Technical Manual

CRISPR editing

Dharmacon™ Edit-R™ DyadGuide™ dual-sgRNA expression systems

Storage: -80°C

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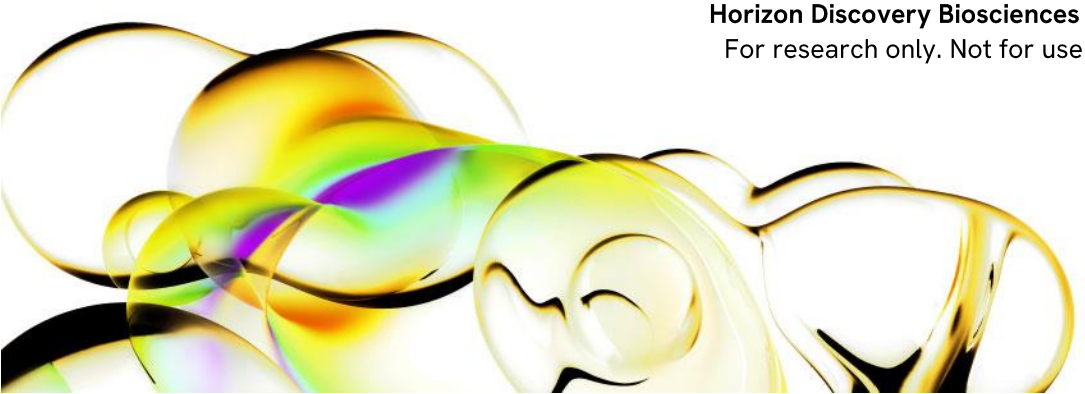
Horizon Discovery Biosciences Ltd. (A Revvity Company)



Contents

1 Introduction: Engineering a CRISPR-Cas9 platform for mammalian genome editing

The CRISPR (clustered regularly interspaced short palindromic repeats) -Cas (CRISPR-associated protein) system is an adaptive bacterial and archaeal defense mechanism that has been adopted as a scientific tool for genome engineering applications in mammalian cells[1]. In particular, using the *Streptococcus pyogenes* CRISPR-Cas9 system, only three components are required for targeted DNA cleavage at specific target sites adjacent to a protospacer adjacent motif (PAM)[2]: (1) The endonuclease Cas9, programmed by (2) a mature crRNA processed from transcription of the CRISPR locus/array which complexes with (3) another CRISPR locus-encoded RNA, the trans-activating CRISPR RNA (tracrRNA, Figure 1A)[3]. Alternatively, the crRNA can be fused to the tracrRNA creating a chimeric structure named single guide RNA (sgRNA, Figure 1B)[2].

Upon site-specific double-stranded DNA cleavage, a mammalian cell can repair such a breakthrough either non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ is often imperfect, resulting in small insertions and deletions (indels) that can cause nonsense mutations resulting in gene disruptions to produce gene knockouts[4,5]. This endogenous DNA break repair process, coupled with the highly tractable *S. pyogenes* CRISPR-Cas9 system, allows for a readily engineered platform to permanently disrupt gene function.

