Protocol

Reverse transfection protocol for Dharmacon™ Edit-R™ and CRISPRmod™ arrayed synthetic guide RNA

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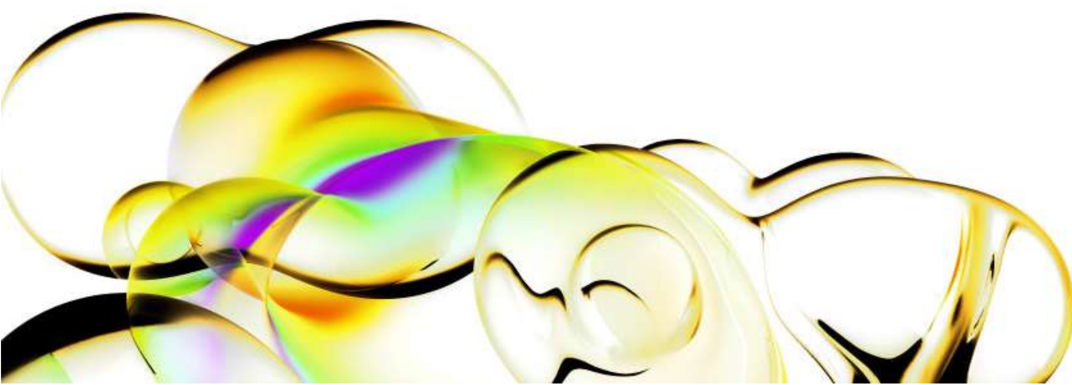
For research only. Not for use in diagnostic procedures.



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1. Reverse transfection of arrayed sgRNAs

Product description

Libraries of predesigned synthetic guide RNAs (sgRNAs) provide an opportunity to utilize the Edit-R or CRISPRmod™ (CRISPRa and CRISPRi) gene systems for analysis gene knockout or functional gene modulation, respectively, in an arrayed format.

Edit-R and CRISPRmod sgRNA Libraries are available as the following:

Catalog libraries of predefined gene family collections for human targets.

- » Three sgRNA per gene as an individual sgRNA or a pool of three sgRNA per well, at 0.1 nmol, 0.25 nmol or 0.5 nmol/well
- » Provided in NUNC Polystyrene 96-well V-bottom plates (Cat #249952), 384-well plates (Thermo Scientific Abgene Cat #AB-0781), with outer columns left open for inclusion of untreated cells and screen-specific positive and non-targeting controls. Plates compatible with the ECHO Liquid Handler (Beckman Coulter Cat# 001-14655) are available upon request

Alternatively, cherry-pick Edit-R, CRISPRa or CRISPRi sgRNA libraries can be created from a custom gene list. Learn more or get started here.

- » Plates of sgRNAs, up to 2 nmol per well in 96-well plates (20 wells minimum), or up to 0.5 nmol per well in 384-well plates (minimum 40 wells)
- » Customizable plate layout; catalog control sgRNAs may be added to any wells within the plate(s)
- » Provided in NUNC Polystyrene 96-well V-bottom plates (Cat #249952), 384-well plates (Thermo Scientific Abgene Cat #AB-0781 non-sterile 384-well plates compatible with the ECHO Liquid Handler (Beckman Coulter Cat# 001-14655)

Materials

- Plates of Edit-R or CRISPRmod sgRNAs, up to 2 nmol per well in 96-well format, or up to 0.5 nmol per well in 384-well format

Additional required materials are listed below and are not provided with sgRNA library purchase:

- DharmaFECT transfection reagent (formulation is dependent on specific cell line of interest)
  - » DharmaFECT™ 1 Cat #T-2001-XX
  - » DharmaFECT™ 2 Cat #T-2002-XX
  - » DharmaFECT™ 3 Cat #T-2003-XX
  - » DharmaFECT™ 4 Cat #T-2004-XX
- 10 mM Tris pH 7.4 nuclease-free buffer solution (Dharmacon™, Cat #B-006000-100)
- 96-well tissue culture plates
- 96-well V-bottom polystyrene standard storage plates or deep well plates (for example, NUNC Cat #249952 or Cat *#12-565 553*)
- Assay for cell viability such as CellTiter-BlueR Cell Viability Assay (Promega Corp., Cat #G8081)
- Assay(s) for detecting gene editing events in a cell population. Typically, Edit-R sgRNA function would be assessed by detecting gene target editing events while CRISPRmod sgRNA function can be assessed by target gene expression assays
- Positive control CRISPRmod sgRNA or Edit-R sgRNA and detection primers for assessment of editing
- Non-targeting control sgRNA (see appendix)
- Assay-specific positive control sgRNA (defined by researcher)
- Growth medium: antibiotic-free cell culture medium (with serum and/ or supplements) recommended for maintenance of the cells of interest
- Serum-free and antibiotic-free cell culture medium for preparation of transfection mix (for example, MEM-RS, HyClone Cat # SH30564)

For phenotypic analysis with the arrayed synthetic sgRNA libraries for gene knockout, we strongly recommend using cell lines that constitutively express the requisite Cas9-based protein. For gene modulation with CRISPRa sgRNA, it is best to transfect RNA guides into a cell line that is constitutively expressing dCas9-VPR. This will result in a higher degree

of gene activation, thus allowing for easier downstream high throughput phenotypic analysis of modulated cell populations.

Note: The phenotypic analysis, including assay optimization and analysis, is cell line and assay-specific and requires optimization by the researcher.

For generation of cells that stably express Cas9, please follow recommendations for Edit-R for CRISPRmod systems:

- Dharmacon Edit-R for gene knockout Technical Manual
- Dharmacon CRISPRmod CRISPRa transcriptional activation system Technical Manual
- Dharmacon CRISPRmod CRISPRi transcriptional interference system Technical manual

Guidelines for resuspension of the sgRNA and generating sgRNA plates

1. The sgRNA libraries are shipped at ambient temperature as dry pellets of RNA in each well and should be stored at –20°C upon arrival in a manual defrost or non-cycling freezer. If necessary, sgRNAs can be stored as dry pellets (unopened) at 4°C for several weeks.
2. Briefly centrifuge plates to ensure that the sgRNA is collected at the bottom of the well.
3. Wipe adhesive foil cover with 70% ethanol or other RNase-decontamination solution.
4. Pierce or carefully peel back the foil seal to gain access to wells. Use caution and avoid shredding the seal.
5. If you are starting with a plate of 0.5 nmol per well, resuspend arrayed sgRNAs to 10 µM solution by adding 50 µL nuclease-free 10 mM Tris pH 7.4 buffer to 0.5 nmol of sgRNA (for different quantities of sgRNAs see **Table 3**).
6. Pipette solution up and down 3-5 times while avoiding introduction of bubbles.
7. Seal the plate and place it on an orbital mixer/shaker for 70-90 minutes at room temperature.
8. Briefly centrifuge plates to collect solution to bottom of the wells.
9. From this master sgRNA plate, generate plates with 1 µM working concentration of sgRNAs using nuclease-free, 10 mM Tris pH 7.4 buffer. This eliminates the subsequent requirement for pipetting of very small volumes.
10. sgRNA plates may now be used immediately, aliquoted into single-use plates or stored at -20°C in a manual defrost or non-cycling freezer.
11. For storage, seal plates with appropriate adhesive or heat seals.

Reverse transfection of arrayed sgRNA

The following is a general protocol for reverse transfection of arrayed sgRNA libraries using stable Cas9 or dCas9-VPR-expressing mammalian cells. For guidelines using arrayed sgRNA plates with forward transfection see appropriate Edit-R, CRISPRa or CRISPRi technical manual. Optimal plating density will depend on growth characteristics of specific cell lines and assay requirements and these parameters should be determined experimentally. Exact parameters for sgRNA transfection in your cells of interest should be empirically determined through careful optimization prior to experimentation see Appendix for guidelines to optimize transfection conditions with sgRNA. Catalog sgRNA library plates are supplied with columns 1 and 12 empty to allow addition of researcher-defined controls. We suggest including the following controls:

- Untreated cells
- Positive control sgRNA
- Negative control: non-targeting sgRNA

Day 1

This protocol is for transfection of one arrayed sgRNA 96-well plate, in triplicate, at final 25 nM concentration of sgRNA. Calculations are done for quadruplicates providing excess for the ease of pipetting.

