TECHNICAL MANUAL

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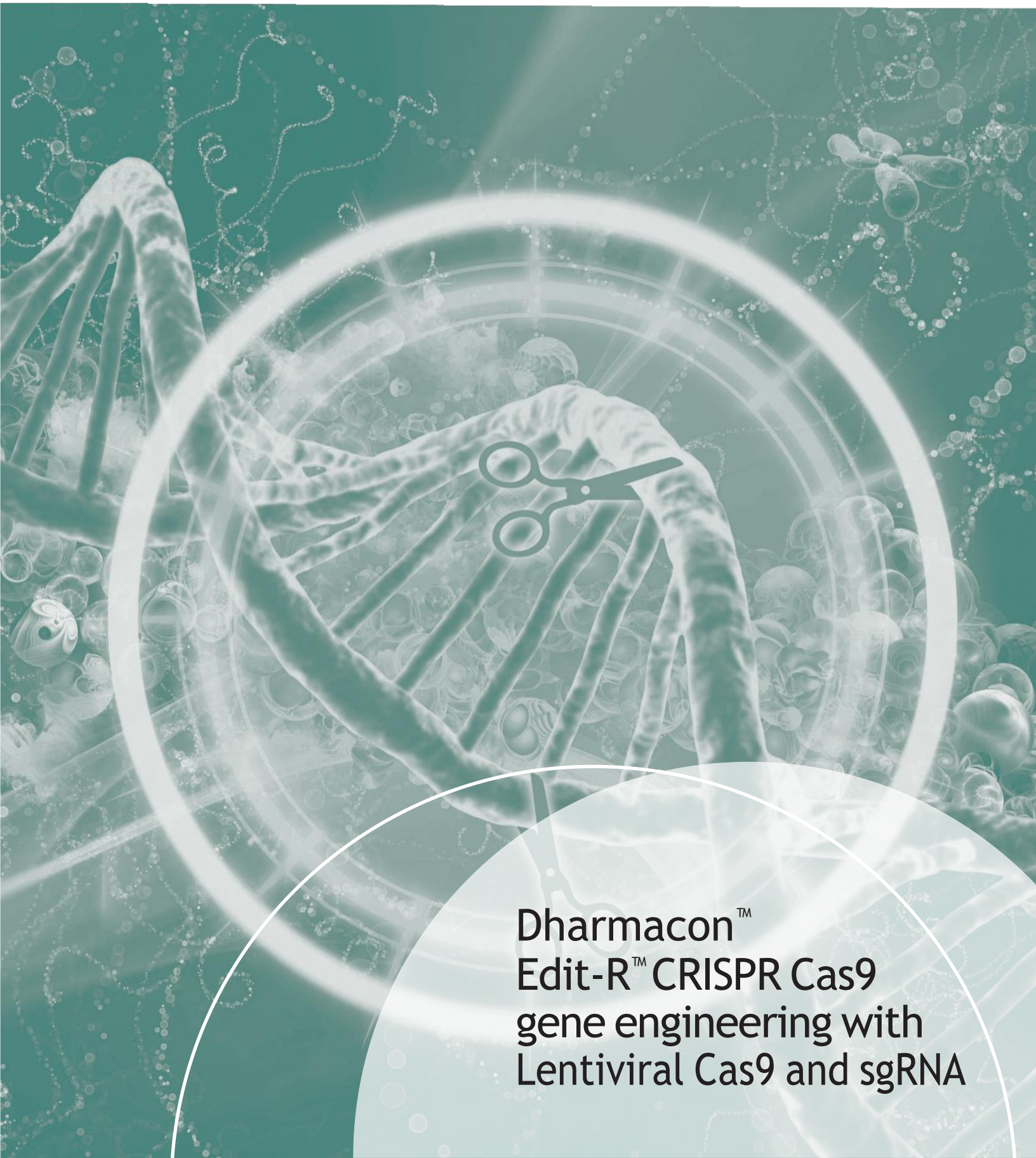

Dharmacon™ Edit-R™ CRISPR Cas9 gene engineering with Lentiviral Cas9 and sgRNA

Contents

1 Introduction to the CRISPR-Cas9 system for gene engineering

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[1].

