Protocol

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

Version 0001

Revision date: 110524

For research only. Not for use in diagnostic procedures.

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:

mRNA encoding effector protein (choose one):

1. For gene activaton (CRISPRa): CRISPRmod Cas9-VPR mRNA, 20 µg (1 µg/µL; #CAS12024, #CAS12025 or #CAS12026, featuring no enrichment option, eGFP or puromycin, respectively)
2. For gene inhibition (CRISPRi): CRISPRmod dCas9-SALL1-SDS3 mRNA, 20 µg (1 µg/ µL; Cat #CAS12224, #CAS12225 or #CAS12226, featuring no enrichment option, eGFP or puromycin, respectively)

Synthetic targeting guide RNA specific for CRISPRa or CRISPRi platform (choose one):

1. CRISPRmod CRISPRa synthetic guide RNA (sgRNA), predesigned for gene of interest in a variety of sizes, or ordered as a custom sgRNA. CRISPRmod CRISPRa crRNA can also be ordered as a custom sequence using the CRISPR Guide RNA Designer paired with tracrRNA (Cat #U-002005-XX).
2. CRISPRmod CRISPRi sgRNA, predesigned for your gene of interest in a variety of sizes, or ordered as a custom sequence using the CRISPR Guide RNA Designer.

Note: 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 for CRISPRa or CRISPRi platform:

1. CRISPRmod CRISPRa sgRNA Non-targeting Control single (Cat Cat#U-009500-XX-02 or U-009500-XX-05) or pooled (Cat#U-009500-10-02 or U-009500-10-05) sgRNAs or crRNA.
2. CRISPRmod CRISPRi Non-targeting Control single (Cat#U-009550-XX-02 or U-009550-XX-05) or pooled (Cat#U-009550-10-02 or U-009550-10-05) sgRNAs or crRNA.

Positive control guide RNA (choose one):

1. CRISPRa synthetic sgRNA positive sgRNA control or similar crRNA
2. CRISPRi synthetic sgRNA positive control

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 sgRNA

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 (2×10^6) 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 reach 70-80% confluency after an overnight incubation. For example, plate 8×10^6 K-562 cells in a 150 mm dish.
3. Incubate cells at 37°C in 5% CO_2 overnight.

Note: Cell densities greater than 80% may reduce electroporation efficiency.

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 samples for electroporation.

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.

For crRNA and tracrRNA:

a. Prepare a 200 µM crRNA stock solution by adding the required volume of Tris buffer to crRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust 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 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.

Table 1. Recommended samples for a gene modulation experiment

Sample	Purpose
CRISPRmod mRNA with Nontargeting control guide RNA	Negative control: (i) ensure absence of gene modulation with nontargeting guide RNA and (ii) cell viability after transfection
CRISPRmod mRNA with Positive control guide RNA	Positive control: ensure efficient transfection of CRISPRmod components by detection effective control target modulation
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

Note: It is recommended to electroporate guide RNA in triplicate along with controls listed in Table 1 for high-confidence experimental results.

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 sgRNA working solution. This will result in 5 µM of guide RNA in the final electroporation mixture.

Note: CRISPRmod mRNA and sgRNA volume to be electroporated should not exceed 11 µL **(or 10% of cell resuspension volume).**

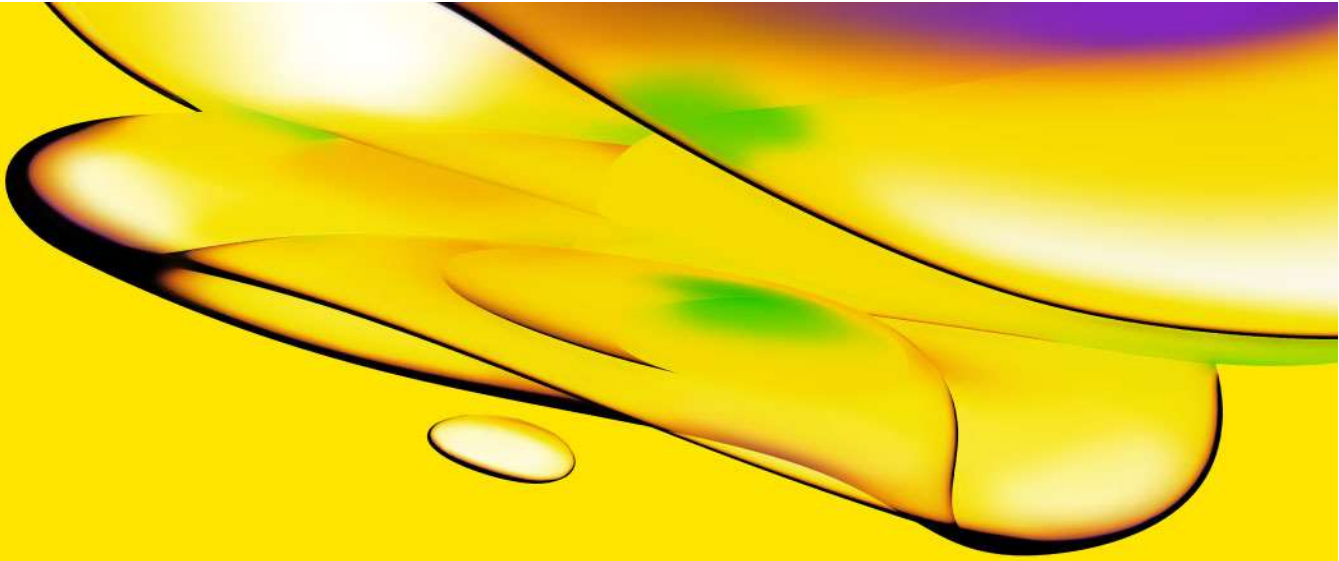
7. Collect 2×10^6 cells for each sample. Centrifuge at ~ 500 × g for 2 minutes at room temperature.

8. Aspirate medium from the cell pellet, wash once with phosphate-buffered saline (PBS) by adding buffer to gently resuspend cells. Re-pellet cells as in step 7.
9. Aspirate PBS from the cell pellet and resuspend in 100 µL of Lonza kit V electroporation buffer.

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

10. Transfer resuspended cells to 1.7 mL tube containing CRISPRmod dCas9 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 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 culture well.
13. Repeat steps 6-12 for remaining samples.
14. 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.

Note: When using Fluorescent CRISPRmod mRNA, we suggest enriching for fluorescent cells with FACS 8-24 hours after electroporation. For more information, refer to Enrichment of transfected cells with Dharmacon™ CRISPRmod CRISPRa Fluorescent dCAS9-VPR mRNA protocol.



Revvity
77 4th Avenue
Waltham, MA 02451 USA

For a complete listing of our global offices, visit www.revvity.com