TECHNICAL MANUAL

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INSPIRED CELL SOLUTIONS

Dharmacon™ Edit-R™ CRISPRa transcriptional activation system with synthetic guide RNA

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1 Introduction to the CRISPR-Cas9 system for transcriptional activation

CRISPR-Cas: an adaptive immunity defense mechanism in bacteria and archaea

The CRISPR (clustered regularly interspaced palindromic repeats)-Cas (CRISPR-associated protein) system is an adaptive bacterial and archaeal defense mechanism that serves to recognize and silence incoming foreign nucleic acids. Upon infection by bacteriophage or other foreign DNA elements, a host organism can incorporate short sequences from the invading genetic material, called protospacers, into a specific region of its genome (the CRISPR locus) between short palindromic DNA repeats of variable lengths. Multiple spacer-repeat units are clustered at the CRISPR locus to form the CRISPR array. The entire locus including the CRISPR array is transcribed by RNA polymerase into a primary transcript, the pre-CRISPR RNA (pre-crRNA), which is then processed into small, mature CRISPR RNAs (crRNAs) such that they include sequences complementary to the foreign, invading DNA. crRNAs guide a multifunctional protein or protein complex (the CRISPR-associated or Cas proteins) to cleave complementary target DNA that is adjacent to short sequences known as protospacer-adjacent motifs (PAMs). Thus, the organism acquires a way to protect itself from subsequent infection (Bhaya, et al., 2011).

CRISPR-Cas9 platform for transcriptional gene activation in mammalian cells

In addition to genome engineering applications in mammalian cells (Jinek et al., 2012), the *Streptococcus pyogenes* CRISPR-Cas9 system has been adapted to technologies for transcriptional regulation (Qi et al., 2013, Gilbert et al., 2013, Cheng et al., 2013). The nuclease activity of the *S. pyogenes* Cas9 was abolished by point mutations introduced into two catalytic residues (D10A and H840A) yielding a deactivated Cas9 (dCas9) that maintains the ability to bind to target DNA when guided by sequence-specific guide RNAs. When the dCas9 is fused to transcriptional regulators and guided to gene promoter regions, it induces RNA-directed transcriptional regulation. CRISPR-Cas9 based technologies for transcriptional regulation include CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa). CRISPRa utilizes dCas9 fused to different transcriptional activation domains (Cheng et al., 2013, Gilbert et al., 2014, Tanenbaum et al., 2014, Konermann et al., 2015; Chavez et al., 2015), which can be directed to promoter regions by specifically designed guide RNA (depicted in Fig 1). The VPR activation system utilizes a fusion of three transcriptional activators (VP64, p65 and Rta) to the C-terminal end of dCas9 and demonstrates a robust gene activation in mammalian systems (Chavez et al., 2015). Unlike other CRISPRa systems that require expression of the transcriptional activators from separate vectors, the VPR system requires delivery of just two components to the cells of interest: dCas9-VPR and a guide RNA, making it easier to utilize across different biological applications.

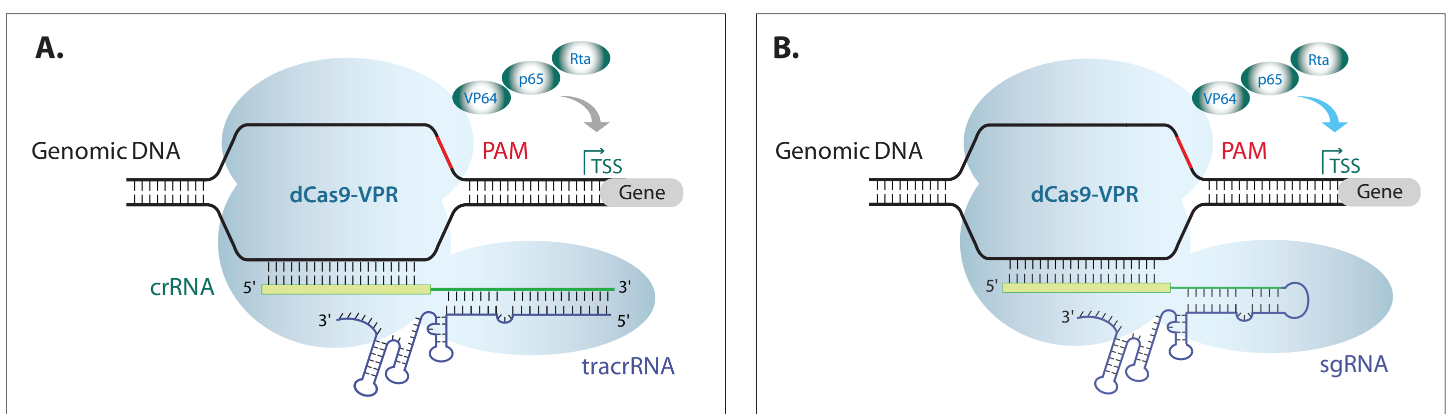

Figure 1. Diagram of dCas9-VPR with crRNA:tracrRNA or sgRNA targeting a gene's promoter region

A CRISPRa guide RNA can be either synthetic CRISPR RNA (crRNA) complexed with a trans-activating CRISPR RNA (tracrRNA), or a single guide RNA (sgRNA) where the crRNA has been fused to the tracrRNA creating a chimeric structure (Figure 1). This protocol provides guidance for the two-part synthetic approach (crRNA complexed with tracrRNA). For CRISPRa using expressed single guide RNA (sgRNA), please see this manual.

2 Edit-R™ CRISPRa workflow

To facilitate rapid generation of cell lines that constitutively express dCas9-VPR, the Edit-R™ lentiviral dCas9-VPR expression vector is packaged into particles, purified and concentrated for direct viral transduction. Subsequent transfection of synthetic or plasmid CRISPRa guide RNA or transduction of lentiviral CRISPRa sgRNA into dCas9-VPR expressing cells results in target gene activation. Figure 2 summarizes the general experimental workflow.

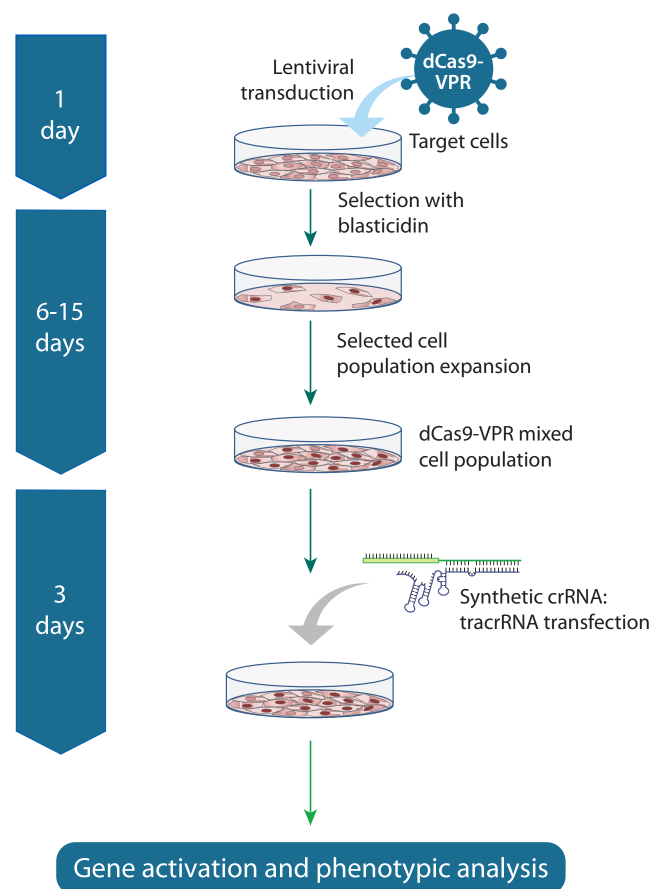

Figure 2. CRISPR activation workflow with lentiviral dCas9-VPR and synthetic crRNA:tracrRNA

Edit-R CRISPRa Lentiviral dCas9-VPR expression vectors

The Edit-R Lentiviral dCas9 -VPR expression vectors contain a human codon-optimized version of the catalytically inactive *S. pyogenes cas9 (csn1)* gene due to point mutations on the RuvC1 and HNH nuclease domains (D10A and H840A) and the blasticidin resistance marker (BlastR). Expression is bicistronic with a 2A peptide sequence and under the control of a single promoter (Figure 3). A brief description of the lentiviral vector elements is provided in Table 1.

Several promoter options are available (Figure 3) enabling the researcher to choose a lentiviral vector with the most active promoter for specific cells of interest. All Edit-R Lentiviral dCas9-VPR expression vectors are supplied as lentiviral particles (≥ 1 × 10^7 TU/mL, ± 20%) or dried down, endotoxin-free plasmid DNA, ready for lentiviral packaging.

Table 1. Elements of the Edit-R Lentiviral dCas9-VPR expression vectors.

Vector element	Utility
dCas9-VPR	*S. pyogenes* dCas9-VPR for gene activation of targeted DNA when programmed with a guide RNA
T2A	Self-cleaving peptide allows simultaneous expression of two proteins from a single transcript
Blast^R	Blasticidin resistance marker permits antibiotic selection of transduced mammalian cells
hCMV	Human cytomegalovirus immediate early promoter
mCMV	Mouse cytomegalovirus immediate early promoter
hEF1α	Human elongation factor 1 alpha promoter
5' LTR	5' Long Terminal Repeat necessary for lentiviral particle production and integration of the construct into the host cell genome
ψ	Psi packaging sequence allows lentiviral genome packaging using lentiviral packaging systems
RRE	Rev Response Element enhances titer by increasing packaging efficiency of full-length lentiviral genomes
WPRE	Woodchuck Hepatitis Post-transcriptional Regulatory Element enhances transgene expression in target cells
3' SIN LTR	3' Self-inactivating Long Terminal Repeat for generation of replication-incompetent lentiviral particles
SV40 pA	Simian virus 40 polyadenylation signal
pUC ori	pUC origin of replication
SV40 ori	Simian virus 40 origin of replication
Amp^R	Ampicillin resistance gene for vector propagation in *E. coli* cultures