Engineering a CRISPR-Cas9 platform for mammalian genome editing

Many bacterial and archaeal CRISPR-Cas systems have been identified with diverse sets of mechanisms, Cas proteins and multi-subunit complexes. In particular, the processes and key components of the *Streptococcus pyogenes* CRISPR-Cas9 system have been well studied and adapted for genome engineering in mammalian cells. In *S. pyogenes*, only three components are required for targeted DNA cleavage at specific target sites adjacent to a 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 termed a single guide RNA **(sgRNA, Figure 1B)**[2].

Upon site-specific double-stranded DNA cleavage, a mammalian cell can repair such a break through either non-homologous end joining (NHEJ) or homology directed repair (HDR). 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.

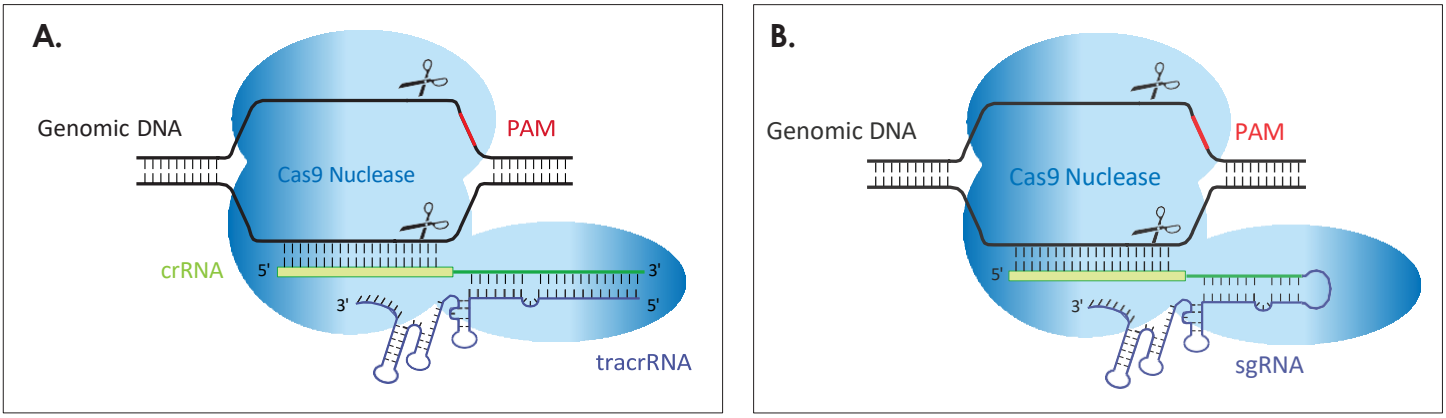

Figure 1. Illustration of CRISPR-Cas9 system. Cas9 nuclease (light blue), programmed by the crRNA (green):tracrRNA (blue) complex (A) or the sgRNA (B), cutting both strands of genomic DNA 5' of the PAM (red).

2 Gene editing using Edit-R Lentiviral Cas9 Nuclease with sgRNA particles

The Edit-R Lentiviral Gene Engineering platform includes two critical components based on the natural *S. pyogenes* system: a vector for Cas9 expression and a gene-specific vector for sgRNA expression designed to the target site of interest. To facilitate rapid generation of cells lines that constitutively express Cas9 nuclease and the sgRNA, the Cas9 and sgRNA vectors are packaged into lentiviral particles, purified and concentrated. Cas9-expressing cells lines can be easily generated with the Edit-R Lentiviral Cas9 Nuclease Expression particles and subsequent gene knockouts can be obtained by an additional transduction with sgRNA lentiviral particles or, alternatively, by transfection with synthetic guide RNA if the cells are amenable to transfection (see Edit-R™ CRISPR-Cas9 Gene Engineering with Lentiviral Cas9 and Synthetic guide RNA for more information and protocols). **Figure 2** summarizes a general experimental workflow to generate stable cell lines expressing Cas9 nuclease followed by transduction with sgRNA lentiviral particles for phenotypic analysis of gene knockout.

