Technical Manual

CRISPRmod

Dharmacon™ CRISPRmod™ DyadGuide™ dual-sgRNA transcriptional modulation expression systems

Storage: -80°C

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Horizon Discovery Biosciences Ltd. (A Revvity Company)

Contents

1 Introduction: CRISPR-Cas9 platform for transcriptional gene modulation in mammalian cells

The CRISPR (clustered regularly interspaced palindromic repeats)-Cas (CRISPR-associated protein) system is an adaptive bacterial and archaeal defense mechanism that has been adopted as a tool in the scientific community for genome engineering applications in mammalian cells[1,2]. Additional advances have adapted the system to create technologies for transcriptional regulation, such as CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi)[3-5].

CRISPRa utilizes dCas9 fused to different transcriptional activation domains[5-9], which can be directed to promoter regions by specifically designed guide RNA (depicted in Figure 1, left). 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[6].

The CRISPRmod 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 for improved transcriptional repression compared to traditional systems[10]. This CRISPRi system was developed to provide robust and consistent gene repression when used in conjunction with synthetic guide RNA, as well as with expressed guide RNA (Figure 1, right).

The DyadGuide dual-sgRNA expression platform is the next generation of advancement for the CRISPR Cas9 toolbox. This platform consists of an engineered, bidirectional U6 promoter which drives expression of two sgRNAs, in a sense- and anti-sense orientation, from a single promoter. This enables researchers to target the same gene with multiple sgRNAs using an expressed system, ensuring the desired genetic perturbation is achieved. Alternatively, the DyadGuide system also enables the targeting of two independent genes for more complex genetic perturbations in a single experiment. Delivering two sgRNAs within a single lentiviral particle reduces the need for multiple rounds of transductions and selections, greatly reducing the time of the experimental workflow and increasing the overall yield of cells with the desired lentiviral integration.

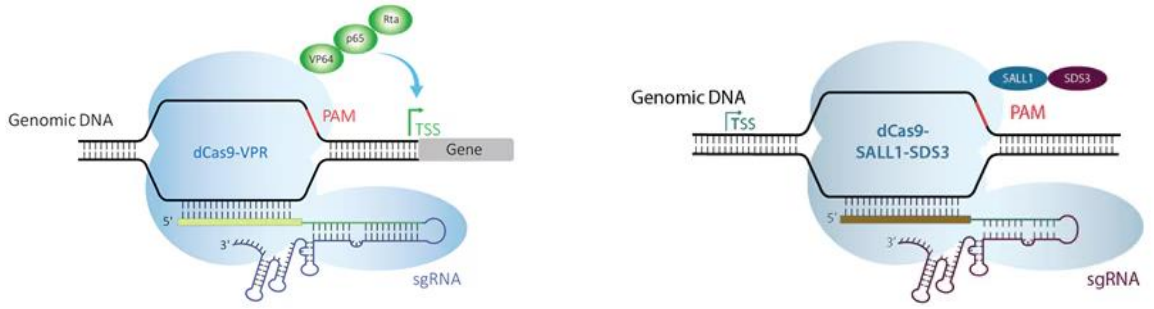

Figure 1. Diagram of dCas9-VPR (A, CRISPRa) or dCas9-SALL1-SDS3 (B, CRISPRi) with sgRNA targeting a gene's promoter region

2 Transcriptional modulation using CRISPRmod Lentiviral DyadGuide dual-sgRNA vectors

Each sgRNA in the CRISPRmod Lentiviral DyadGuide dual-sgRNA vector is specific to the transcriptional start site (TSS) of the gene of interest. CRISPRmod sgRNAs are designed based on a published algorithm[11] and target genomic DNA in the proximity of TSS. When more than one TSS exists for a gene, a second set of sgRNA reagents is available (labeled P2). 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*. The optimized sgRNA scaffolds used in the CRISPRmod Lentiviral DyadGuide dual-sgRNA vector can further improve efficiency.