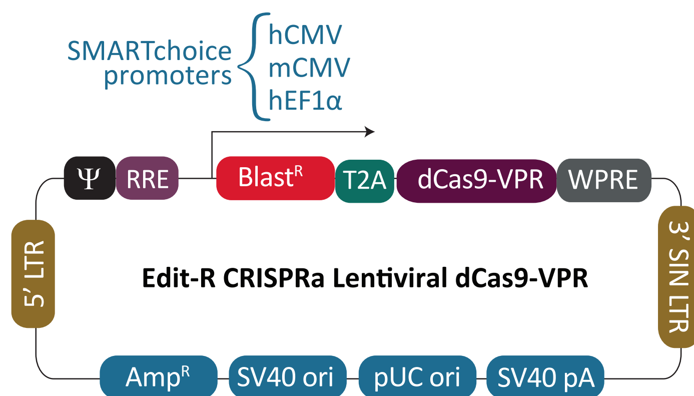

Figure 3. Schematic diagram of the Edit-R Lentiviral dCas9-VPR expression vectors.

Edit-R CRISPRa synthetic guide RNA

Edit-R CRISPRa RNA (crRNA) for transcriptional activation

Edit-R CRISPRa crRNA is comprised of a 20-nucleotide synthetic RNA, identical to the genomic DNA target site, or protospacer, followed by a fixed *S. pyogenes* repeat sequence that interacts with the tracrRNA. It is modified for nuclease resistance and must be used with Edit-R tracrRNA. Edit-R CRISPRa crRNAs are pre-designed, based on a published CRISPRa v2 algorithm (Horlbeck et al., 2016) and target genomic regions in the proximity of a transcriptional start site (TSS). The crRNAs are available as four individual crRNAs or a pool of four crRNA for human and mouse protein coding genes. When more than one TSS exists for a gene, a second set of crRNA reagents is available (labeled P2) to target the alternative start site.

Edit-R trans-activating CRISPR RNA (tracrRNA)

Edit-R tracrRNA is a chemically synthesized and HPLC-purified RNA based on a published *S. pyogenes* tracrRNA sequence (Jinek, 2012). It is modified for nuclease resistance and can be used with Edit-R CRISPRa crRNA for gene activation or Edit-R crRNA for gene editing.

3 CRISPRa protocol for transcriptional gene activation

In this workflow, lentiviral dCas9-VPR particles are utilized to generate cells stably expressing dCas9-VPR in a population of cells or in isolated clonal cell lines. These cells are then transfected with synthetic guide RNAs to obtain transcriptional target gene activation.

Edit-R CRISPRa materials required

Edit-R Lentiviral dCas9-VPR

Dharmacon Edit-R Lentiviral dCas9-VPR expression vectors are provided as concentrated, purified lentiviral particles for immediate transduction or as endotoxin-free plasmid DNA for direct transfection into a packaging cell line and production of your own lentiviral particles. Select the lentiviral dCas9-VPR nuclease vector with the most active promoter in your cell line based on empirical testing or known promoter activity.

- Edit-R CRISPRa Lentiviral dCas9-VPR particles with your choice of promoter:
  - Edit-R™ CRISPRa Lentiviral hCMV-Blast-dCas9-VPR particles (Cat # VCAS11918)
  - Edit-R™ CRISPRa Lentiviral mCMV-Blast-dCas9-VPR particles (Cat # VCAS11920)
  - Edit-R™ CRISPRa Lentiviral hEF1α-Blast-dCas9-VPR particles (Cat # VCAS11922)
- Edit-R™ CRISPRa Lentiviral dCas9 -VPR Expression plasmids with your choice of promoter:
  - Edit-R™ CRISPRa Lentiviral hCMV-Blast-dCas9-VPR plasmid (Cat # CAS11914)
  - Edit-R™ CRISPRa Lentiviral mCMV-Blast-dCas9-VPR plasmid (Cat # CAS11915)
  - Edit-R™ CRISPRa Lentiviral hEF1α -Blast-dCas9-VPR plasmid (Cat # CAS11916)

Edit-R CRISPRa guide RNA

- Edit-R CRISPRa synthetic crRNA: Predesigned crRNA for activation of your gene of interest
  - Edit-R Human CRISPRa crRNA pool (Cat# P-HUMAN-XX) 5, 10, 20 nmol tubes
  - Edit-R Human CRISPRa Set of 4 crRNA (Cat# PQ-HUMAN-XX) 2, 5, 10, 20 nmol tubes
  - Edit-R Human CRISPRa crRNA (Cat# CA-HUMAN-XX), 2, 5, 10, 20 nmol tubes
  - Edit-R Mouse CRISPRa crRNA pool (Cat# P-MOUSE-XX) 5, 10, 20 nmol tubes
  - Edit-R Mouse CRISPRa Set of 4 crRNA (Cat# PQ-MOUSE-XX) 2, 5, 10, 20 nmol tubes
  - Edit-R Mouse CRISPRa crRNA (Cat# CA-MOUSE-XX), 2, 5, 10, 20 nmol tubes

  The above catalog numbers are agnostic; actual products have gene-specific catalog numbers. Search for your gene.
- Edit-R tracrRNA, 5, 20 or 50 nmol (Cat #U-002005-05, -20, -50)

DharmaFECT Transfection Reagent for transfection of crRNA:tracrRNA

Each DharmaFECT formulation is chemically distinct to optimize delivery and viability across a wide variety of cell types. DharmaFECT 1 is the most universal reagent that works well across a wide variety of cell lines. Optimal conditions for siRNA may be used for crRNA:tracrRNA in a given cell line.