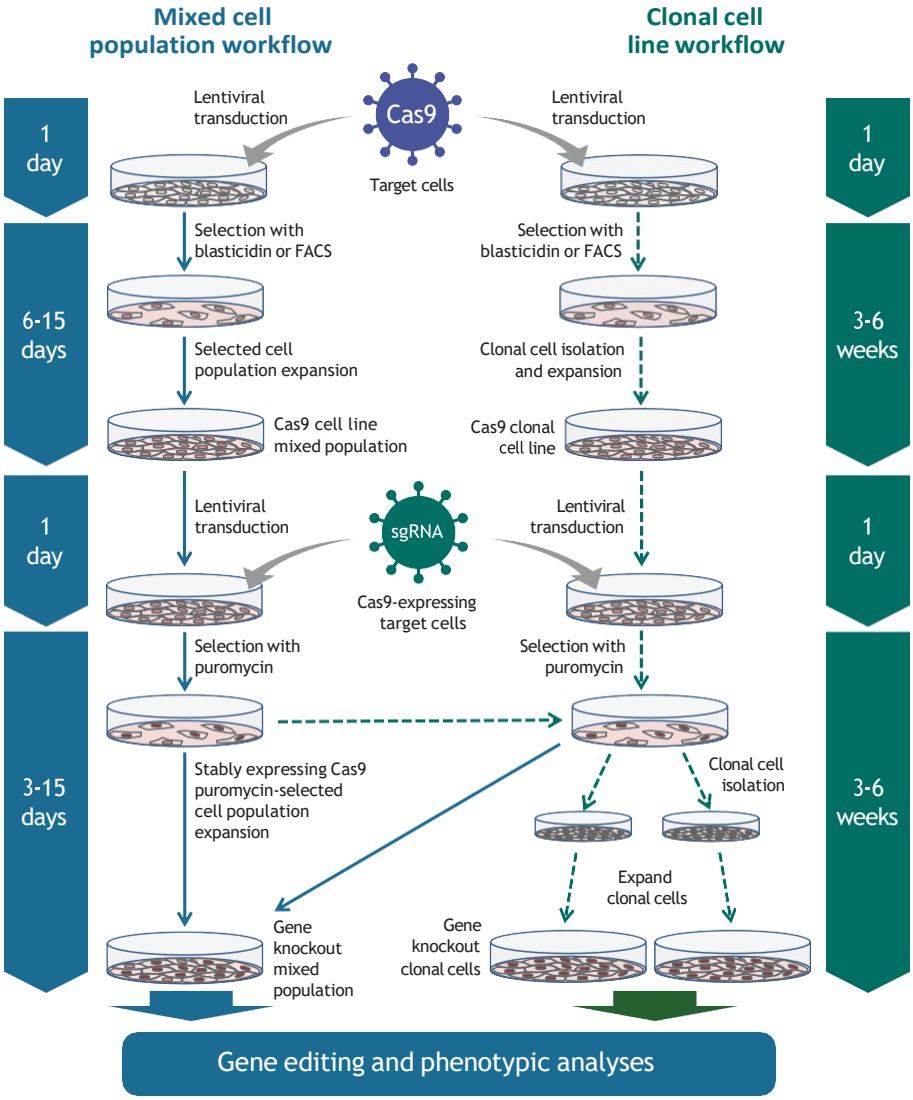

Figure 2. Gene knockout workflow using the Edit-R Lentiviral Cas9 Nuclease with sgRNA system. Gene editing with Edit-R Lentiviral Cas9 Nuclease and sgRNA can be done following a mixed cell populations approach (left side) typically for gene knockout screenings or on isolated clonal cells lines (right side) when a defined genotype is desired or required on each step for the phenotypic analyses.

Edit-R Lentiviral Cas9 Nuclease and sgRNA Expression vectors

The Edit-R Lentiviral Cas9 Nuclease Expression vectors contain a human codon-optimized version of the *S. pyogenes cas9* (*csn1*) gene and the blasticidin resistance marker (Blast^R) or fluorescent reporter (mKate2 or TurboGFP). In both vectors, expression is bicistronic with a 2A peptide sequence linker and under the control of a single promoter **(Figure 3)**. Reporter genes Blast^R are placed upstream of the Cas9 coding region rather than on the 3' end so that no extra amino acids are added to the C-terminus, thus preserving the full Cas9 endonuclease activity. A brief description of the lentiviral vector elements are listed in Table 1. Multiple promoter options are available **(Figure 3A and 3B)** for selection of a Cas9 nuclease vector with the most active promoter for specific cells of interest.

In the Edit-R Lentiviral sgRNA vector backbone **(Figure 3C)**, the gene-specific sgRNA is expressed under the control of a human U6 promoter, while expression of the puromycin resistance marker (Puro^R) is driven from the mouse CMV promoter and allows for rapid selection of cells with integrated sgRNA.

All Edit-R Lentiviral Cas9 Nuclease and sgRNA vectors are supplied as concentrated, purified lentiviral particles (≥ 1 × 10^8 TU/mL).

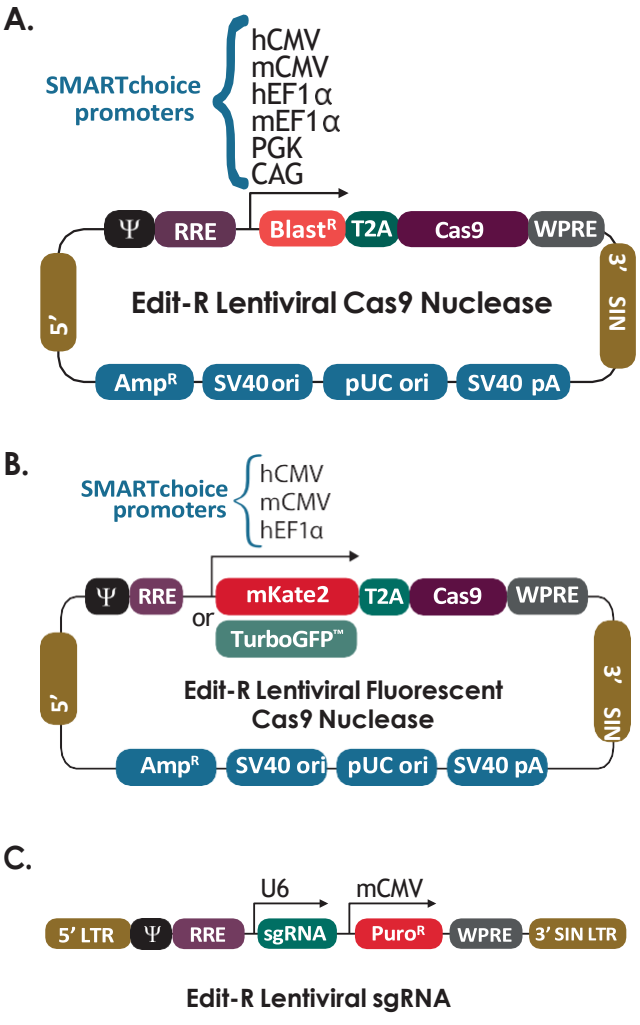

Figure 3. Schematic map of the Edit-R Lentiviral Cas9 Nuclease Expression (A) Edit-R Lentiviral Fluorescent Cas9 Nuclease (B) and sgRNA (C) vectors.

Table 1. Elements of the Edit-R Lentiviral Cas9 Nuclease Expression and sgRNA vectors.

Vector element	Utility
Cas9	Human codon-optimized *S. pyogenes* Cas9 nuclease for cleavage of targeted DNA when programmed with a sgRNA
T2A	Self-cleaving peptide allows for simultaneous expression of blasticidin resistance and Cas9 protein from a single transcript
Blast^R	Blasticidin resistance marker permits antibiotic selection of transduced mammalian cells
mKate2	Red fluorescent protein reporter enables selection of transduced mammalian cells by FACS
TurboGFP	Green fluorescent protein reporter enables selection of transduced mammalian cells by FACS
hCMV	Human cytomegalovirus immediate early promoter
mCMV	Mouse cytomegalovirus immediate early promoter
hEF1α	Human elongation factor 1 alpha promoter
mEF1α	Mouse elongation factor 1 alpha promoter
PGK	Mouse phosphoglycerate kinase promoter
CAG	Human cytomegalovirus, chicken β-actin hybrid promoter
U6	Human RNA polymerase III promoter U6
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
WPRE	Woodchuck Hepatitis Post-transcriptional Regulatory Element enhances transgene expression in target cells
3' SIN LTR	3' Self-inactivating Long Terminal Repeat for generation of replication-incompetent lentiviral particles
SV40 pA	Simian virus 40 polyadenylation signal
pUC ori	pUC origin of replication
SV40 ori	Simian virus 40 origin of replication
Amp^R	Ampicillin resistance gene for vector propagation in *E. coli* cultures

Each Edit-R Lentiviral sgRNA is specific to the gene or genomic site of choice. The crRNA region of the sgRNA is comprised of 19-20 nucleotides identical to the genomic DNA target site, or protospacer, followed by the non-variable sgRNA scaffold containing the tracrRNA sequence from *S. pyogenes*. The chosen genomic DNA target sequence must be immediately upstream of a PAM. The predominant *S. pyogenes* PAM nucleotide sequence is NGG. Pre-designed gene-specific sgRNAs can be ordered by searching for genes of interest on horizondiscovery.com, or custom-designed using the Dharmacon CRISPR Design Tool.