CRISPRmod Lentiviral DyadGuide dual-sgRNA expression vectors are supplied as lentiviral particles or glycerol stocks (see Appendix F). Predesigned, gene-specific CRISPRmod Lentiviral DyadGuide dual-sgRNA particles can be ordered via a webtool through which the CRISPR modality (CRISPRa, CRISPRi), promoter, selection marker, guide RNA 1, and guide RNA 2 can be chosen.

A. CRISPRmod All-in-one Lentiviral DyadGuide dual-sgRNA vectors

The CRISPRmod All-in-one Lentiviral DyadGuide dual-sgRNA vector includes all components necessary for your CRISPRa or CRISPRi experiment in one convenient construct (Figure 2). 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) allow for selection of positive clones. The gene-specific sgRNAs are expressed under the control of a bidirectional engineered U6 promoter, specifically optimized to recruit RNA polymerase III and drive transcription of sgRNAs from both the sense and antisense strands. The activity of both sgRNAs expressed from this engineered promoter have been shown to be roughly equivalent to one another. Moreover, the bidirectional promoter in the DyadGuide dual-sgRNA expression platform has been shown to outperform conventional dual sgRNA expression strategies (ex: sequential RNA polymerase III promoters driving expression of a single sgRNA) with respect to gene perturbation efficiency.

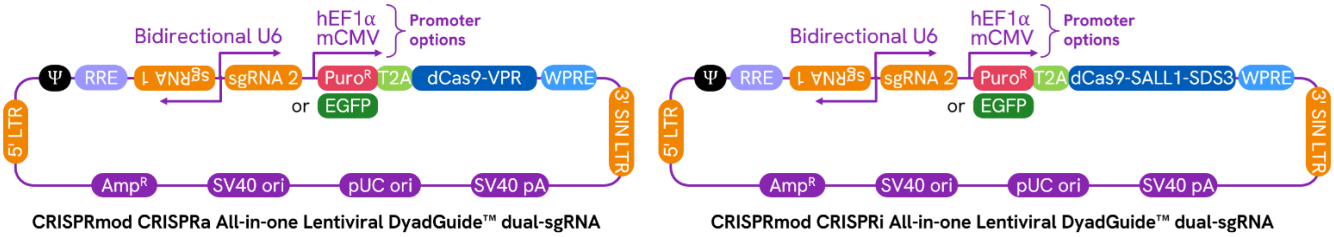

Figure 2. Schematic maps of the CRISPRa and CRISPRi All-in-one Lentiviral DyadGuide dual-sgRNA vectors.

B. CRISPRmod Lentiviral DyadGuide dual-sgRNA vectors

CRISPRmod Lentiviral DyadGuide dual-sgRNA vectors containing gene-specific CRISPRmod sgRNAs are recommended for applications where separate dCas9 delivery is preferred (dCas9-integrated cell line, temporal control, etc). Utilizing the same optimized guide design strategy as the All-in-One format, dual guide RNAs are expressed under the control of a bidirectional engineered U6 promoter for superior activity. Puromycin resistance marker (Puro^R) is driven by the mouse CMV promoter and allows for rapid selection of cells with integrated sgRNA (Figure 3).

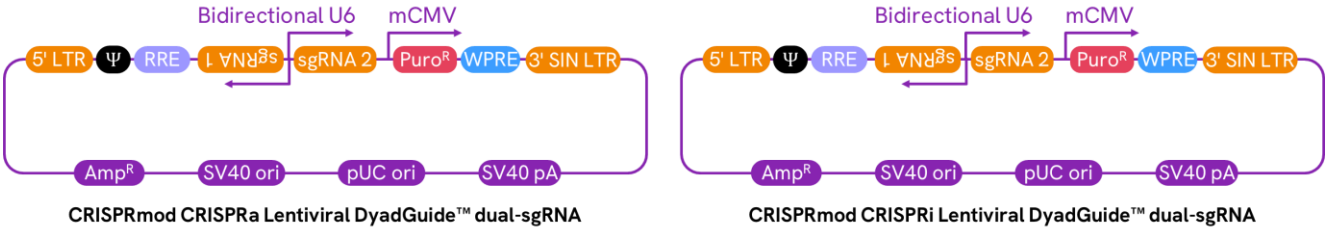

Figure 3. Schematic map of the CRISPRmod Lentiviral DyadGuide dual-sgRNA vector.

Table 1. Elements of the CRISPRmod Lentiviral DyadGuide dual-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
hEF1α	Human elongation factor 1 alpha short promoter
mCMV	Mouse cytomegalovirus immediate early promoter
T2A	Self-cleaving peptide allows for simultaneous expression of selectable marker and dCas9 proteins from a single transcript
WPRE	Rev Response Element enhances titer by increasing packaging efficiency of full-length lentiviral genomes
Bidirectional U6	Modified Human RNA polymerase III promoter U6, engineered to express two sgRNA transcripts
sgRNA	Optimized single guide RNA, a fusion of gene-specific crRNA with the tracrRNA scaffold
EGFP	Green fluorescent protein reporter enables selection of transduced mammalian cells by FACS Mouse cytomegalovirus immediate early promoter
Puro^R	Puromycin resistance marker permits antibiotic selection of transduced mammalian cells
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
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

C. Overview of gene modulation workflows with CRISPRmod Lentiviral DyadGuide dual-sgRNA systems

The CRISPRmod Lentiviral DyadGuide dual-sgRNA particles are available as All-in-one Lentiviral sgRNA vectors which include all critical components required for gene modulation in one product (dCas9 fusion protein and two gene-specific sgRNAs), or as particles containing the two sgRNAs alone. Figure 4 diagrams the workflows for each.

