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TECHNICAL MANUAL

CRISPRmod transcriptional modulational with All-in-one Lentiviral dCas9-VPR or dCas9-SALL1-SDS3 and sgRNA expression systems

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Contents

1 Introduction to the CRISPR-Cas9 system for transcriptional modulation

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 modulation 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 Figure 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.

Conversely, CRISPRi systems initially utilized the Krüppel associated box (KRAB) domain from zinc finger protein 10 (KOX1) as a transcriptional repressor (Gilbert et al., 2013, Gilbert et al., 2014), but recent publications have demonstrated improved transcriptional repression with a variety of different effectors (Yeo et al.,2018, Moghadam et al.,2020, Alerasool et al.,2020). Horizon's CRISPRi system utilizes a novel fusion protein comprised of repressor domains from two human transcriptional repressors, Sal-like protein 1 (SALL1) and Sin3 histone deacetylase corepressor complex component SDS3 (SDS3), fused to the C-terminal end of dCas9 (Mills et al., 2022). Horizon's CRISPRi system was developed to provide robust and consistent gene repression when used in conjunction with synthetic guide RNA.

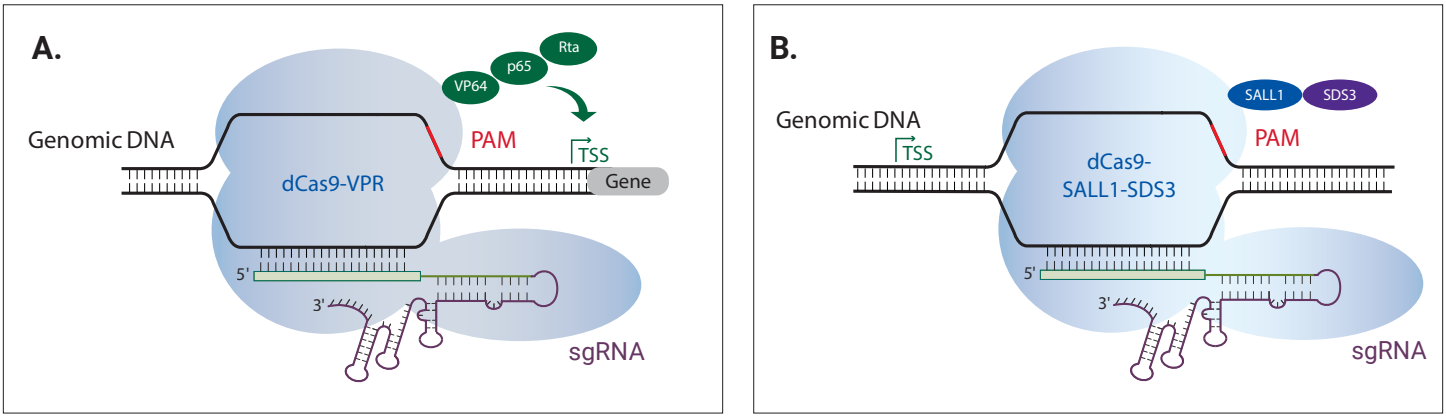

Figure 1. Diagram of dCas9-VPR (A, CRISPRa) or dCas9-SALL1-SDS3 (B, CRISPRi) with sgRNA targeting a gene's promoter region

2 Transcriptional modulation using CRISPRmod All-in-one Lentiviral sgRNA

The CRISPRmod All-in-one Lentiviral sgRNA vector includes the two critical components based on the natural *S. pyogenes* system, the mutated dCas9 fused to a transcriptional activator (VPR) or a transcriptional repressor (SALL1-SDS3) along with the gene-specific sgRNA. To facilitate rapid generation of cell lines that constitutively express dCas9 transcriptional effectors and the sgRNA, these vectors are packaged into lentiviral particles, purified, and concentrated. Cell lines stably overexpressing or suppressing a target gene can be obtained with the CRISPRmod All-in-one Lentiviral sgRNA by transduction with lentiviral particles followed by selection with puromycin or enrichment by FACS sorting, depending on lentiviral vector selected. Figure 2 summarizes a general experimental workflow to generate stable cell lines expressing dCas9 transcriptional effectors and sgRNA following transduction with CRISPRmod All-in-one Lentiviral sgRNA lentiviral particles for phenotypic analysis of gene modulation.

