1-SDS3 mRNA and synthetic guide RNA for gene modulation

Successful electroporation of CRISPRmod mRNA and synthetic guide RNA (single guide RNA (sgRNA) or crRNA complexed with tracrRNA) with subsequent gene activation or repression, 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

CRISPRmod materials for gene modulation can be ordered at horizondiscovery.com

1. CRISPRmod Cas9-VPR mRNA, 20 µg (1 µg/µL; #CAS12024, #CAS12025, or #CAS12026)
2. CRISPRmod dCas9-SALL1-SDS3 mRNA, 20 µg (1 µg/ µL; Cat #CAS12224, #CAS12225, #CAS12226)

- Synthetic targeting guide RNA (choose one):
  1. CRISPRmod synthetic CRISPRa crRNA and tracrRNA oligos:
     a. CRISPRa crRNA, predesigned for your gene of interest in a variety of sizes, or ordered as a custom sequence using the Custom CRISPR Ordering Tool
     b. tracrRNA, 5, 20 or 50 nmol (Cat #U-002005-XX)
     or
  2. CRISPRmod synthetic CRISPRi sgRNA, predesigned for your gene of interest in a variety of sizes, or designed and ordered using the Custom CRISPR Ordering Tool.

**We recommend testing three guide RNA designs per gene of interest to identify the most active guide RNA or using predesigned pooled guide RNA.**

- Non-targeting control guide RNA (choose one):
  1. CRISPRmod CRISPRa crRNA Non-targeting Control (Cat #U-009500-01-XX or #U-009500-10-XX)
     or
  2. CRISPRmod CRISPRi sgRNA Non-targeting Control (Cat#U-009550-XX-02 or U-009550-XX-05)

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 activation or repression in a cell population
- 10 mM Tris pH 7.4 nuclease-free buffer (Tris buffer) solution (Cat #B-006000-100)

## General protocol for electroporation of CRISPRmod mRNA and synthetic guide RNA

The following example provides a general protocol using electroporation to deliver CRISPRmod mRNA and 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 ($2 \times 10^6$) 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. Count cells using a hemocytometer or other automated method.
2. Plate cells to achieve 70-80% confluence the next day. For example, plate $8 \times 10^6$ 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 modulation experiment

| Sample | Purpose |
|---|---|
| CRISPRmod mRNA with Non-targeting control guide RNA | Negative control: CRISPRmod mRNA without targeting guide RNAs |
| CRISPRmod mRNA with gene-specific guide RNA | Gene modulation sample: CRISPRmod nuclease programmed by guide RNAs for targeted transcriptional modulation of 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 crRNA and tracrRNA:**
   a. 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.
   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.

   **For synthetic sgRNA:**
   a. Prepare a 100 µM synthetic sgRNA working 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.
6. Prepare each sample to be electroporated in a 1.7 mL microcentrifuge tube by mixing 5 µg CRISPRmod mRNA (5 µL) with 5.4 µL of the 100 µM crRNA:tracrRNA or synthetic sgRNA working solution. This will result in 5 µM of guide RNA in the final electroporation mixture.

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

7. Collect $2 \times 10^6$ 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, 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.**

9. Transfer resuspended cells to 1.7 mL tube containing CRISPRmod 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.
10. Electroporate sample with program T-016.
11. Use a transfer pipette to remove pre-incubated medium from one well of a 6-well plate (Step 4) and gently layer it on top of electroporated cells. Gently aspirate cells from the bottom of the cuvette and transfer to the well.
12. Repeat steps 6-11 for remaining samples.
13. Incubate cells at 37 °C in 5% $CO_2$ for a total of 48 to 72 hours after electroporation; proceed with gene activation or gene repression analysis.

**When using Fluorescent CRISPRmod, we suggest enriching for positive fluorescent cells using FACS 8-24 hours after electroporation. Refer to the protocol for using CRISPRmod mrna electroporation protocol 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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V7-0221