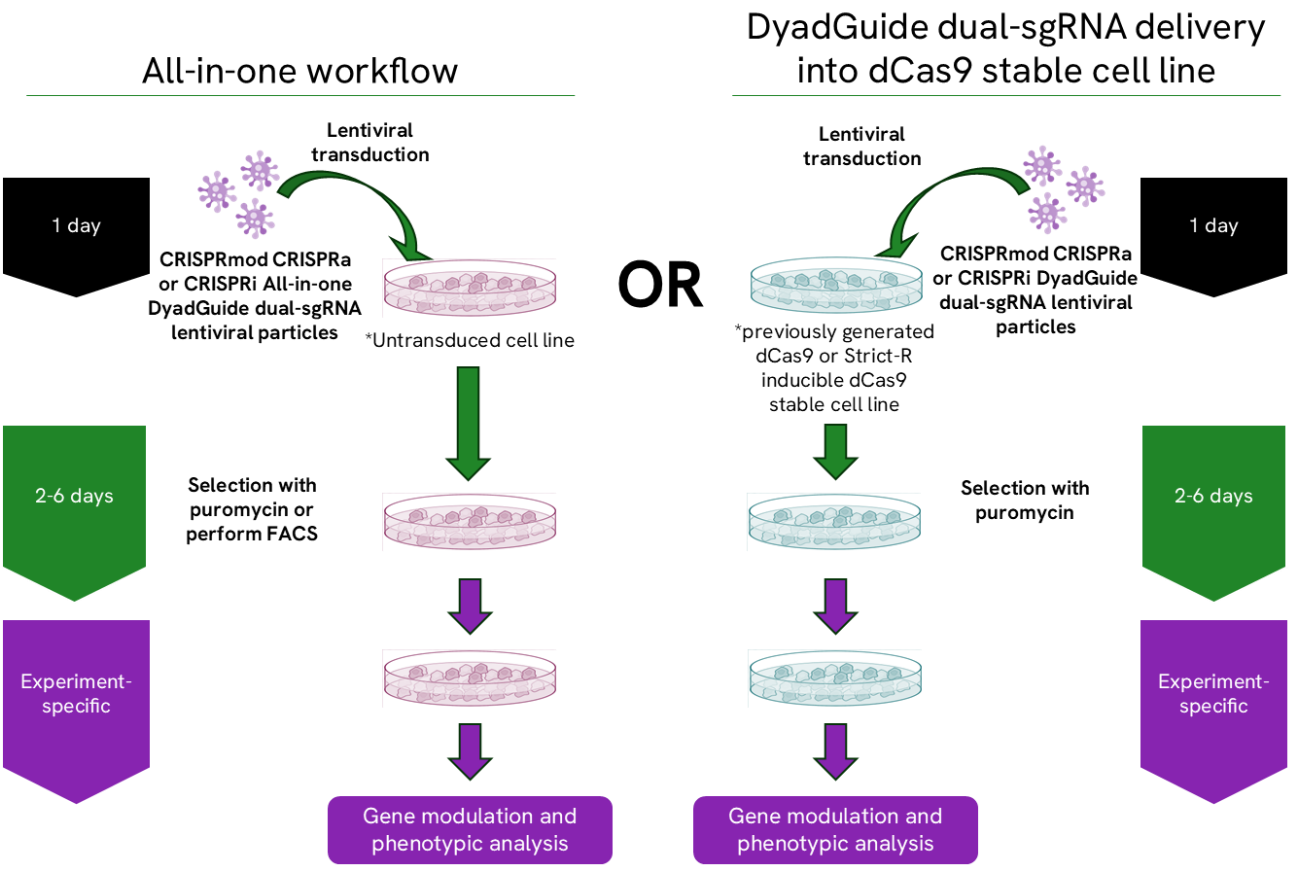

Figure 4. Gene modulation workflow using CRISPRmod CRISPRa or CRISPRi Lentiviral All-in-one DyadGuide dual-sgRNA particles (left) or CRISPRmod Lentiviral DyadGuide dual-sgRNA particles (right).

3 Required materials for gene modulation with the CRISPRmod DyadGuide dual-sgRNA system

A. Materials required

- Dharmacon™ CRISPRmod All-in-one Lentiviral DyadGuide™ dual-sgRNA particles
  ≥ 1 × 10^7 TU/mL, ± 20%, see CoA for specific titer
  - DyadGuide dual-sgRNA Puro^R hEF1α dCas9-VPR All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA Puro^R mCMV dCas9-VPR All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA EGFP hEF1α dCas9-VPR All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA EGFP mCMV dCas9-VPR All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA Puro^R hEF1α dCas9-SALL1-SDS3 All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA Puro^R mCMV dCas9-SALL1-SDS3 All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA EGFP hEF1α dCas9-SALL1-SDS3 All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA EGFP mCMV dCas9-SALL1-SDS3 All-in-one Lentiviral Particles

OR

- CRISPRmod Lentiviral DyadGuide dual-sgRNA particles
  ≥ 1 × 10^8 TU/mL, ± 20%, see CoA for specific titer
  - CRISPRmod CRISPRa Lentiviral DyadGuide dual-sgRNA particles
  - CRISPRmod CRISPRi Lentiviral DyadGuide dual-sgRNA particles
- Dharmacon CRISPRmod dCas9 expressing cells
  - CRISPRmod CRISPRa dCas9-VPR solutions
  - CRISPRmod CRISPRi dCas9-SALL1-SDS3 solutions

B. 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 or similar) for Puro^R constructs
- Choice of CRISPRmod All-in-one Lentiviral DyadGuide dual-sgRNA controls:
- Choice of CRISPRmod DyadGuide dual-sgRNA lentiviral controls:
- Transduction enhancer such as LentiBOOST® Cat# SB-P-LV-101-10, or Polybrene
- Live/Dead indicator such as Zombie Dyes (BioLegend®, https://www.biolegend.com/en-us/live-dead), Resazurin cell viability reagent or similar metabolic assay.
- Assay(s) for detecting gene modulation (RT-qPCR or equivalent, see Appendix A)

4 Assay optimization

A. Optimization of lentiviral transduction

While lentiviral particles exhibit broad cell tropism, the conditions for successful and efficient delivery can vary significantly. Parameters that may influence the efficiency of lentiviral transduction include, but are not limited to:

Transduction medium: When possible, the transduction of cells with lentiviral particles should be performed in a small volume of low-serum (0.5-2%) or serum-free medium. For cells sensitive to low serum conditions, transduction optimization can be performed in complete medium.

Transduction duration: Incubation time can vary between 4 and 24 hours and will depend on the cells of interest.

Transduction medium additives: Cationic polymers such as hexadimethrine bromide (Polybrene) or transduction enhancers such as LentiBOOST® may be added to enhance lentiviral particle binding to the cell surface. We recommend testing a range of concentrations for identification of optimal transduction efficiency with minimal or no cell toxicity.

Cell density at transduction: The density at which cells are seeded may also influence transduction efficiency. We recommend seeding cells at a range of densities for optimization of transduction efficiency.

CRISPRmod Lentiviral DyadGuide dual-sgRNA particles co-expressing Puro^R do not contain a fluorescent reporter. Therefore, CRISPRmod Lentiviral DyadGuide dual-sgRNA particles co-expressing EGFP may be substituted for rapid optimization of transduction conditions.

B. Selection optimization

Puromycin selection

CRISPRmod All-in-one Lentiviral DyadGuide dual-sgRNA Puro^R particles or CRISPRmod Lentiviral DyadGuide dual-sgRNA particles confer resistance to puromycin in transduced cells. Before transducing cells, generate a puromycin kill curve to determine the minimum concentration of puromycin required to kill non-transduced cells between three and ten days. The puromycin concentration range for many mammalian cells is 1-10 µg/mL.

5 Protocol for transduction of DyadGuide dual-sgRNA particles for CRISPR modulation

A. Transduction of CRISPRmod Lentiviral DyadGuide dual-sgRNA particles

The protocol below provides the basic steps for transduction of the lentiviral particles into U2OS cells (as an example) using serum-free medium in a 24-well plate format (see Appendix B for guidelines on other plate formats). Adjust proportionally the number of cells, volumes, and reagent quantities when using a different sized culture dish. Permissivity to lentiviral delivery and optimal transduction conditions vary widely amongst cell types and must be determined empirically for each cell line of interest. Associated protocols can be found in Appendix C.

Day 1:
1. Plate 5 × 10^4 cells per well in a 24-well plate using standard culture medium (specifics may vary for the cell type of interest, and separate establishment of dCas9 expression will be required prior to this step for sgRNA-only vector format. See Appendix C for associated protocols).
2. Incubate cells at 37 °C in a humidified CO_2 incubator overnight.

Day 2:
1. Thaw the CRISPRmod Lentiviral DyadGuide dual-sgRNA or CRISPRmod All-in-one Lentiviral DyadGuide dual-sgRNA particles on ice.
2. Once thawed, to prepare the transduction medium, gently mix the appropriate volume of titered CRISPRmod Lentiviral DyadGuide Dual-sgRNA particles (to generate an MOI 0.3, see appendix D) by pipette into the calculated volume of base cell culture medium (without serum) such that the total volume is 0.25 mL per well (see Appendix B for guidelines on other plate formats).
3. Remove the cell culture maintenance medium from the well and add 0.25 mL of the transduction medium containing the lentiviral particles prepared in step 2.
4. Incubate cells at 37 °C in a humidified CO_2 incubator for 4-6 hours.
5. At 4-6 hours post-transduction, add an additional 0.75 mL of culture medium per well and resume incubation at 37 °C in a humidified CO_2 incubator. If toxicity occurs with your cells, replace the medium after 4-6 hours with fresh culture medium.

Days 3-7:
1. At 24-48 hours post-transduction, replace the culture medium with selection medium containing the appropriate concentration of puromycin.
2. Replace the selection medium every 2-3 days and monitor the accumulation of dead cells daily.
3. Once the cells are growing normally in selection medium, expand the cells to freeze enough aliquots for your experimental project and/or proceed with detection of transcriptional modulation and/or phenotypic characterization.

Record the passage number and avoid working with stable cell populations from frozen stock with passage numbers higher than 10.