DharmaFECT 1 Transfection Reagent (Cat #T-2001-01, 02, 03, 04)
DharmaFECT 2 Transfection Reagent (Cat #T-2002-01, 02, 03, 04)
DharmaFECT 3 Transfection Reagent (Cat #T-2003-01, 02, 03, 04)
DharmaFECT 4 Transfection Reagent (Cat #T-2004-01, 02, 03, 04)

Additional materials required

The following additional materials are required but not supplied:

- 10 mM Tris pH 7.4, nuclease-free buffer (Tris buffer) solution (Dharmacon, Cat #B-006000-100)
- Multi-well tissue culture plates or tissue culture dishes
- Blasticidin S (Fisher Scientific, Cat #BP2647-25; InvivoGen, Cat #ant-bl-1)
- Positive control CRISPRa crRNA reagent:
  - Edit-R CRISPRa Human POU5F1 crRNA (Cat #U-009200-01-05 or -20)
  - Edit-R CRISPRa Human POU5F1 crRNA pool (Cat #U-009200-10-05 or -20)
  - Edit-R CRISPRa Human TTN crRNA (Cat #U-009100-01-05 or -20)
  - Edit-R CRISPRa Human TTN crRNA pool (Cat #U-009100-10-05 or -20)
  - Edit-R CRISPRa Mouse Pou5f1 crRNA (Cat #U-009200-02-05 or -20)
  - Edit-R CRISPRa Mouse Pou5f1 crRNA pool (Cat #U-009200-20-05 or -20)
  - Edit-R CRISPRa Mouse Ttn crRNA (Cat #U-009100-02-05 or -20)
  - Edit-R CRISPRa Mouse Ttn crRNA pool (Cat #U-009100-20-05 or -20)
- Negative control crRNA reagent:
  - Edit-R CRISPRa Non-targeting crRNA (Cat #U-009500-01-05 or -20)
  - Edit-R CRISPRa Non-targeting crRNA Pool (Cat #U-009500-10-05 or-20)
- Base Medium: Appropriate antibiotic-free cell culture medium without serum
- Growth Medium: Appropriate antibiotic-free cell culture medium (with serum and/or supplements) recommended for maintenance of the cells of interest
- Selection Medium: Growth Medium supplemented with the appropriate concentration of antibiotics

Additional recommended materials:

- Materials for RNA isolation and quantitative RT-qPCR for gene expression analysis
- Assay for assessing cell viability

Generation of stable cell line expressing dCas9-VPR

The protocol described here is designed for rapid generation of a cell population where most of the cells have single integration of a lentiviral dCas9-VPR proviral sequence in the genome.

Determining blasticidin concentration for selection of transduced cells

The Edit-R Lentiviral dCas9-VPR expression vectors confer resistance to blasticidin in transduced cells. Before transducing cells, determine the minimum concentration of blasticidin required to kill non-transduced cells (in 3 to 10 days) by generating a blasticidin kill curve. The blasticidin concentration range for many mammalian cells is 2-15 µg/mL.

Transduction of cells with Edit-R CRISPRa Lentiviral dCas9-VPR expression particles

The protocol below describes the basic steps for transduction of the lentiviral particles into U2OS cells (as an example) using serum-free medium in a 24-well plate. Optimal transduction conditions vary widely between cell types and must be determined empirically for each cell line of interest.

If a different sized culture dish is used, adjust the number of cells, volumes, and reagent quantities in proportion to the change in surface area (see Appendix for suggested volumes of Transduction Medium per surface area of culture dishes).

Day 1:

1. Plate 5×10^4 cells per well in a 24-well plate using Growth Medium.
2. Incubate cells at 37 °C in a humidified CO2 incubator overnight.

Optimal cell number for plating will vary with growth characteristics of specific cells and should be determined experimentally. Typically, cells should be at 60-80% confluency on the day of transduction.

Day 2:

1. Equilibrate the Base Medium to 37 °C.
2. Calculate the volume of lentiviral particles needed for transduction at MOI = 0.3.

The functional titer of Edit-R Lentiviral dCas9-VPR expression particles (in HEK293T cells, as determined by qPCR) is reported on the Certificate of Analysis (C of A). We recommend an MOI ≤ 0.3 (adjusted for relative transduction efficiency in your cell type) to ensure single integration of the lentiviral dCas9-VPR. The relative transduction efficiency of your cell type will likely be lower than that of HEK293T cells.