3 Protocol for gene engineering using Edit-R Lentiviral Cas9 Nuclease and sgRNAs

The Edit-R Lentiviral Gene Engineering system utilizes Cas9 nuclease and the sgRNA in a two-step process. First, Edit-R Lentiviral Cas9 Nuclease Expression particles are utilized to generate cell lines stably expressing Cas9 nuclease. These cells can subsequently be transduced with Edit-R Lentiviral sgRNA particles to achieve efficient gene editing for phenotypic analyses in a population of cells or in isolated clonal cell lines.

Edit-R CRISPR-Cas9 gene engineering materials required

Edit-R Lentiviral Cas9 Nuclease Expression vectors are provided as concentrated, purified lentiviral particles for immediate transduction or as endotoxin-free plasmid DNA for direct transfection into a packaging cell line and production of your own lentiviral particles. Edit-R Lentiviral sgRNA particles are produced upon ordering and provided as concentrated, purified lentiviral particles for direct transduction.

- Edit-R™ Lentiviral Cas9 Nuclease (choose one):

Table 2. Edit-R Lentiviral Cas9 Nuclease particles with SMARTchoice promoter options and selection options

Promoter	Selection	Particles Cat. No.
hCMV	Blast^R	VCAS10124
	mKate2	VCAS11869
	TurboGFP™	VCAS11868
mCMV	Blast^R	VCAS10125
	mKate2	VCAS11863
	TurboGFP™	VCAS11862
hEF1 α	Blast^R	VCAS10126
	mKate2	VCAS11865
	TurboGFP™	VCAS11864
mEF1α	Blast^R	VCAS10127
PGK	Blast^R	VCAS10128
CAG	Blast^R	VCAS10129

- Edit-R™ Lentiviral sgRNA particles:
  - Custom sgRNA designed using the Dharmacon CRISPR Design Tool or
  - Pre-designed Edit-R sgRNA

Additional materials required

The following additional materials are required but not supplied:

- Multi-well tissue culture plates or tissue culture dishes
- Blasticidin S (Fisher Scientific, Cat #BP2647-25; InvivoGen, Cat #ant-bl-1) or a flow cytometer
- Puromycin (Fisher Scientific, Cat #BP2956-100; InvivoGen, Cat #ant-pr-1)
- Resazurin cell viability reagent or similar
- Assay(s) for detecting gene engineering events in a cell population
- Positive control sgRNA lentiviral particles
- Edit-R Lentiviral sgRNA Non-targeting Controls
- **Growth Medium**: antibiotic-free cell culture medium (with serum and/or supplements) recommended for maintenance of the cells of interest.
- **Transduction Medium**: the base cell culture medium containing lentiviral particles (with transduction additives and serum, if necessary).
- **Selection Medium**: Growth Medium supplemented with the appropriate concentration of blasticidin and/or puromycin.

Generation of a stable cell line expressing Cas9 nuclease

The protocol described here is designed for rapid generation of a cell population where most of the cells have a single copy of a lentiviral Cas9 nuclease proviral sequence in the genome.

Select the lentiviral Cas9 nuclease vector with the most active promoter in your cell line based on empirical testing or known promoter activity.

Blasticidin selection

The Edit-R Lentiviral Cas9 Nuclease Expression vectors confer resistance to blasticidin in transduced cells. Before transducing cells, determine the minimum concentration of blasticidin required to kill non-transduced cells between three and ten days by generating a blasticidin kill curve. The blasticidin concentration range for many mammalian cells is 2-15 µg/mL.

FACS selection

The Edit-R Lentiviral Fluorescent Cas9 Nuclease vectors allow creation of stable cell lines through selecting for positive fluorescent cells using Fluorescence-Activated Cell Sorting (FACS). For optimal Cas9 gene editing, cell populations with high fluorescence should be collected. Additionally, single cells with high fluorescence can be sorted into 96-well plates for clonal cell line generation.

Transduction of cells with Edit-R Lentiviral Cas9 Nuclease Expression 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. Permissivity to lentiviral delivery and optimal transduction conditions vary widely amongst cell types and must be determined empirically for each cell line of interest.