6 Appendix

A. 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.

B. Volume of transduction medium per surface area in culture dishes

Table 2. Suggested volumes of transduction medium per surface area per well of adherent cells.

Cell culture dish	Surface area per well (cm^2)	Suggested total 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

C. Associated protocols

CRISPRmod CRISPRi transcriptional repression system with expressed sgRNA technical manual

Dharmacon™ CRISPRmod CRISPRa transcriptional activation system with expressed sgRNA technical manual

Edit-R™ Cas9 and CRISPRmod CRISPRa dCas9-VPR stable cell lines technical manual

Transcriptional repression with the Dharmacon™ Strict-R™ Inducible CRISPRi Lentiviral System technical manual

Gene activation with the Dharmacon™ Strict-R™ Inducible CRISPRa Lentiviral System technical manual

D. Multiplicity of Infection (MOI)

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

E. Stability and storage

CRISPRmod Lentiviral DyadGuide dual-sgRNA particles are shipped on dry ice and must be stored at -80 °C. Under these conditions, lentiviral particles are stable for at least 12 months from receipt. 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.

F. Glycerol stock guidelines

CRISPRmod Lentiviral DyadGuide dual-sgRNA vectors are optimized for use as lentiviral particles, but bacterial glycerol stocks may be purchased for self-creation of lentiviral particles.

Culture conditions for individual plasmid preparations

For plasmid preparation, grow all DyadGuide dual-sgRNA Lentiviral vector glycerol stocks at 37 °C in LB broth medium plus 100 µg/mL carbenicillin.

Day 1:

1. Upon receiving your glycerol stock(s) containing the sgRNA(s), store at -80 °C until ready to begin.
2. To prepare plasmid DNA, first thaw your glycerol stock culture on ice and pulse vortex to resuspend any *E. coli* that may have settled to the bottom of the tube. Alternatively, keep glycerol stock culture on ice, propagate by scraping with sterile pipette to minimize the number of freeze/thaw cycles.
3. Take a 10 µL inoculum from the glycerol stock into 3-5 mL of LB broth medium with 100 µg/mL carbenicillin. Return the glycerol stock(s) to -80 °C.

If a large culture volume is desired, incubate the 3-5 mL culture for 8 hours at 37 °C with shaking and use as a starter inoculum. Dilute the starter culture 1:500-1:1000 into the larger volume

4. Incubate at 37 °C for 18-19 hours with vigorous shaking.

Day 2:

1. Pellet the culture and begin preparation of plasmid DNA. Plasmid DNA can be isolated using a plasmid miniprep kit of your choice.

Packaging lentiviral particles

DyadGuide Lentiviral dual-sgRNA constructs are Tat-dependent, so a packaging system expressing the *tat* gene is required. We recommend the Trans-Lentiviral shRNA packaging kit (Cat #TLP5912 or TLP5917). The Trans-Lentiviral packaging system allows creation of replication-incompetent, HIV-1-based lentiviral particles that can be used to deliver and express your sgRNAs of interest in either dividing or non-dividing mammalian cells, and it is based on the trans-lentiviral system developed by Kappes (Kappes and Wu 2001). For protocols and information on packaging Edit-R Lentiviral sgRNA constructs with the Trans-Lentiviral packaging system, please see the product manual available on our website.

7 Frequently asked questions

How do I choose between the two CRISPRmod All-in-one Lentiviral DyadGuide dual-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 DyadGuide dual-sgRNA Positive Controls. For more information, please consult our promoter selection guide.

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 only been tested in mammalian cells. Additionally, the CRISPRa guide RNAs are predesigned to activate mouse or human genes while CRISPRi guide RNAs are predesigned to inhibit 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. 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 Lentiviral DyadGuide dual-sgRNA or CRISPRmod All-in-one Lentiviral DyadGuide dual-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 documentation shipped with purchase, and can be found online under your account Order History at horizondiscovery.com.

What are the maximum excitation and emission wavelengths and laser/filter requirements for detecting and sorting EGFP?