The equation to calculate a volume of lentiviral stock for a given MOI is:

$$V = MOI \times CN \div VT \times 1000$$

Where:

V = volume of lentiviral stock in µL

MOI = desired multiplicity of infection

CN = number of cells in the well at transduction

VT = lentiviral titer in TU/mL (indicated in the Certificate of Analysis) and multiplied by 1000 to convert the volume from mL to µL

For example, for a desired MOI of 0.3 and:

- Cell density of 100 000 cells per well at time of transduction
- Lentiviral titer = 1×10^7 TU/mL

Then,

$V = 0.3$ TU/cell $\times$ 100 000 cells/well $\div 1 \times 10^7$ TU/mL $\times 1000 = 3$ µL of lentiviral stock per well.

3. Thaw the Edit-R CRISPRa Lentiviral dCas9-VPR particles on ice.

Lentiviral particles are shipped on dry ice as 25 µL aliquots and must be stored at -80 °C. Under these conditions, lentiviral particles are stable for at least 12 months. Repeated freeze-thaw cycles should be avoided, as this is expected to negatively affect titer. Once thawed, unused lentiviral particles should be kept on ice, divided into smaller aliquots (if necessary) and immediately returned to -80 °C.

4. Once thawed, gently mix and pipette the calculated volume of lentiviral particles into 0.25 ml of the Base Medium (no serum) to create the Transduction Medium.
5. Remove the Growth Medium from the well and add the Transduction Medium containing the lentiviral particles (see Appendix for guidelines on other plate formats).
6. Incubate cells at 37 °C in a humidified CO_2 incubator for 4-6 hours.
7. At 4-6 hours post-transduction, add an additional 0.75 mL of Growth Medium (with serum) and resume incubation at 37 °C in a humidified CO_2 incubator.

If toxicity occurs with your cells, in step 7, replace the medium after 4-6 hours with fresh Growth Medium (with serum).

Days 3-15:

Generation of stably expressing dCas9-VPR cell lines with blasticidin selection

1. At 24-48 hours post-transduction, replace the Transduction Medium with Selection Medium (Growth Medium containing the appropriate amount of blasticidin).

The appropriate antibiotic concentration is specific to each cell line and should be determined experimentally prior to selection using a kill curve. Blasticidin usually kills cells between 3 and 10 days, slow growing cells may take longer. If the cells become confluent, split the cells into a larger dish to allow proper blasticidin selection (for example, split cells from 24-well to 6-well culture dishes).

2. Once the selected cells are growing normally, expand accordingly to freeze enough aliquots for your experimental project. These cells will be a mixed population that on average have a single integration of Cas9 in their genomes.

Record the passage number and avoid working with stable cell populations that exceed 10 passages from frozen the stock.

Utilize the mixed population of dCas9-VPR expressing cell line obtained above for transfection with synthetic CRISPRa guide RNAs or transduction of lentiviral CRISPRa sgRNAs for activation of your gene of interest. If clonal cell lines are required for your application, we recommend that you isolate clonal cell lines for downstream experiments using protocols appropriate for your cells of interest.

Transfection of synthetic CRISPRa guide RNAs

The following is an example protocol for delivery of Edit-R CRISPRa synthetic crRNA complexed with tracrRNA into adherent U2OS cells stably expressing dCas9-VPR. The protocol is provided for transfection in 96-well plates and the volumes are given for one well and a final 25 nM concentration of the crRNA:tracrRNA complex. We suggest performing the transfection in triplicate wells and adjusting the volumes accordingly providing excess for the ease of pipetting. Transfection conditions vary between cell lines and should be determined empirically. For alternative plating formats see Table 3 for volume recommendations.

Day 1:

1. Plate 1×10^4 U2OS-dCas9-VPR cells per well in a 96-well plate using Growth Medium.
2. Incubate cells at 37 °C in a humidified CO_2 incubator overnight.

Optimal cell number for plating will vary with growth characteristics of specific cells and should be determined experimentally.

Day 2:

1. Prepare 1 µM solution of Edit-R CRISPRa synthetic crRNA and tracrRNA from previously prepared 10 µM stocks.
2. In nuclease-free microcentrifuge tubes (or deep-well 96-well plates for multiple targets) prepare the synthetic crRNA:tracrRNA complex by adding 2.5 µL of 1 µM crRNA and 2.5 µL of 1 µM tracrRNA to 5 µL of serum-free medium.
3. Prepare a DharmaFECT transfection reagent working solution in a separate tube by diluting 0.2 µL of DharmaFECT 4 reagent in 9.8 µL of serum-free medium and mix gently. Incubate tube for 5 minutes at room temperature.

The optimal DharmaFECT Transfection Reagent formulation and concentration varies between cell lines and is affected by the cell density. Easy-to-transfect cells and lower cell densities typically require lower amount of DharmaFECT Transfection Reagent. For replicates, prepare sample volumes sufficient for the number of replicates and extra to account for pipetting errors.