1. Transfer 10 µL of 1 µM working sgRNA solution to each well of a 96-well V-bottom transfection mixture plate (NUNC polystyrene 96 well V- bottom plates Cat #249952 or other appropriate plates may be used).

Note: Positive and negative sgRNA controls can be added to empty wells of the V-bottom transfection mixture plate (columns 1 and 12 in the catalog libraries) and 12 in ca).

2. Add 30 µL of serum-free medium to 10 µL of sgRNA, prepared in step 1. This will bring the concentration of the sgRNA to 250 nM.
3. Prepare transfection reagent working solution by diluting the transfection reagent stock solution in serum-free medium for a total volume of 5 mL. This volume will allow preparation of one sgRNA library plate in triplicate and includes excess for ease of pipetting. For example, if the optimal amount of transfection reagent was determined to be 0.05 µL per well of cells, add 25 µL of transfection reagent stock solution to serum-free medium for a total volume of 5 mL. For preparations of other transfection reagent concentrations see **Table 1**. Incubate diluted transfection reagent for 5 minutes at room temperature.
4. Add 40 µL of transfection reagent working solution to each well of 96-well V-bottom transfection mixture plate containing sgRNA. This brings the total volume to 80 µL.
5. Immediately mix by pipetting gently up and down and incubate for 20 minutes at room temperature.
6. During this incubation time, cells may be prepared for seeding. Trypsinize cells, spin down, remove medium and resuspend in cell growth medium such that when 80 µL are added per well it will result in the previously determined optimal cell density for your transfection. For example, if optimal cell density was determined to be 10,000 cells per well, prepare 28 mL of cell suspension at 1.25×10^5 cells/mL for three plates.
7. Briefly mix (by pipetting) the transfection mix prepared in step 6 and add 20 µL of the transfection mixture to each well of three 96-well tissue culture plates.
8. Add 80 µL of the cell suspension prepared in step 6 to each well of the three 96-well tissue culture plates. This will bring the volume to 100 µL and the final concentration of sgRNA to 25 nM.
9. Incubate transfected cells at 37°C in a humidified CO_2 incubator for 48-72 hours before proceeding with the phenotypic assay or gene editing analysis.

Table 1. Transfection reagent amounts for transfection of one 96-well sgRNA library plate in triplicate.

Transfection reagent volume per well of cells (µL)	Transfection reagent volume (µL)	Serum-free medium volume (mL)
0.01	5	5.00
0.025	12.5	4.99
0.05	25	4.98
0.1	50	4.95
0.2	100	4.90

2. Appendix

Optimization of transfection conditions for sgRNA delivery

To obtain the highest transfection efficiency of sgRNA with minimal effect on cell viability, we recommend carefully optimizing transfection conditions for each cell line using a positive Edit-R or CRISPRmod sgRNA control. Transfection optimization can be easily performed in a 96-well previously been optimized for siRNA delivery are a reasonable starting point for sgRNA transfection optimization. Cell type-specific guidelines for DharmaFECT transfection reagent formulations can be found in the DharmaFECT Cell Type Guide.

Note: The transfection reagent amounts for reverse transfection are typically different from forward transfection, and optimal conditions should be empirically determined for your cells.

The optimization experiment should include two to three cell densities and a range of DharmaFECT transfection reagent volumes. Our recommendations for the different components in the transfection optimization experiment are as follows:

- 0.01 to 0.4 µL/well of DharmaFECT 1, 2, 3, or 4 transfection reagent in a 96-well plate.
- 25 nM positive control sgRNA per well (recommended range 20 nM-50 nM).

Use **Table 2** for guidance in preparation of samples for 96-well transfection optimization.

At 48-72 hours post-transfection, perform a cell viability assay to determine the highest lipid concentration that has minimal cell toxicity (≥ 80% cell viability is preferred). After assaying for cell viability, we recommend that you carefully wash the cells once with PBS and proceed with a gene editing or expression assay (see below) to determine the condition that produces the optimal effect. Use these optimal conditions for subsequent transfection of your selected stable cell lines with target sgRNA.