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

Day 1:

1. Plate 5 × 10^4 cells per well in a 24-well plate using Growth Medium

Optimal cell number for plating will vary with growth characteristics of specific cells and should be determined experimentally. Typically cells should be at 40-80% confluence on the day of transduction.

2. Incubate cells at 37 °C in a humidified CO_2 incubator overnight.

Day 2:

1. Prepare the Transduction Medium and by equilibrating the base cell culture medium to 37 °C.
2. Calculate the volume of lentiviral particles needed for transduction at MOI = 0.3.

The functional titer of Edit-R Lentiviral Cas9 Nuclease Expression particles (in HEK293T cells, as determined by qPCR) is reported on the Certificate of Analysis (C of A). We recommend an MOI of 0.3 (adjusted for relative transduction efficiency in your cell type) to ensure single integration of the lentiviral Cas9 nuclease. The relative transduction efficiency of your cell type will likely be lower than that of HEK293T cells. Thus, using an MOI of 0.3 based on the HEK293T titer reported on your C of A may be acceptable for generating a stable cell population with a single Cas9 integration.

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 × 10^8 TU/mL

Then,

V = 0.3 TU/cell × 100 000 cells/well ÷ 1 × 10^8 TU/mL × 1000 = 0.3 µL of lentiviral stock/well.

3. Thaw the Edit-R Lentiviral Cas9 Nuclease Expression 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 base cell culture 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).

Days 3-15:

Generation of stably expressing Cas9 cell lines can be achieved by two different methods, depending on the Edit-R Cas9 lentivirus used during the experiment:

Method 1 using Edit-R Lentiviral Fluorescent Cas9 Nuclease

1. At 72 hours post-transduction expand cells into a larger dish and continue to passage until enough cells are collected for cell sorting (500,000-10 million cells depending on generation of clonal cell line or population).
2. Prepare cells for FACS analysis according to commonly used protocols.
3. Sort cells as desired.

We recommend sorting the cells into fractions with low, medium, and high fluorescence expression levels. Additionally, ensure that each fraction has enough of the total cell population to be easily expanded for downstream applications and testing. Alternatively, single cells with high fluorescence can be sorted into 96-well plates for clonal cell line generation.

4. Once the sorted cells are growing normally, expand accordingly to freeze enough aliquots for your experimental project. Unless single cells were isolated, these cell lines will be a mixed population that on average have a single integration of Cas9 in their genomes.

Method 2 using Edit-R Lentiviral Cas9 Nuclease with blasticidin selection

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

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

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

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

Utilize the mixed population of Cas9-expressing cell line obtained above for transduction with Edit-R Lentiviral sgRNA particles for phenotypic analysis by knockout of your gene of interest, or isolate clonal cell lines for downstream experiments using protocols appropriate for your cells of interest if clonal cell lines are desired.

Transduction of cells with Edit-R Lentiviral sgRNA particles

The protocol below is an example of Edit-R Lentiviral sgRNA Expression particles transduction at an MOI of 0.3 into adherent U2OS cells stably expressing Cas9 nuclease in 24-well plate format. Permissivity to lentiviral delivery and optimal transduction conditions vary widely amongst cell types and must be determined empirically for each cell line of interest.

Puromycin selection

The Edit-R Lentiviral sgRNA vector confers resistance to puromycin in transduced cells. Similar to blasticidin selection, 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.

Day 1:

1. Plate 5 × 10^4 Cas9 Nuclease-expressing cells per well in a 24-well plate using Growth Medium.
2. Incubate cells at 37 °C in a humidified CO_2 incubator overnight.

Day 2:

1. Prepare the Transduction Medium by equilibrating the base cell culture medium to 37 °C.
2. Calculate the volume of lentiviral particles needed for transduction at MOI = 0.3 (see Note above).
3. Thaw the Edit-R Lentiviral sgRNA particles on ice.
4. Once thawed, gently mix and pipette the calculated volume of lentiviral particles into the base cell culture 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.

Days 3-7:

1. At 24-48 hours post-transduction, replace the medium with Selection Medium containing the appropriate amount of puromycin.

Since the Cas9-expressing cells are resistant to blasticidin, addition of blasticidin to the Selection Medium containing puromycin is optional. If the cells become confluent, split the cells into a larger dish to allow proper antibiotic selection (for instance, split cells from 24-well to 6-well culture dishes).