4. Add 10 µL of DharmaFECT 4 working solution to each sample tube containing the crRNA:tracrRNA complex. This brings the total volume to 20 µL.
5. Immediately mix by pipetting gently up and down and incubate for 20 minutes at room temperature.
6. Prepare the Transfection Medium by adding 80 µL antibiotic-free complete Growth Medium to each sample to bring the total volume in each tube to 100 µL.
7. Remove medium from the wells of the 96-well tissue culture plate with cells and replace with 100 µL of the appropriate Transfection Medium in each well.
8. Incubate cells at 37 °C in a humidified CO_2 incubator for 48 to 72 hours before proceeding with the phenotypic assay or gene expression analysis (see Appendix).

Table 2. Suggested volumes per well for transfection of dCas9-VPR-expressing cells with 25 nM synthetic crRNA:tracrRNA

		Tube 1: crRNA:tracrRNA working solution (µL/well)			Tube 2: DharmaFECT transfection reagent working solution (µL/well)			
plating format (wells/plate)	well surface area (cm²/well)	1 µM synthetic tracrRNA (µL)	1 µM synthetic crRNA (µL)	Base Medium (serum free) (µL)	DharmaFECT (µL)	Base Medium (serum free) (µL)	Growth Medium (µL/well)	Final transfection volume (µL/well)
96	0.3	2.5	2.5	5	0.2	9.8	80	100
24	2	12.5	12.5	25	1	49	400	500
12	4	25	25	50	2	98	800	1000
6	10	50	50	100	5	195	1600	2000

Packaging Edit-R Lentiviral dCas9-VPR plasmids into particles

Edit-R CRISPRa Lentiviral dCas9-VPR expression plasmids are Tat dependent and require a packaging system that expresses the *tat* gene. For packaging of lentiviral constructs, we recommend the Dharmacon Trans-Lentiviral ORF Packaging System. For packaging protocols and additional information please consult the product manual.

Edit-R CRISPRa Lentiviral Blast-dCas9-VPR Plasmids do not express a fluorescent protein reporter, therefore, after packaging of plasmid DNA, we recommend titering the lentiviral particles using a functional lentiviral titration protocol such as limiting dilution with cell viability assay by crystal violet staining or genomic qPCR assay.

4 Appendix

Optimization of transfection conditions for delivery of Edit-R CRISPRa synthetic guide RNA

To obtain the highest transfection efficiency of the synthetic guide RNA with minimal effects on cell viability, we recommend carefully optimizing transfection conditions for each cell line using a control crRNA. The transfection optimization can be easily performed in a 96-well format allowing testing of multiple transfection conditions Transfection conditions that have previously been optimized for small RNA delivery are a reasonable starting point for guide RNA complex transfection. The optimization experiment should include two to three cell densities and a range of DharmaFECT Transfection Reagent volumes.

Our recommended ranges for transfection components are as follows:

- 0.05 to 0.8 µL/well of a 96-well plate DharmaFECT 1, 2, 3 or 4
- 25 nM control crRNA:tracrRNA complex per well

At 48 to 72 hours post-transfection, perform a cell viability assay to determine the highest lipid concentration that has minimal cell toxicity (≥ 70% of cell viability is preferred). After assaying for cell viability, we recommend to carefully wash the cells once with Phosphate Buffered Saline (PBS) and proceed with gene expression analysis to determine the condition that produces highest gene activation. Use the determined optimal conditions for subsequent transfection of your selected dCas9-VPR expressing cell lines with the Edit-R CRISPRa synthetic guide RNA.

Gene expression analysis recommendations

RNA can be isolated 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 for normalization of the expression of the gene of interest. Follow best practices for RT-qPCR analysis with appropriate number of technical replicates and proper controls.

Volume of medium per surface area in culture dishes

Table 4. Suggested volumes of Transduction Medium for different plate formats.

Tissue culture dish	Surface area per well (cm^2)	Suggested total serum-free medium volume per well (mL)
100 mm	56	5
6 well	9.4	1
12 well	3.8	0.5
24 well	1.9	0.25
96 well	0.3	0.05

Stability and storage

Lentiviral particles

Edit-R CRISPRa Lentiviral dCas9-VPR Expression particles are shipped on dry ice as 25 µL aliquots and must be stored at -80 °C. Under these conditions, lentiviral particles are stable for at least 12 months. Repeated freeze-thaw cycles should be avoided, as this is expected to negatively affect titer. Once thawed, unused lentiviral particles should be kept on ice, divided into smaller aliquots (if necessary) and immediately returned to -80 °C.

Plasmid DNA

Edit-R Edit-R CRISPRa Lentiviral dCas9-VPR Expression plasmid DNA are shipped as dried pellets at room temperature. Under these conditions, they are stable for at least four weeks. Upon receipt, plasmid DNA should be stored at -20 °C to -80 °C. Under these conditions, the reagents are stable for at least one year. Always dissolve plasmid in nuclease-free solution, such as Tris buffer.

Synthetic crRNA and tracrRNA

Edit-R CRISPRa synthetic guide RNA reagents are shipped as dried pellets at room temperature. Under these conditions, they are stable for at least four weeks. Upon receipt, synthetic guide RNA should be stored at -20 °C to -80 °C. Under these conditions, the reagents are stable for at least one year. Always resuspend synthetic guide RNA in nuclease-free Tris buffer. In solution and stored at -20 °C, the reagent aliquots are stable for at least one year. Avoid multiple freeze-thaw cycles. We recommend not exceeding four to five freeze-thaw cycles to ensure RNA integrity.