Table 2. Preparing samples for 96-well transfection optimization. Mix the diluted sgRNA and diluted transfection regent and incubate the transfection mixture for 20 minutes. Transfer the transfection mixture to a well of a tissue culture plate and add 80 µL of cell suspension in growth medium at the desired cell density. This will bring the total volume per well to 100 µL and the concentration of sgRNA complex to 25 nM. Volumes (µL) are shown per ONE well of a 96-well plate; for triplicate wells multiply all values by 3.5 to have sufficient volume for three wells and to account for pipetting error. For the diluted transfection reagent prepare a larger volume to enable accurate pipetting of the small volumes that are required per one well.

Transfection condition	Tube 1: Diluted sgRNA		Tube 2: Diluted DharmaFECT transfection reagent		Final reagent volumes		
DharmaFECT transfection reagent amount per well	Serum-free medium (µL)	Volume of 1 µM sgRNA (µL)	Serum-free medium (µL)	DharmaFECT transfection reagent volume (µL)	Transfection mixture volume (µL)	Cell suspension in Growth medium (µL)	Total volume per 96-well (µL)
0.01 µL/well	7.5	2.5	9.99	0.01	20	80	100
0.025 µL/well	7.5	2.5	9.997	0.025	20	80	100
0.05 µL/well	7.5	2.5	9.95	0.05	20	80	100
0.1 vL/well	7.5	2.5	9.9	0.1	20	80	100
0.2 µL/well	7.5	2.5	9.8	0.2	20	80	100
0.3 µL/well	7.5	2.5	9.7	0.3	20	80	100
0.4 µL/well	7.5	2.5	9.6	0.4	20	80	100
Untreated (0 µL/well)	10	0	10	0	20	80	100

Gene editing assay recommendations

Assessment of gene expression using CRISPRmod sgRNA libraries can be achieved with RT-qPCR following RNA isolation. Quantitative RT-qPCR analysis can be performed using gene expression assays according to manufacturer's instructions. Use the expression of a housekeeping gene to normalize expression of gene of interest. Follow best practices for RT-qPCR analysis with appropriate number of technical replicates and proper controls.

When assessing gene knockout using Edit-R sgRNA, a commonly used method is the T7 Endonuclease I (T7E1) assay. This mismatch detection assay can rapidly determine the presence of insertions and deletions (indels) at the gene target locus and can be performed on either purified genomic DNA or whole cell lysate. See provided protocol for more details on the T7E1 assay, or other gene editing analyses.

3. Frequently asked questions

How should I store my sgRNAs?

RNA oligonucleotides should be stored at -20°C or -80°C in a non-frost-free freezer, either as a dried pellet or resuspended in an RNase-free 10 mM Tris solution buffered to pH 7.4 to help with stability during freeze-thaw cycles. We recommend that RNA oligonucleotides be resuspended to a convenient stock concentration (**Table 3**) and stored in small aliquots to avoid multiple freeze thaw cycles. RNA oligonucleotides should not go through more than four to five freeze thaw cycles. If degradation is a concern, the integrity of the RNA oligonucleotides can be evaluated on an analytical PAGE gel.

Table 3. Making stock solutions of sgRNA.

sgRNA amount (nmol)	Volume (mL) of 10 mM Tris pH 7.4 to be added for desired final concentration	
	100 mM stock	10 mM stock
0.5	-	50
2	20	200
5	50	500
20	200	2000
50	500	Exceeds tube volume *

*When tube volume is exceeded, make a 100 µM stock and dilute it 1:10 to obtain the 10 µM stock.

What is the stability of the Edit-R/CRISPRmod sgRNAs?

Dried RNA oligonucleotide pellets are stable at room temperature for two to four weeks, but should be placed at -20°C or -80°C for long-term storage. Under these conditions, the dried sgRNA will be stable for at least one year. Once resuspended the RNAs are stable for at least one year when stored at -20°C or -80°C in a non-frost-free freezer. Maintaining sterile, RNase- and DNase-free conditions is always recommended as a critical precaution.

Can I use my siRNA transfection protocols to transfect Edit-R/CRISPRmod sgRNAs?

Previously optimized protocols to transfect synthetic siRNA into your cells of interest can be a good starting point for transfection of synthetic sgRNA.

Can I use a different transfection reagent other than DharmaFECT transfection reagents to deliver the Edit-R components into my cells?

We cannot predict the performance of other transfection reagents, nor can we troubleshoot experiments performed with any reagent other than DharmaFECT transfection reagents. However, other suitable transfection reagents designed for RNA transfection could be utilized provided transfection conditions are carefully optimized for each cell line of interest.

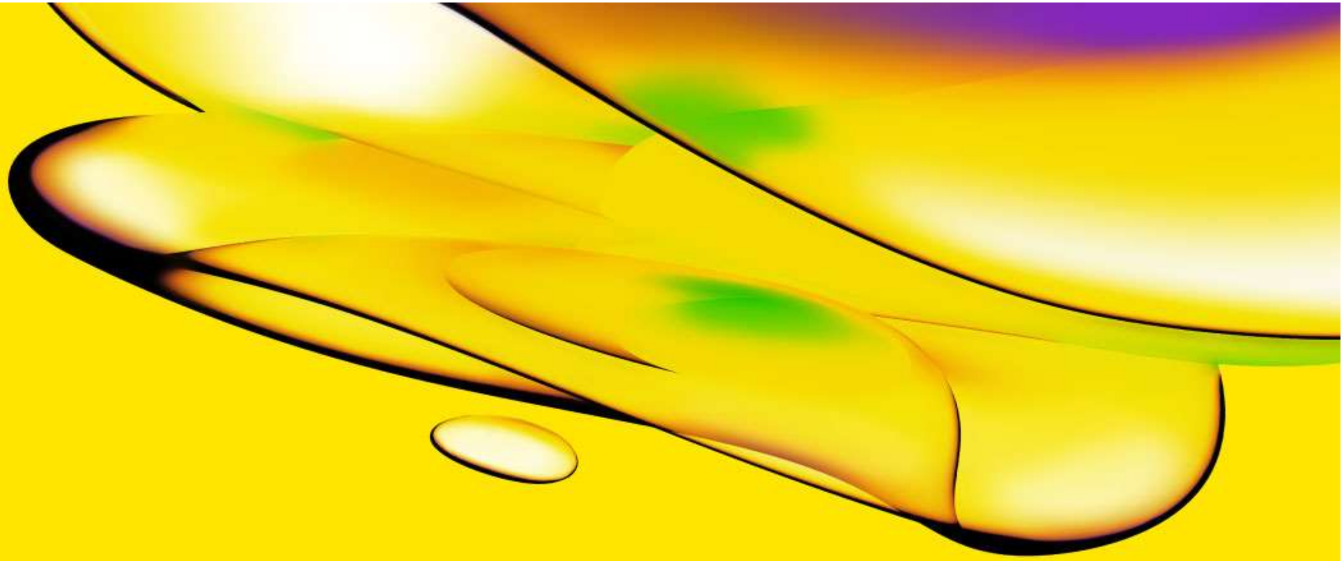
Can I co-transfect arrayed synthetic sgRNAs with a Cas9 expression plasmid?

You can perform genome engineering by transient transfection of the synthetic sgRNA with Cas9 Nuclease Expression plasmids using DharmaFECT Duo transfection reagent. For performing phenotypic analysis in the cell population in a high throughput manner, we have found that sgRNA transfection in a cell line that stably expresses Cas9 nuclease produces higher efficiency gene editing in the cell population with lower toxicity associated with the transfection.

Can I co-transfect arrayed synthetic Edit-R or CRISPRmod sgRNA with the Edit-R Cas9 or CRISPRmod dCas9 mRNAs?

Yes. Cas9 or dCas9 mRNA can be used with the appropriate arrayed synthetic Edit-R/CRISPRmod sgRNA in cells not stably expressing Cas9/dCas9.

If you have any questions, contact
https://horizondiscovery.com/en/contact-us
For technical questions, please contact
technical@horizondiscovery.com



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