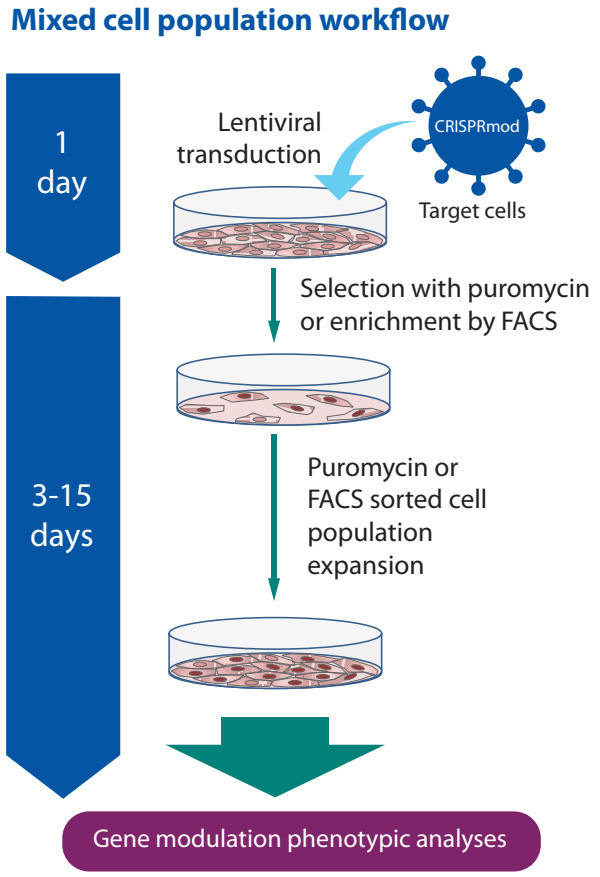

Figure 2. Gene modulation workflow using the CRISPRmod All-in-one Lentivral sgRNA system. Gene modulation with CRISPRmod All-in-one Lentiviral sgRNA produces a mixed cell population for CRISPRa or CRISPRi screening.

CRISPRmod All-in-one Lentiviral sgRNA vectors

The CRISPRmod All-in-one Lentiviral sgRNA vector contains a human codon-optimized version of the *S. pyogenes* dCas9 gene fused to transcriptional modulators (CRISPRa – VPR; CRISPRi – SALL1-SDS3) and either the puromycin resistance marker ($Puro^R$) or Enhanced Green Fluorescent Protein (EGFP). The gene-specific sgRNA is expressed under the control of a human U6 promoter. CRISPRmod All-in-one Lentiviral sgRNAs are predesigned, based on a published CRISPRa v2.0 and CRISPRi v2.1 algorithms (Horlbeck et al., 2016) and target genomic DNA in the proximity of transcriptional start site (TSS). The optimized sgRNA scaffold used in the CRISPRmod All-in-one lentiviral sgRNA can further improve gene modulation efficiency. A brief description of the lentiviral vector elements is listed in Table 1. Two promoter options are available (Figure 3) for selection of a dCas9 transcriptional modulation vector with the most active promoter for specific cells of interest.

Each CRISPRmod All-in-one Lentiviral sgRNA is specific to the gene of choice. The crRNA region of the sgRNA is comprised of 19-20 nucleotides identical to the genomic DNA target site, followed by the non-variable sgRNA scaffold containing the tracrRNA sequence from *S. pyogenes*. Ten pre-designed lentiviral sgRNAs per transcriptional start site are available for human protein coding genes. When more than one TSS exists for a gene, a second set of sgRNA reagents is available (labeled P2). All CRISPRmod All-in-one Lentiviral sgRNA expression vectors are supplied as lentiviral particles (≥ 1×10^7 TU/mL, ± 20%, see CoA for specific titer). Predesigned, gene-specific CRISPRmod All-in-one Lentiviral sgRNA particles can be ordered by searching for genes of interest (CRISPRa , CRISPRi).

Table 1. Elements of the CRISPRmod All-in-one Lentiviral sgRNA vectors.