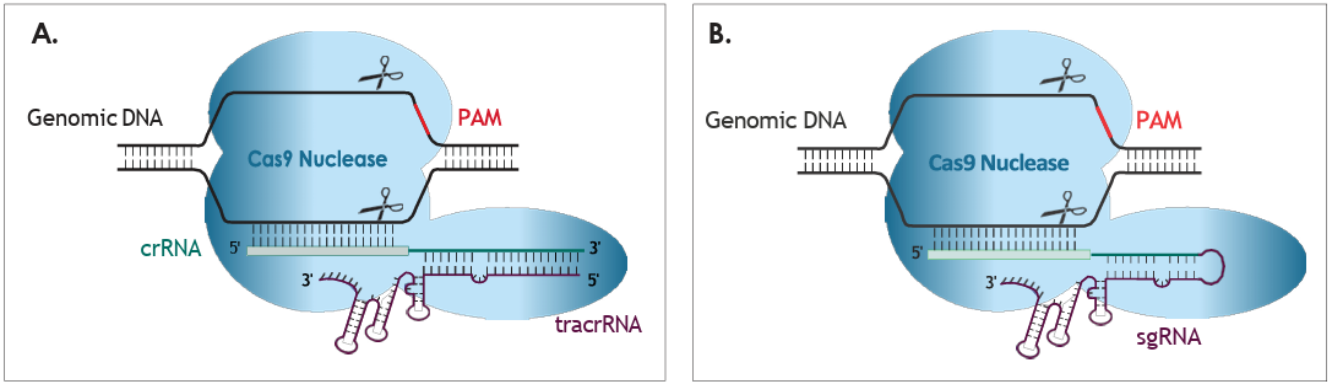


Figure 1. Illustration of CRISPR-Cas9 system. Cas9 nuclease (light blue), programmed by the crRNA (green): tracrRNA (blue) complex (A) or the sgRNA (B), cutting both strands of genomic DNA 5′ of the PAM (red).

2 Genome editing using the Edit-R Lentiviral DyadGuide dual-sgRNA vectors

Each sgRNA in the Edit-R Lentiviral DyadGuide dualsgRNA vector is specific to the genomic site of interest. The target-specific region of the sgRNA is comprised of 19-20 nucleotides identical to the genomic DNA (gDNA) target site, or protospacer, followed by the non-variable sgRNA scaffold. All Edit-R DyadGuide dual-sgRNA expression vectors are supplied as lentiviral particles or glycerol stocks (See Appendix F). Predesigned, gene-specific Edit-R Lentiviral DyadGuide dual-sgRNA particles can be ordered via a webtool through which the CRISPR modality (Edit-R, CRISPRa, CRISPRi), promoter, selection marker, guide RNA 1, and guide RNA 2 can be chosen.

A. Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA vectors

The Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA vector includes all critical components for gene editing in one convenient construct (Figure 2). In addition to the two gene-specific sgRNAs, the vector contains a human codon-optimized version of the *S. pyogenes* Cas9 and either the puromycin resistance marker (Puro^R) or Enhanced Green Fluorescent Protein (EGFP). 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.

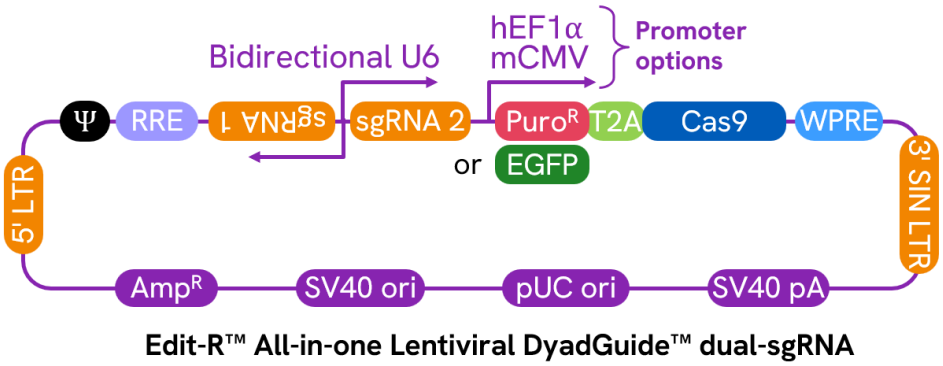


Figure 2. Schematic map of the Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA vector.

B. Edit-R Lentiviral DyadGuide dual-sgRNA vectors

Edit-R Lentiviral DyadGuide dual-sgRNA vectors containing gene-specific Edit-R sgRNAs are recommended for applications where separate Cas9 delivery is preferred (Cas9-integrated cell line, temporal control, etc). Utilizing the same optimized guide design strategy as the All-in-one format, dual guides RNAs are expressed under control of the 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).