2. Replace the Selection Medium every 2-3 days and monitor the presence of dead cells daily.
3. Once the cells are growing normally in Selection Medium, expand the cells to freeze a sufficient number of aliquots for your experimental project or proceed to isolation of clonal cell lines.
    a. Expansion of the puromycin/blasticidin-resistant cells at this stage will generate a mixed population of cells having a single integration of the sgRNA in their genomes, in addition to the single integration of Cas9 nuclease. This mixed population will carry a variety of insertions and deletions (indels) and can be used for phenotypic analysis of your gene knockout.
    b. If a defined mutation genotype is required, proceed to isolation of clonal cell lines using protocols appropriate for your cells of interest and determine the exact genotype by DNA sequencing.

4 Packaging Edit-R Lentiviral Cas9 Nuclease plasmids

Edit-R Lentiviral Cas9 Nuclease Expression plasmids are Tat-dependent and require a packaging system that expresses the *tat* gene. For packaging lentiviral constructs, we recommend the Dharmacon™ Trans-Lentiviral™ ORF Packaging System. For packaging protocols and additional information please consult the product manual.

Edit-R Lentiviral Blast-Cas9 Nuclease Plasmids do not express a fluorescent protein reporter, therefore, after packaging plasmid DNA, we recommend titering the lentiviral particles produced using a functional lentiviral titration protocol such as limiting dilution with cell viability assay by crystal violet staining or genomic qPCR assay[14]. Edit-R Lentiviral Fluorescent Cas9 Nuclease Expression plasmids have a fluorescent protein reporter and therefore, can be used to determine functional lentiviral titer in cells through observing fluorescence.

5 Appendix

Gene editing assay recommendations

The most commonly used method for detection of insertions and deletions (indels) in a cell population is a mismatch detection assay such as T7 Endonuclease I[11,12,13]. When edited cells are expanded and clonal populations isolated, the most commonly used method for confirming gene editing is Sanger sequencing[11].

Volume of Transduction Medium per surface area in culture dishes

Table 3. 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

Edit-R Lentiviral Cas9 Nuclease Expression and 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.

Plasmid DNA

Edit-R Lentiviral Cas9 Nuclease Expression plasmid DNA reagents are shipped as dried pellets at room temperature. Under these conditions, they are stable for at least four weeks. Upon receipt, plasmid DNA should be stored at -20 °C to -80 °C. Under these conditions, the reagents are stable for at least one year. Always dissolve plasmid in nuclease-free solution, such as nuclease-free 10 mM Tris pH 7.4.

6 Frequently asked questions

How do I choose between the various Edit-R Lentiviral Cas9 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 using multiple lentiviral promoter-Cas9 constructs or selecting the best promoter empirically using the Dharmacon SMARTchoice Promoter Selection Plate (Cat #SP-001000-01).

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 the Edit-R Lentiviral Cas9 system be used for gene knockout in non-mammalian organisms, such as flies or worms?

We have designed the Edit-R Cas9 plasmids and lentiviral particles for mammalian expression and thus have only tested in mammalian cells. We cannot predict the efficacy of using Edit-R Lentiviral Cas9 Nuclease and sgRNA Expression particles, nor can we troubleshoot experiments performed in non-mammalian systems.

Can I order the Edit-R Lentiviral sgRNA Expression vector as plasmid DNA?

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

Can I co-transduce the Edit-R Lentiviral Cas9 Nuclease Expression and my sgRNA particles in my cell lines to generate my gene knockout faster?

Although this is not the recommended protocol, transduction of both the Edit-R Lentiviral Cas9 Nuclease Expression and the sgRNA particles can be performed at the same time. If co-transduction is necessary, it is recommended to extend the dual antibiotic selection to the longest selection time period previously determined for the individual antibiotics in your cells. We cannot predict the outcome, nor troubleshoot any issues, resulting from co-transduction of Cas9 and sgRNA lentiviral particles.

What filters are recommended for sorting cells using mKate2 or TurboGFP™?

mKate2 has an excitation maximum of 588nm and emission maximum of 633nm. Recommended Omega Optical filter sets are QMAX-Red, XF102-2, Texas red, or similar. TurboGFP™ has an excitation maximum of 482nm and emission maximum of 502nm. Recommended filter sets are EGFP, FITC, and other green dyes. Recommended Omega Optical filter sets are QMAX-Green, XF100-2, XF100-3, XF115-2, and XF116-2.

7 References

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8 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 technical support team.

Horizon Discovery Group
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 (Recombinant DNA Advisory Committee (RAC) guidelines);
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).

9 Limited use licenses

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t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

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