Vector element	Utility
dCas9-VPR	*S. pyogenes* dCas9-VPR for CRISPRa gene activation of targeted DNA when programmed with a guide RNA
dCas9-SALL1-SDS3	*S. pyogenes* dCas9-SALL1-SDS3 for CRISPRi gene repression of targeted DNA when programmed with a guide RNA
T2A	Self-cleaving peptide allows for simultaneous expression of puromycin resistance and Cas9 protein from a single transcript
$Puro^R$	Puromycin resistance marker permits antibiotic selection of transduced mammalian cells
EGFP	Enhanced Green Fluorescent Protein permits identification and enrichment of transduced cells
mCMV	Mouse cytomegalovirus immediate early promoter
hEF1α	Human elongation factor 1 alpha short promoter
U6	Human RNA polymerase III promoter U6
sgRNA	Optimized single guide RNA, a fusion of gene-specific crRNA with the tracrRNA scaffold
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

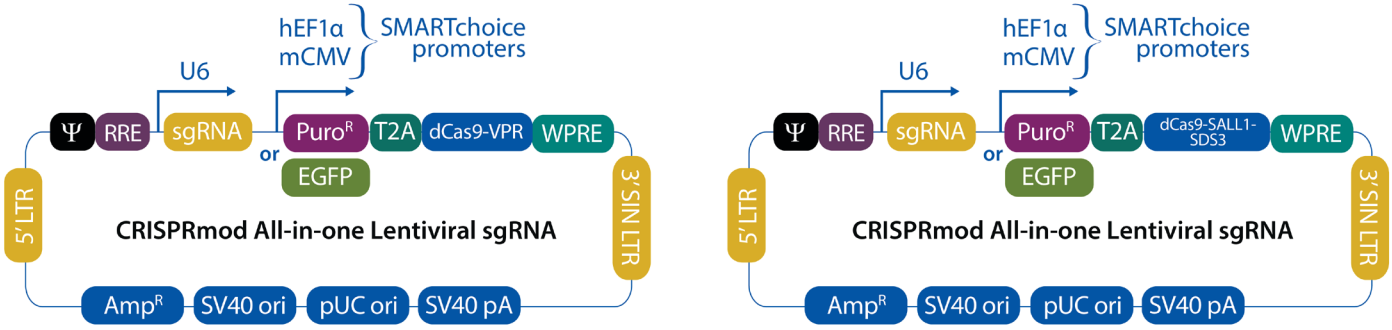

Figure 3. Schematic map of the CRISPRmod All-in-one Lentiviral sgRNA vectors.

3 CRISPRmod transduction protocol for transcriptional modulation

The CRISPRmod All-in-one Lentiviral sgRNA transcriptional modulation system utilizes dCas9 fused to transcriptional modulation domains and the sgRNA in a single-step process to achieve efficient gene modulation for phenotypic analyses in a population of cells. If required by the downstream assay and gene of interest, isolated clonal cell lines can be produced by clonal selection after these steps.

CRISPRmod All-in-one Lentiviral sgRNA transcriptional modulation materials required

CRISPRmod CRISPRmod All-in-one Lentiviral sgRNA
CRISPRmod All-in-one Lentiviral sgRNA vectors are provided as concentrated, purified lentiviral particles for immediate transduction.

1. CRISPRmod All-in-one Lentiviral sgRNA with SMARTchoice promoter options and predesigned sgRNA particles.
   - » CRISPRmod CRISPRa Human All-in-one hEF1α Lentiviral sgRNA
   - » CRISPRmod CRISPRa Human All-in-one mCMV Lentiviral sgRNA
   - » CRISPRmod CRISPRi Human All-in-one hEF1α Lentiviral sgRNA
   - » CRISPRmod CRISPRi Human All-in-one mCMV Lentiviral sgRNA

Additional materials required

The following additional materials are required but not supplied:

- Multi-well tissue culture plates or tissue culture dishes
- Puromycin (Fisher Scientific, Cat #BP2956-100; InvivoGen, Cat #ant-pr-1)
- Resazurin cell viability reagent or similar
- Assay(s) for detecting gene modulation in a cell population
- CRISPRmod All-in-one Lentiviral sgRNA Positive Controls particles
- CRISPRmod All-in-one Lentiviral sgRNA Negative Controls particles
- **Growth Medium:** antibiotic-free cell culture medium (with serum and/or supplements) recommended for maintenance of the cells of interest.
- **Transduction Medium:** the serum-free base cell culture medium containing lentiviral particles. Depending on the cell type, transduction additives, such as polybrene, and serum may be added.
- **Selection Medium:** Growth Medium supplemented with the appropriate concentration of puromycin.