If fluorescence-activated cell sorting (FACS) is to be performed, please ensure that the lasers and filters are suitable for the EGFP marker, an example laser and filter that would work for this are provided.

Fluorescent reporter	Excitation wavelength	Emission wavelength	Cytometer laser	Bandpass filter
EGFP	488 nm	507 nm	488 nm blue	e.g. 510/20 nm or 530/30 nm

How do I know if the dCas9 fusion protein is expressed after introducing the CRISPRmod All-in-one Lentiviral DyadGuide dual-sgRNA vectors?

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. Additionally, we recommend performing a functional assay, or RT-qPCR, to determine the active presence of the CRISPRmod dCas9 fusion protein.

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

The CRISPRmod All-in-one Lentiviral DyadGuide dual-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 DyadGuide dual-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.

Are Dharmacon lentiviral particle products safe to use in the laboratory? What precautions should be taken when handling lentiviral particles?

Lentiviral delivery systems have been employed in many research laboratories worldwide without incident. Handling lentiviral products requires extensive experience with cell culture techniques. It is vital that the protocols provided by Revvity and the safety guidelines described for appropriate handling and storage are fully understood and followed precisely (see Section 9, lentiviral particle product safety level information).

8 References

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9 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 to 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 Scientific Support team.

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Tel: +1 800 235 9880; 303 604 9499 (United States)
Fax: +1 800 292 6088; 303 604 9680 (United States)
Email: technical.horizon@revvity.com (North America and Europe)

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 (https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf);
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) (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf)

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 above-mentioned 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).

10 Limited use licenses

The 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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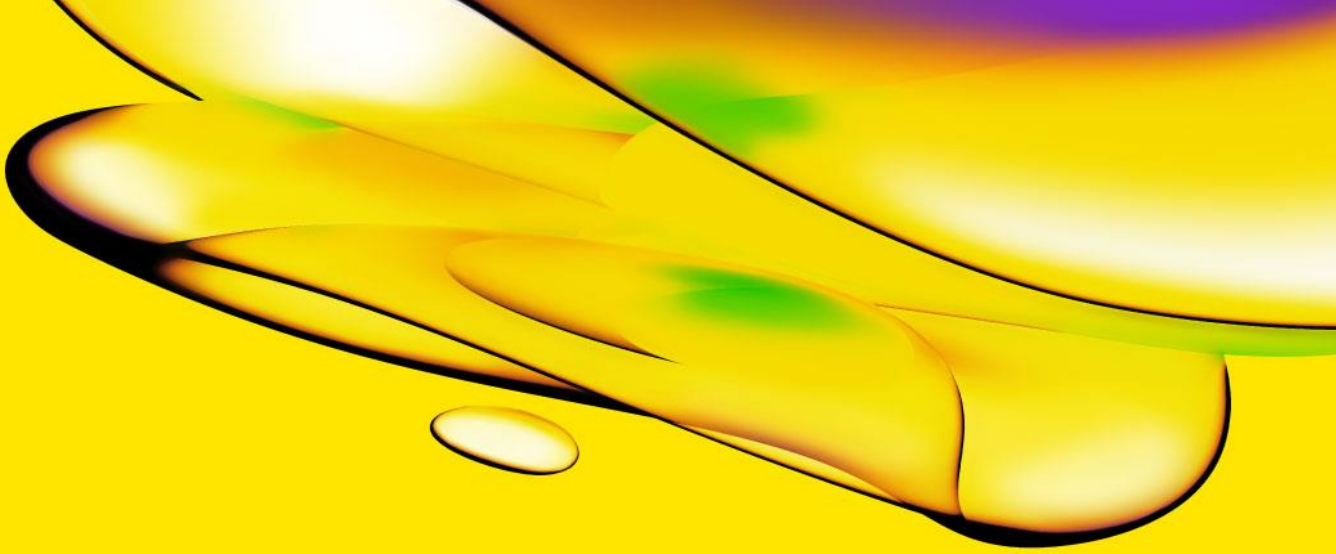

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