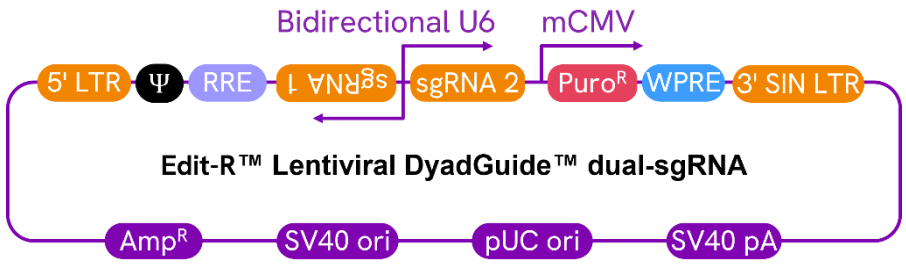


Figure 3. Schematic map of the Edit-R Lentiviral DyadGuide dual-sgRNA vector.

Table 1. Elements of the Edit-R Lentiviral DyadGuide dual-sgRNA vectors.

Vector element	Utility
Cas9	*S. pyogenes* Cas9 for cleavage of targeted DNA when programmed with a sgRNA
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 Cas9 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 pol III transcripts
sgRNA	Optimized single guide RNA, a fusion of gene-specific crRNA with the tracrRNA scaffold
EGFP	Enhanced Green Fluorescent Protein permits identification and enrichment of transduced cells
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 editing workflows with Edit-R Lentiviral DyadGuide dual-sgRNA systems

The Edit-R Lentiviral DyadGuide dual-sgRNA particles are available as All-in-one Lentiviral sgRNA vectors which include all critical components required for gene editing in one product (Cas9 nuclease and two gene-specific sgRNAs), or as particles containing the two sgRNAs alone. Figure 4 diagrams the workflows for each.

