GenTarget Inc

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SaCas9 endonuclease Expression Lentivirus for CRISPR

Cat#	Product Name	Amounts
LVP1790	SaCas9 (CMV, Puro), Concentrated Lentivirus	200ul x (1 x 10^8 IFU/mL)
LVP1791	SaCas9 (CMV, Bsd), Concentrated Lentivirus	
LVP1792	SaCas9 (CMV, Neo), Concentrated Lentivirus	
LVP1793	SaCas9 (CMV, Hygro), Concentrated Lentivirus	
LVP1794	SaCas9 (CMV, Zeo), Concentrated Lentivirus	
LVP1795	SaCas9 (CMV, RFP-Bsd), