Protocol

Dharmacon™ CRISPRmod™ CRISPRa sgRNA Libraries

A protocol for transfection of arrayed CRISPRa sgRNA plates for transcriptional activation

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Guidelines for using arrayed CRISPRa sgRNA plates for transcriptional activation

Product description

Libraries of predesigned synthetic guide RNAs (sgRNAs) provide an opportunity to apply the CRISPR-Cas9 activation system (dCas9-VPR) for transcriptional gene activation, in an arrayed format. Dharmacon™ CRISPRa sgRNA libraries for functional gene activation consist of predesigned synthetic CRISPRa sgRNAs which target dCas9-VPR to a target gene's promoter region to result in activation, or upregulation of target gene transcription. Dharmacon's CRISPRa guide designs use a published CRISPRa v2 algorithm[1] developed via machine learning techniques and were refined by a systematic study of guide RNAs next to transcriptional start sites (TSS) to identify features that contribute to CRISPRa activity.

CRISPRmod™ CRISPRa 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 (one column for 96-well and two columns for 384-well plate formats) left open for inclusion of untreated cells and screen-specific positive and non-targeting controls
  - » ECHO qualified plates are available upon request

- Cherry-pick sgRNA libraries based on a customer's gene list. Learn more or get started
  - » Multiple individual sgRNAs or a sgRNA pool, minimum 20 wells in a 96-well plate
  - » Customizable plate layout; CRISPRa sgRNA catalog control sgRNAs may be added to any wells within the plate(s)
  - » Provided in NUNC Polystyrene 96-well V-bottom plates (Cat #249952) or 384-well plates (Thermo Scientific Abgene Cat #AB-0781) or non-sterile 384-well plates compatible with ECHO Liquid Handler (Beckman Coulter Cat #001-14655)

Materials

- Plates of synthetic sgRNAs, up to 2 nmol per well in 96-well plates or up to 0.5 nmol per well in 384-well plates

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 assessing cell viability such as CellTiter-Blue® Cell
- Viability Assay (Promega Corp., Cat #G8081)
- Positive control CRISPRa sgRNA
- Non-targeting control CRISPRa sgRNA
- Assay-specific positive control CRISPRa 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 CRISPRa sgRNA libraries for gene activation we strongly recommend using cell lines that constitutively express dCas9-VPR protein. Transfection of CRISPRa sgRNA into a cell line that is constitutively expressing dCas9-VPR results in a higher degree of gene activation, thus allowing for easier downstream high throughput phenotypic analysis of the modulated cell populations. For generation of cells that stably express dCas9-VPR, please follow recommendations in the CRISPRmod CRISPRa transcriptional activation system with synthetic guide RNA technical Manual.

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

Guidelines for resuspension of the sgRNA and generating sgRNA plates

1. CRISPRa 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. Make 10 µM working concentration of sgRNA in Tris pH 7.4 buffer as noted in **Table 1.**
3. Briefly centrifuge sgRNA plates to ensure that the sgRNA is collected at the bottom of the well.
4. Wipe adhesive foil cover with 70% ethanol or other RNase decontamination solution.
5. Pierce or carefully peel back the foil seal to gain access to wells. Use caution and avoid shredding the seal.
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 the master sgRNA plate, generate daughter sgRNA plates with 2 µM working concentration 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 (5 µL/well is recommended for direct use in lipid transfection—see next section) or stored at –20 °C in a manual defrost or non-cycling freezer.
11. For storage, seal plates with appropriate adhesive or heat seals.

Table 1. Making stock solutions of sgRNA

sgRNA (nmol)	Volume (µL) of 10 mM Tris pH 7.4 to be added for desired final concentration		
	100 µM stock	10 µM stock	2 µM stock
0.1	*	*	50
0.25	*	25	125
0.5	*	50	250
2	20	200	1000
5	50	500	*
20	200	2000	*
50	500	*	*
200	2000	*	*

* not recommended due to either small volume required for proper mixing or large volume that exceeds the tube (well) volume. When tube volume is exceeded, make the larger stock and make the dilution with 10 mM Tris buffer pH 7.4 in a separate tube (plate).

Transfection protocol of arrayed sgRNA plates

The following is a general protocol for transfection of arrayed sgRNA libraries using stable dCas9-VPR-expressing mammalian cells in 96-well plates in triplicate at 25 nM final concentration of sgRNA. Optimal plating density for each transfection method will depend on growth characteristics of specific cell lines and assay requirements, which should be determined experimentally. Exact parameters for sgRNA transfection in cells of interest should be empirically determined through careful optimization prior to experimentation (see Appendix for Optimization of 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:

1. Untreated cells
2. Positive sgRNA control
3. Non-targeting sgRNA (negative control)

The protocol is provided for transfection of one arrayed sgRNA plate in triplicate for a final 25 nM concentration of the sgRNA. Calculations are done for quadruplicates providing excess volume for the ease of pipetting. This protocol is written for direct use of the daughter plates containing 5 µL of 2 µM sgRNA to prepare the transfection mix. This protocol uses the least amount of pipetting and liquid-handling steps.

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

Protocol

1. Prepare transfection reagent working solution by diluting the transfection reagent stock solution in serum-free medium. Preparing 9 mL volume will allow for transfection of one 96-well 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 0.1 µL per well of cells, add 48 µL of transfection reagent stock solution to serum-free medium for a total volume of 9 mL See **Table 2** for additional volumes in plate format, or **Table 3** for individual well recommendations.

Table 2. Preparation of different concentration working solution of transfection reagent for transfection of one arrayed sgRNA plate in triplicate. The highlighted row indicates the experimental conditions used in steps 1–3, above.

Transfection reagent volume per well of cells (µL)	Transfection reagent volume (µL)	Serum-free medium volume (µL)
0.01	6	9
0.025	12	9
0.5	24	9
0.1	48	9
0.2	96	8.9
0.3	144	8.9
0.4	192	8.8
0.5	240	8.8
0.6	288	8.7

2. Add 75 µL of transfection reagent working solution to each well of 96-well V-bottom daughter plate containing 5 µL of 2 µM sgRNA complex. This brings the total volume to 80 µL and the concentration of the sgRNA to 125 nM.
3. Immediately mix by pipetting gently up and down and incubate for 20 minutes at room temperature.
4. Briefly mix the transfection solution in the V-bottom plates after 20 min incubation by gently pipetting up and down.
5. Add transfection mix to your cells.
    a. For the Forward transfection method, seed cells a day in advance. Before transfection, replace the media with 80 µL of fresh cell media and add 20 µL of transfection mix from the 96-well V-bottom transfection mix prepared in previous steps to corresponding wells of the 96-well tissue culture plate. This will bring the volume to 100 µL and the final concentration of sgRNA to 25 nM. Repeat this step for the other two plates to obtain triplicates.
    b. For the Reverse transfection method, add 20 µL of the transfection mixture to each well of three new 96-well tissue culture plates and add 80 µL of cell suspension 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.
6. Incubate transfected cells at 37°C in a humidified CO_2 incubator for 48–96 hours before proceeding with the phenotypic assay or assessment of target gene expression.

Table 3. Preparing samples for 96-well transfection optimization. Volumes (µL) are shown per ONE well of a 96-well plate; for triplicate wells, multiply all values by 4 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.

Appendix

Optimization of transfection conditions for delivery of sgRNA.

To obtain the highest transfection efficiency of CRISPRa sgRNA with minimal effects on cell viability, we recommend carefully optimizing transfection conditions for each cell line using a positive sgRNA control. The transfection optimization can be easily performed in a 96-well format allowing for testing of multiple transfection conditions. Transfection conditions that have previously been optimized for siRNA delivery are a reasonable starting point for sgRNA transfection optimization. Cell-type specific guidelines for the four DharmaFECT formulations can be found in the DharmaFECT Cell Type Guide. 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:

Transfection Condition [DharmaFECT volume (µL/well)]	Tube 1: sgRNA (µL/well)		Tube 2: Diluted DharmaFECT Transfection Reagent (µL/well)		Final Reagent Volumes		
	2 µM sgRNA (µL)	Serum-free medium (µL)	DharmaFECT Transfection Reagent (µL)	Serum-free medium (µL)	Transfection Mix volume (µL)	Growth Medium or cell suspension (µL)	Total volume per well (µL)
0.01	1.25	8.75	0.01	9.99	20	80	100
0.025	1.25	8.75	0.025	9.98	20	80	100
0.05	1.25	8.75	0.05	9.95	20	80	100
0.1	1.25	8.75	0.1	9.90	20	80	100
0.2	1.25	8.75	0.2	9.80	20	80	100
0.3	1.25	8.75	0.3	9.70	20	80	100
0.4	1.25	8.75	0.4	9.60	20	80	100
0.5	1.25	8.75	0.5	9.50	20	80	100
0.6	1.25	8.75	0.6	9.40	20	80	100
Untreated	0	10.00	0	10	20	80	100

- 0.05 to 0.8 µL/well of DharmaFECT 1, 2, 3, or 4 in a 96-well plate
- 25 nM CRISPRa positive control (TTN or POU5F1) sgRNA per well (recommended range 20 nM to 50 nM).

Refer to Table 3 for guidance to prepare 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 RT-qPCR analysis for CRISPRa libraries to determine the conditions that produce the best transcriptional activation (see assay recommendation below). Use these optimized conditions for subsequent transfection of your selected dCas-VPR-expressing cell lines with CRISPRa sgRNA.

Gene expression analysis recommendations for CRISPRa libraries

RNA can be isolated from cells using different methods per manufacturer's instructions. 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.

Frequently Asked Questions

How should I store my sgRNA?

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 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 1) 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.

What is the stability of CRISPRa sgRNA?

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. Maintaining sterile, RNase- and DNase-free conditions is always recommended as a critical precaution.

Can I use my siRNA transfection protocols to transfect CRISPRa sgRNA?

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

Can I use a different transfection reagent other than DharmaFECT Transfection Reagents to deliver CRISPRa reagents 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 CRISPRa sgRNA with the Edit-R dCas9-VPR mRNA?

Yes. dCas9-VPR mRNA can be used with arrayed synthetic CRISPRa sgRNA for activation in cells not stably expressing the dCas9-VPR system.

Can I use CRISPRmod CRISPRa sgRNA with the dCas9-SAM system?

No. Synthetic CRISPRmod CRISPRa crRNA connot be used with the SAM system. Our sgRNAs have a canonical S. Pyrogenes Cas9 repeat that is compatible with our dCas9-VPR system. One would need to use crRNAs combined with a MS2 tracrRNA for activation in cells expressing the dCas9-SAM system.

What plate layout can I expect for my library?

Catalog 96-well libraries are fulfilled with the following plate layout:

80-wells per plate, columns 1 and 12 left empty.

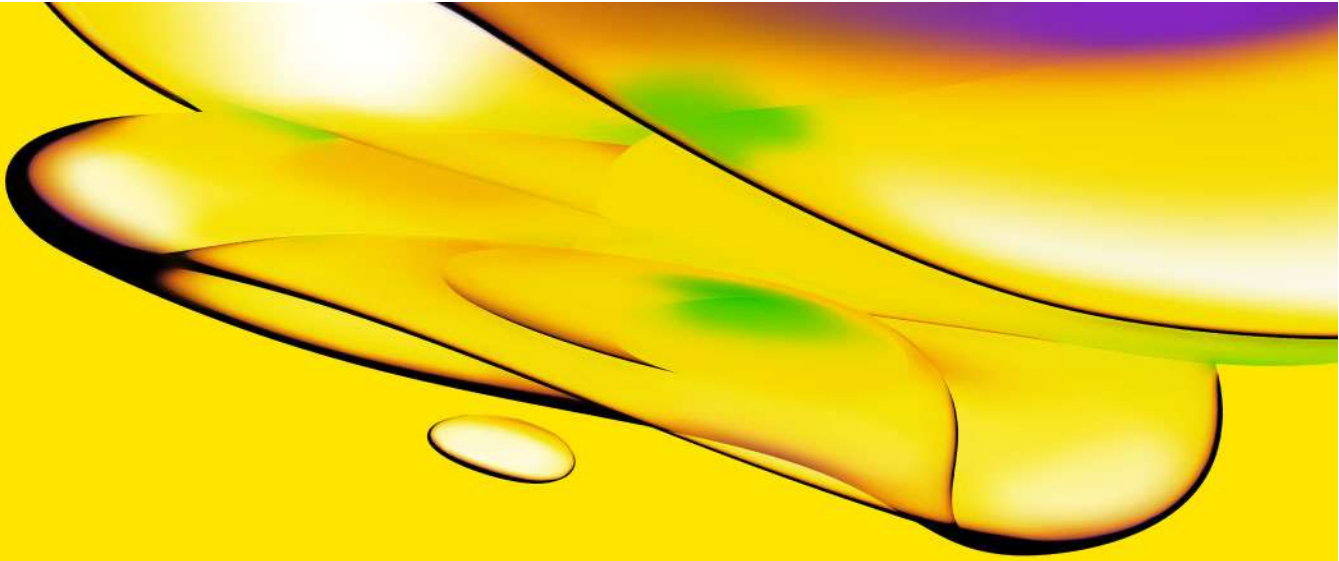
Catalog 384-well libraries are fulfilled the following plate layouts:

320-wells per plate, columns 1, 2 and 23, 24 left empty.

NOTE: Please refer to the platemap provided with order for your precise layout and contact Technical Support with any questions.

References

1. Max A Horlbeck, Luke A Gilbert, Jacqueline E Villalta, Britt Adamson, Ryan A Pak, Yuwen Chen, Alexander P Fields, Chong Yon Park, Jacob E Corn, Martin Kampmann, Jonathan S Weissman (2016) Compact and highly active next-generation libraries for CRISPR-mediated gene repression and activation eLife 5:e19760



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