Generation of a stable cell line expressing CRISPRmod All-in-one Lentiviral sgRNA

The protocol described here is designed for rapid generation of a cell population where most of the cells have a single copy of the CRISPRmod All-in-one Lentiviral sgRNA viral sequence in the genome.

Select the CRISPRmod All-in-one Lentiviral sgRNA vector with the most active promoter in your cell line based on empirical testing or known promoter activity. Positive controls are available with both promoter options for empirical testing.

Puromycin selection

The CRISPRmod All-in-one Lentiviral sgRNA vectors containing the puromycin resistance gene confer resistance to puromycin in transduced cells. Before transducing cells, determine the minimum concentration of puromycin required to kill non-transduced cells by generating a puromycin kill curve. The puromycin concentration range for many mammalian cells is 0.5-10 µg/mL and it usually kills cells between two and seven days.

Enrichment using fluorescent markers

CRISPRmod All-in-one Lentiviral sgRNA vectors containing EGFP will display green fluorescence after transduction. This fluorescence allows for the identification of transduced cells. If fluorescence-activated cell sorting (FACS) is to be performed, please ensure that the lasers and filters are suitable for the EGFP marker (see maximum excitation and emission wavelengths for EGFP in Section 6 Frequently Asked Questions).

Transduction of cells with CRISPRmod All-in-one Lentiviral sgRNA particles

The protocol below provides the basic steps for transduction of the lentiviral particles into mammalian cells using serum-free medium in a 24-well plate. Permissivity to lentiviral delivery and optimal transduction conditions vary widely amongst cell types and must be determined empirically for each cell line of interest.

Day 1:

1. Based on experimentally determined growth characteristics of the cell line being used, plate cells so they are at 40-80% confluency on the day of transduction.
2. Incubate cells at 37 °C in a humidified CO_2 incubator overnight.

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 CRISPRmod All-in-one Lentiviral sgRNA particles is reported on the Certificate of Analysis (CoA). We recommend an MOI of 0.3 (adjusted for relative transduction efficiency in your cell type) to ensure single integration of the dCas9 transcriptional modulation fusion protein and sgRNA.

3. Thaw the CRISPRmod All-in-one Lentiviral sgRNA 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 the Transduction Medium.
5. Remove the Growth Medium from the well and add 0.25 mL of 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. Approximately 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).

For puromycin resistance-containing constructs

Days 3-15:

1. At 24-48 hours post-transduction, replace the Transduction Medium with Selection Medium (Growth Medium containing the appropriate amount of puromycin).
2. Replace the Selection Medium every 2-3 days and monitor the presence of dead cells daily
   - If the cells become confluent, split the cells into a larger dish to allow proper puromycin selection (for example, split cells from 24-well to 6-well culture dishes).
3. 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 CRISPRmod All-in-one Lentiviral sgRNA cassette in their genomes.
   - Expansion of the puromycin-resistant cells at this stage will generate a mixed population of cells having a single integration of the dCas9 transcriptional modulation fusion protein and sgRNA in their genomes. This mixed population will be integrated in different places in the genome and may have different levels activation/repression. This mixed population can be used for phenotypic analysis. If uniform levels of activation/repression are required for a specific assay, clonal cell populations can be grown from these stocks.
4. Record the passage number and avoid working with stable cell populations at passage numbers exceeding 10 from frozen stock.

For EGFP containing constructs

Days 3-5:

1. At 24-72 hours post-transduction, check the cells for expression of EGFP.
   - Cells can be subjected to enrichment by FACS or studied individually.

Visualizing the cells in media specifically designed for imaging may reduce the background fluorescence.

2. If cells have been subjected to enrichment of the fluorescent population, expand the cells to freeze a sufficient number of aliquots for your experimental project or proceed to isolation of clonal cell lines.
    - Expansion of the fluorescent cells at this stage will generate a mixed population of cells having a single integration of the dCas9 transcriptional modulation fusion protein and sgRNA in their genomes. This mixed population will be integrated in different places in the genome and may have different levels activation/repression. This mixed population can be used for phenotypic analysis. If uniform levels of activation/repression are required for a specific assay, clonal cell populations can be grown from these stocks.
3. Gene modulation can be assessed in cells 48-72 hours post transduction. If freezing, record the passage number and avoid working with stable cell populations at passage numbers exceeding 10 from frozen stock.