5 Frequently asked questions

How should I store my synthetic guide RNAs?

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. You may use our 10 mM Tris-HCl Buffer pH 7.4 for resuspension. We recommend that RNA oligonucleotides be resuspended to a convenient stock concentration (Synthetic guide RNA resuspension protocol) and stored in small aliquots to avoid multiple freeze-thaw cycles. RNA oligonucleotides should not go through more than five freeze-thaw cycles. If degradation is a concern, the integrity of the RNA oligonucleotides can be evaluated on an analytical PAGE gel.

Can I use my siRNA transfection protocols to transfect Edit-R CRISPRa synthetic guide RNAs?

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

Can I use a transfection reagent other than DharmaFECT to deliver the crRNA:tracrRNA into my cells?

We cannot predict the transfection ability 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.

What is the best way to confirm that my gene is activated?

We suggest using RT-qPCR to measure the relative change in target gene expression levels between samples treated with a non-targeting control and CRISPRa guide RNAs. RT-qPCR analysis can be completed with either the SYBR green method or probe-based gene expression assays. Follow manufacturer's instructions for RNA isolation and RT-qPCR set up and use best practices to avoid contaminations during the RNA isolation, cDNA synthesis and qPCR set up. Use proper controls for RT-qPCR analysis (include no RNA samples, no reverse transcriptase samples, no cDNA samples negative controls). Additionally, when performing RT-qPCR for gene activation the expression level may go from not detectable to expressed. In this case, when using the ΔΔCq method of analysis, an arbitrary value representing the detection limit of the qPCR instrument is used as a place holder for "non-detectable" as a non-zero value is necessary to perform the calculation. In most cases this value will be between 35 and 40 depending on the number of programmed cycles and the instrument Cq determination method. We recommend adding additional cycles (up to 45 total) to standard qPCR cycling conditions.

Can the Edit-R CRISPRa system be used for gene activation in non-mammalian organisms, such as flies or worms?

Edit-R CRISPRa system is designed for mammalian expression and have been tested in mammalian cells. The guide RNAs are predesigned to activate human and mouse genes. Custom guide RNAs could be ordered that target promoter regions of other species, however we cannot predict the efficacy of using Edit-R CRISPRa lentiviral dCas9-VPR particles and Edit-R CRISPRa synthetic guide RNA components, nor can we troubleshoot experiments performed in non-mammalian systems.

Can I co-transfect the Edit-R CRISPRa crRNA:tracrRNA components with the Edit-R CRISPRa Lentiviral dCas9-VPR expression plasmid?

We do not recommend doing a co-transfection of Edit-R Lentiviral dCas9-VPR Expression plasmids with synthetic guide RNAs due to the transient nature of transcriptional activation with the non-expressed guide RNAs and the longer time necessary to perform blasticidin selection (typically > 5 days) to remove the non-transfected cells. For transient expression experiments, or to avoid the use of lentiviral particles, we recommend doing a co-transfection of the Edit-R sgRNA plasmid with the Lentiviral dCas9-VPR expression plasmid which then allows for selection of both components.

What are the average molecular weight and extinction coefficient of crRNA and tracrRNA?

Molecule	Molecular weight	Extinction coefficient (E260)
crRNA	13,500 g / mol	423000 L/mol×cm
tracrRNA	23,820 g / mol	757800 L/mol×cm

Where can I obtain the Edit-R CRISPRa Lentiviral dCas9-VPR vector maps?

dCas9-VPR plasmid maps can be obtained upon request from Technical Support.

What is the size of the dCas9-VPR protein and what antibody do you recommend for confirmation of expression of the dCas9-VPR?

The VPR activators add additional 536 amino acids to dCas9 which shift the molecular weight of the dCas9-VPR to approximately ~220 kDa. The protein could be detected using Cas9 antibodies (for example: Novus Biologicals cat #NBP2-36440).

Can I use the Edit-R guide RNA predesigned for gene knockout in CRISPRa experiments?

The guide RNA designs for CRISPRa are different than the Edit-R guide RNAs for CRISPR-Cas9 knockout experiments. The guide RNA designs for CRISPRa are required to bind upstream of the transcriptional start site and are based on a published CRISPRa algorithm. Pre-designed Edit-R guide RNAs are optimized for functional gene knockout with the Dharmacon algorithm and target the gene's coding region.

What if a gene has more than one transcriptional start site?

The published CRISPRa v2 algorithm (Horlbeck et al., 2016) used FANTOM and Ensembl databases to predict more accurately the transcriptional start site (TSS). Some genes (6.8%) were identified as having alternative transcriptional start sites. The publication lists 10 gRNA designs per TSS. For the Edit-R CRISPRa predesigned guide RNAs, we offer the top four guide RNAs for the primary TSS, and, when applicable, four guide RNAs for the secondary TSS. These are labeled as P1 and P2, respectively. If the CRISPRa guide RNAs for your gene do not have a P2 designation, only a single start site is designated for that gene. If your gene has both P1 and P2 guide RNAs, it might be beneficial to test both in your experiment as which TSS is active and to what level depends on your cell line. For a small number of genes (0.1%) Horlbeck et al., 2016 identified more than two TSS. We only offer P1 and P2 designs as catalog items, but we can generate the additional guide RNAs as a custom request based on designs from the published algorithm.

How specific are the gRNAs in targeting the gene of interest?

Several publications have shown CRISPRa to be highly specific by RNA seq expression analysis, but CRISPRa is a new technology and off-targeting still needs to be explored in more detail. Keep in mind that for CRISPRa off-targeting, the guide RNA needs to bind to the promoter region of another gene in order to have an off-target effect, which dramatically decreases the potential off-target space. Furthermore, the guide RNAs are designed based on a published algorithm that incorporates chromatin, nucleosome position, and sequence features to accurately predict highly effective guide RNAs and also applies a filter for off-target binding.

However, there might be examples of genes where the promoter region for one gene is in close proximity to another gene's promoter region. Investigation of the genomic location for your gene of interest and performing expression analysis to confirm activation of the target gene without having effects on other proximal genes might be important for proper interpretation of the phenotypic analysis.

Can I use the Edit-R CRISPRa guide RNAs with the SunTag system?

Yes. The Edit-R CRISPRa system uses canonical guide RNAs and they can be used with other similar systems that use canonical guide RNAs, like the SunTag system.

Can I use the Edit-R CRISPRa guide RNAs with the SAM system?

The Edit-R guide RNAs can not be used with CRISPRa systems that utilize guide RNAs modified with aptamer sequences to bring the activators to the dCas9-gRNA binding site, which is the case for the SAM system. The functionality of algorithm-designed guide RNAs is transferable between different CRISPRa systems, so the target region of an Edit-R CRISPRa guide RNA could be utilized in a guide that is designed for use with SAM.

What level of overexpression should I expect?

The level of gene activation correlates with the basal expression of the gene, and is therefore cell-line specific. For genes that are either not expressed or expressed at a low level, we generally see high activation (100 to 10,000 fold over NTC treated cells). But for genes that are expressed at a high level, CRISPRa causes lower activation (2 -100 fold). Therefore, knowing the level of expression of the gene of interest in your cells can help you gauge the expectation for the level of activation.

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7 Lentiviral particle product safety level information

This lentiviral particle product safety level information constitutes Product Documentation according to clause 1 of the Product Terms and Conditions. It is applicable to all Dharmacon lentiviral particle products.

Any investigator who purchases Dharmacon lentiviral particle products is responsible for consulting with their institution's health and biosafety personnel for specific guidelines on the handling of lentiviral vector particles. Furthermore, each investigator is fully responsible for obtaining the required permissions for research use and the acceptance of replication-incompetent SIN lentiviral vectors and replication-defective lentiviral particles into their local jurisdiction and institution.

The Products are solely for internal research use (as set forth in the Product Terms and Conditions) in laboratories where the containment measures stated below and in applicable laws and regulations are met. Products may not be used for diagnostic, therapeutic or other commercial purposes and may not be administered to humans for any purpose or to animals for therapeutic purposes. The Products are replication-incompetent, self-inactivating (SIN) and non-pathogenic (do not cause infectious human disease).

For questions concerning the design or production of the products, please contact our technical support team.
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In Germany:

For US guidance on containment for lentiviral vectors, please refer to:

1. The Recombinant DNA Advisory Committee (RAC) guidelines for research with lentiviral vectors.
2. The U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health, Biosafety in Microbiological and Biomedical Laboratories (BMBL);
3. The NIH Guidelines For Research Involving Recombinant DNA Molecules (NIH Guidelines), April 2016

In the EU:

For the EU directives, please consult the following:

1. Council Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. (revised version of Directive 90/219/EEC of the European Parliament and of the Council of 23 April 1990 on the contained use of genetically modified micro-organisms, amended by Council Directive 98/81/EC of 26 October 1998); and
2. Council Directive 2001/18/EC of the European Parliament and of the Council of 12 March on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

In Germany:

Required Containment Measures: The containment requirements as stated in the German Genetic Safety Ordinance (Gentechnik-Sicherheitsverordnung) of Safety Level 2* or higher have been assigned to the handling of the abovementioned lentiviral vector particles. Please note a higher Security Level might be required if the lentiviral vector particles are used for genetic engineering operations with other products which require a higher Security Level. *Safety Level 2: activities of low risk for human health and the environment by the state of scientific knowledge (Stand der Wissenschaft).

For the German regulations, please consult the following:

1. German Genetic Engineering Act (Gentechnikgesetz - GenTG); and
2. Genetic Engineering Safety Ordinance (Gentechnik-Sicherheitsverordnung - GenTSV).

8 Limited use licenses

The gene editing Products, use and applications, are covered by pending and issued patents. Certain Label licenses govern the use of the products; these can be found at Dharmacon Licensing Statements. It is each Buyer's responsibility to determine which intellectual property rights held by third parties may restrict the use of Products for a particular application. Please review the Label Licenses governing all use of the Products.

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