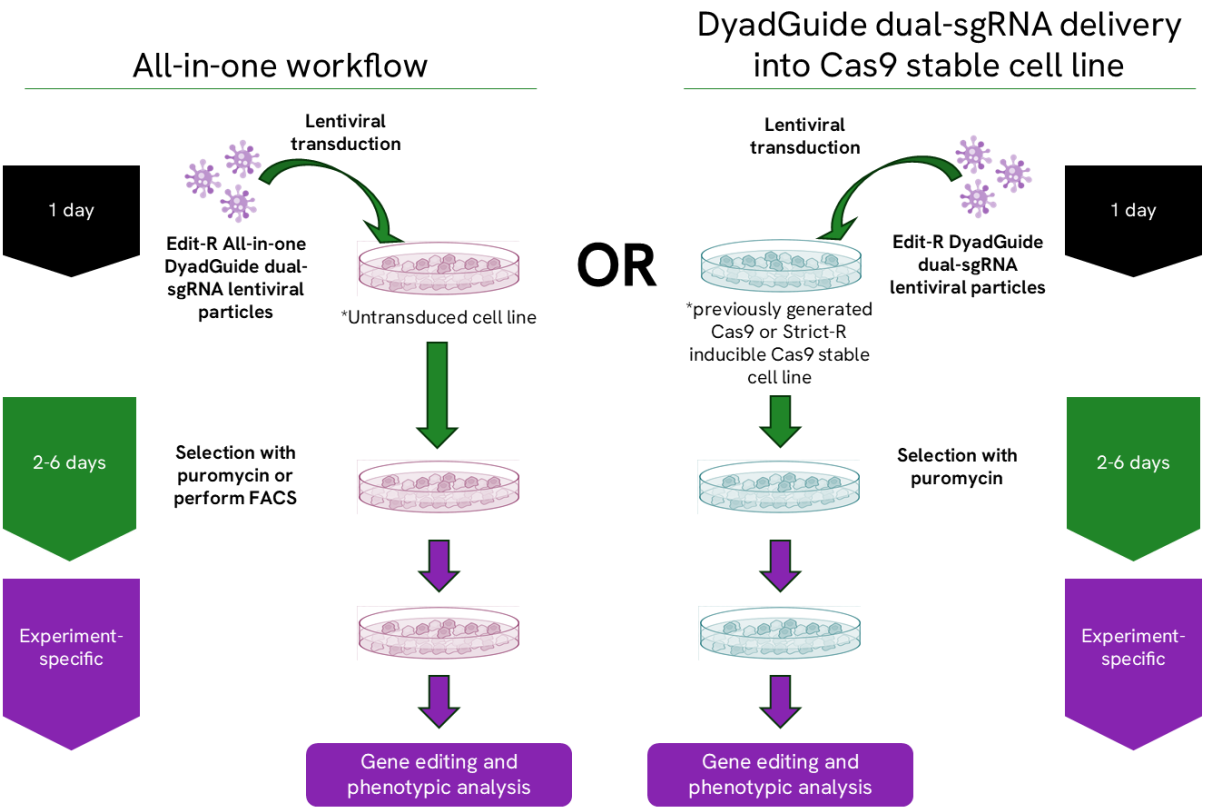


Figure 4. Gene editing workflow using Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA particles (left) or Edit-R Lentiviral DyadGuide dual-sgRNA particles (right).

3 Required materials for gene editing with the Edit-R DyadGuide dual-sgRNA system

A. Materials required

- Dharmacon™ Edit-R™ 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 Cas9 All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA Puro^R mCMV Cas9 All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA EGFP hEF1α Cas9 All-in-one Lentiviral Particles
  - DyadGuide dual-sgRNA EGFP mCMV Cas9 All-in-one Lentiviral Particles

OR

- Edit-R Lentiviral DyadGuide dual-sgRNA particles
  ≥ 1 × 10^8 TU/mL, ± 20%, see CoA for specific titer
- Dharmacon Edit-R Cas9 expressing cells
  - Edit-R Cas9 nuclease 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 Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA controls:
- Choice of Edit-R 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 editing events in a cell population

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.

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

B. Selection optimization

Puromycin selection

Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA Puro^R particles or Edit-R 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 gene editing

A. Transduction of Edit-R 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 Edit-R Lentiviral DyadGuide dual-sgRNA or Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA particles on ice.
2. Once thawed, to prepare the transduction medium, gently mixing the appropriate volume of titered Edit-R 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 gene editing/knockout detection 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 editing assay recommendations

The most commonly used method for detection of insertions and deletions (indels) in a cell population requires PCR-amplification of the genomic region surrounding the target edit site. The PCR amplicon using predesigned primers can be subjected to a mismatch detection assay such as T7 Endonuclease I (T7EI or TIDE) or next generation sequencing (NGS) to verify the efficiency of gene editing. When edited cells are expanded and clonal populations are obtained, the most commonly used method for confirming gene editing is Sanger sequencing.[6,7] After preliminary optimization and editing at the sequence level has been confirmed, expanded or clonal cell populations may also be assessed by phenotypic assays, such as western blot or flow cytometry, for target gene knockout.

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

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

Transcriptional repression with the Dharmacon™ Strict-R™ Inducible Edit-R 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

Edit-R 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

Edit-R 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 Edit-R 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 Edit-R All-in-one Lentiviral sgRNA Positive Controls. For more information, please consult our promoter selection guide.

What is the best way to confirm that my gene is knocked out?

Mismatch detection assays tell you that editing occurred in the cell population. Clonal cell isolation followed by DNA sequencing of the region of interest and determination of the protein functionality are necessary to confirm the gene knockout.

Can I order the Edit-R DyadGuide dual-sgRNA or Edit_R All-in-one DyadGuide dual-sgRNA as plasmid DNA?

No. Our Edit-R Lentiviral DyadGuide dual-sgRNA vectors are made to each specific customer order and are sold exclusively as lentiviral particles or glycerol stocks.

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.

Can I use any 2nd generation packaging system with the Edit-R All-in-One Lentiviral DyadGuide dual-sgRNA or Edit-R Lentiviral DyadGuide dual-sgRNA vectors?

The Edit-R All-in-One Lentiviral DyadGuide dual-sgRNA and Edit-R Lentiviral DyadGuide dual-sgRNA vectors are Tat dependent, so a packaging system that expresses the tat gene is required.

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

Can the Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA system be used for gene knockout in non-mammalian organisms, such as flies or worms?

Our predesigned Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA vectors are designed and intended for mammalian expression and thus have only been tested in mammalian cells.

How do I know if Cas9 is expressed after introducing the Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA vectors?

We recommend performing a functional assay, such as a T7EI mismatch assay, to determine the presence of Cas9. For initial optimization, we recommend using one of our positive control kits (including detection primers) and performing a mismatch assay to assess the editing efficiency.