For maximal gene modulation, it may be necessary to test MOIs higher than 0.3, and this needs to be empirically determined. If enrichment for EGFP positive cells is not performed, or fluorescent cells not specifically analyzed, gene modulation may not be detectable in cell populations transduced with relatively low MOIs.

4 Appendix

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.

Multiplicity of Infection (MOI)

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

V = MOI × CN ÷ VT × 1000

Where:
V = volume of lentiviral stock in µL

MOI = desired multiplicity of infection

CN = number of cells in the well at transduction

VT = Viral 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 × 100 000 cells/well ÷ 1×10^7 TU/mL × 1000 = 3 µL of lentiviral stock/well.

Volume of medium per surface area in culture dishes

Table 2. Suggested volumes of Transduction Medium per surface area per well of adherent cells.

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

CRISPRmod All-in-one Lentiviral sgRNA 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.

5 Frequently asked questions

How do I choose between the two CRISPRmod All-in-one Lentiviral sgRNA promoter options?

Choose the promoter option that has been demonstrated, either by your own experimental observations or through references in the published literature, to actively express a transgene in your cells of choice. For optimal experimental confidence (or if such information is not available), consider testing both options using the CRISPRmod All-in-one Lentiviral sgRNA Positive Controls.

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

We suggest using RT-qPCR to measure the relative change in target gene expression levels between samples treated with non-targeting control and CRISPRmod CRISPRa or CRISPRi 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 cross-contamination 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 CRISPRmod CRISPRa or CRISPRi system be used for gene modulation in non-mammalian organisms, such as flies or worms?

The CRISPRmod CRISPRa and CRISPRi systems are designed for mammalian expression and have been tested in mammalian cells. The guide RNAs are predesigned to activate human genes.

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

The published CRISPRa v2 and CRISPRi V2.1 algorithms (Horlbeck et al., 2016) used FANTOM and Ensembl databases to predict the transcriptional start site (TSS) more accurately. Some genes (6.8%) were identified as having alternative transcriptional start sites. The publication lists 10 gRNA designs per TSS. For the CRISPRmod predesigned guide RNAs, we offer the top four (CRISPRa) or top three (CRISPRi) guide RNAs for the primary TSS, and, when applicable, four (CRISPRa) or three (CRISPRi) guide RNAs for the secondary TSS. These are labeled as P1 and P2, respectively. If the CRISPRmod guide RNAs for your gene do not have a P2 designation, then only a single start site is predicted for that gene.

If your gene has both P1 and P2 guide RNAs, it might be beneficial to test both for your experiment. 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 All-in-one lentiviral items.

How can I identify the best transduction conditions of CRISPRmod All-in-one Lentiviral sgRNA particles in my cells?

Successful transduction of cells depends on cell type, cell density, passage number, MOI during transduction, purity of the lentiviral preparation and the presence and/or absence of reagents that facilitate transduction (for example, polybrene). If you do not know what the best conditions for transduction of your cell type of interest are, optimization can be performed in a 96-well plate format prior to your experiments, testing cell density, presence or absence of serum, presence or absence of transduction additives (polybrene) and the duration of transduction (6 hours or overnight) prior to addition of growth medium to your cells.

How specific are the CRISPRmod sgRNAs in targeting the gene of interest?

Several publications have shown CRISPRa and CRISPRi to be highly specific by RNA seq expression analysis, but CRISPRa and CRISPRi are new technologies and off-targeting still needs to be explored in more detail. Keep in mind that for CRISPRmod 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 published algorithms that incorporate 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.

Where can I find the sequence of an individual sgRNA construct?

The target sequence of an individual sgRNA construct is provided on the product insert and can be found online under your account Order History at horizondiscovery.com.

Fluorescent reporter	Excitation wavelength	Emission wavelength
EGFP	488 nm	507 nm

What are the maximum excitation and emission wavelengths for EGFP?
How do I know if the dCas9 fusion protein is expressed after introducing the CRISPRmod All-in-one Lentiviral sgRNA vectors?

We recommend performing a functional assay, such as RT-qPCR, to determine the presence of the CRISPRmod dCas9 fusion protein. For initial optimization, we recommend using one of our positive control kits and performing RT-qPCR to determine gene modulation efficiency.

We strongly recommend performing RT-qPCR assays when using any gene-specific CRISPRmod All-in-one Lentiviral sgRNA to determine gene modulation efficiency for all experiments, even after the delivery conditions have been optimized.

Additionally, as the dCas9 fusion protein is under the same promoter as EGFP/puromycin resistance marker, expression of EGFP or puromycin resistance would indicate the simultaneous expression of the dCas9 fusion protein. We routinely select and enrich for dCas9 fusion protein expressing cells using antibiotic resistance or EGFP expression, respectively.

How do I choose between CRISPRmod All-in-one Lentiviral sgRNA containing Puromycin and EGFP?

The CRISPRmod All-in-one Lentiviral sgRNA containing PuroR will confer resistance to puromycin in transduced cells and long-term selection is used to generate stable cell lines. As the amount of puromycin and time needed for selection may vary between cell lines, performing a puromycin kill curve before the transducing cells is recommended. CRISPRmod All-in-one Lentiviral sgRNA vectors containing EGFP display green fluorescence in transduced cells and assays such as FACS can be used to enrich for cells containing successful lentiviral construct integration. The equipment being used (e.g lasers, filters, etc) should be suitable for detecting EGFP (see “excitation and emission wavelengths for EGFP”).

Depending on the cell type, both EGFP expression and Puromycin resistance should appear 24-72 hours post-transduction. As FACS enrichment can be done in a matter of hours while puromycin selection can take several days, vectors containing EGFP may be preferable if the cells of interest have a short lifespan.

What is the size of the CRISPRmod CRISPRa and CRISPRi fusion proteins?

The VPR activators add additional 536 amino acids to dCas9 which shift the molecular weight of the dCas9-VPR to approximately ~220 kDa. The SALL1-SDS3 repressor domains add an additional 533 amino acids to dCas9 which shift the molecular weight of dCas9-SALL1-SDS3 to approximately ~220 kDa.

6 References

1. D. Bhaya, *et al.*, CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. *Annu. Rev. Genet.* **45**, 273-297 (2011).
2. M. Jinek, *et al.,* A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial Immunity. *Science.* **337**, 816-821 (2012).
3. Qi, L.S., *et al.*, Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*, **152**(5): p. 1173-83 (2013)
4. L.A.Gilbert, *et al.*, CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell*, **154**(2): p. 442-51 (2013).
5. A.W. Cheng, *et al.*, Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system. *Cell Res*, **23**(10): p. 1163-71 (2013).
6. A. Chavez *et al.*, Highly efficient Cas9-mediated transcriptional programming. *Nat. Methods.* **12**, 326–328 (2015).
7. M. A. Horlbeck *et al.*, Compact and highly active next-generation libraries for CRISPR-mediated gene repression and activation. eLife. 5, e19760 (2016).
8. L. A. Gilbert *et al.*, Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. *Cell.* **159**, 647–661 (2014).
9. M. E. Tanenbaum, L. A. Gilbert, L. S. Qi, J. S. Weissman, R. D. Vale, A protein-tagging system for signal amplification in gene expression and fluorescence imaging. *Cell.* **159**, 635–646 (2014).
10. S. Konermann *et al.*, Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature.* **517**, 583–588 (2015).
11. NC. Yeo, et al., An enhanced CRISPR repressor for targeted mammalian gene regulation. *Nat Methods.* **15**(8): p. 611-616 (2018).
12. F. Moghadam, et al., Synthetic immunomodulation with a CRISPR super-repressor in vivo. *Nat Cell Biol.* **22**(9): p. 1143-1154 (2020).
13. N. Alerasool, et al., An efficient KRAB domain for CRISPRi applications in human cells. *Nat Methods.* **17**(11): p. 1093-1096 (2020).
14. C. Mills, et al., A novel CRISPR interference effector enabling functional gene characterization with synthetic guide RNAs. *CRISPR J.* (2022).
15. M. A. Horlbeck et al., Compact and highly active next-generation libraries for CRISPR-mediated gene repression and activation. *eLife.* **5**, e19760 (2016).

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.

Horizon Discovery
2650 Crescent Drive
Lafayette, CO 80026 USA
Tel. +1 800 235 9880; +1 303 604 9499
Fax: +1 800 292 6088; +1 303 604 9680
Email: technical@horizondiscovery.com

In the US:

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.

For more information

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Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

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