Additionally, as the Cas9 nuclease is under the same promoter as EGFP/puromycin resistance marker, expression of EGFP or puromycin resistance would indicate the simultaneous expression of Cas9 nuclease. We routinely select and enrich for Cas9 expressing cells using antibiotic resistance or EGFP expression, respectively.

How do I choose between Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA containing Puromycin and EGFP?

The Edit-R All-in-one Lentiviral DyadGuide dual-sgRNA containing Puro^R 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. Edit-R 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.

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).

How should lentiviral particle products be stored?

All lentiviral particle products must be stored at -80 °C. If necessary, the particles can be aliquoted upon the first thaw to convenient volumes and the aliquots stored at -80 °C to minimize the number of future freeze-thaws. However, we recommend avoiding multiple freeze-thaw cycles as much as possible.

How are lentiviral particle products shipped?

Lentiviral particle products are shipped on dry ice for overnight domestic delivery or priority international for delivery outside of the U.S.

8 References

1. Bhaya, D., 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. Jinek, M., et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science.* **337**, 816-821 (2012).
3. Deltcheva, E., et al. CRISPR RNA maturation by trans-encoded small RNA and host factor Nuclease III. Nature 471, 602-607 (2011).
4. Mali, P., et al. RNA-guided human genome engineering via Cas9. Science 339, 823-826 (2013).
5. T.R. Sampson, T.R., and Weiss, D.S. Exploiting CRISPR/Cas systems for biotechnology. Bioessays 36, 34-38 (2014).

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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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.

To find the contact information in your country for your technology of interest, please visit us at horizondiscovery.com/contact-us
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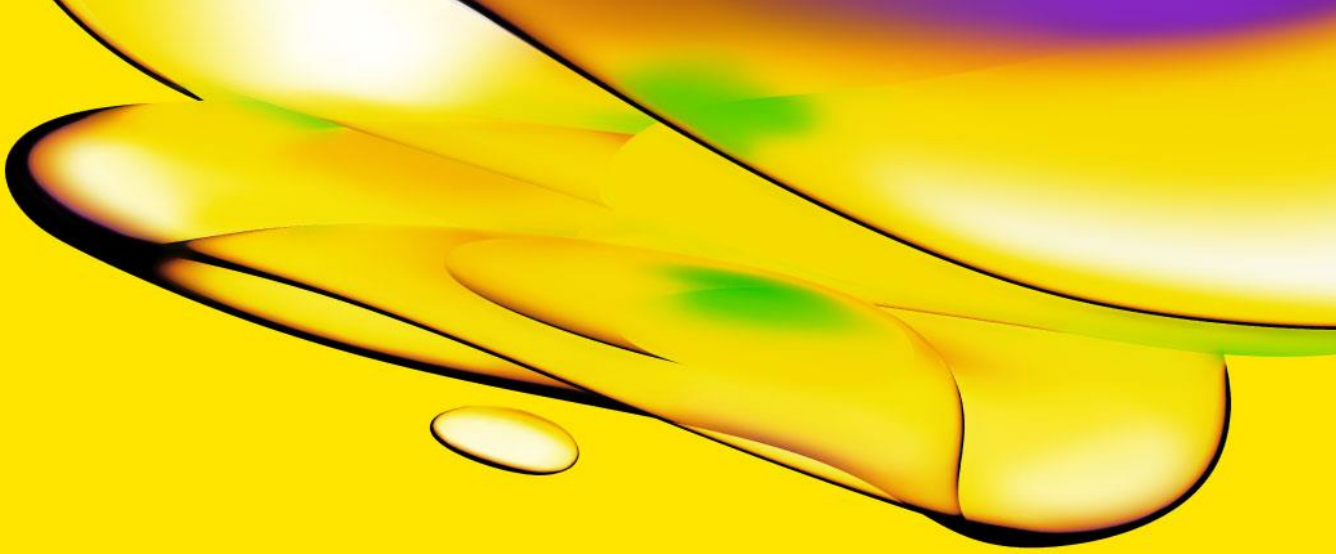

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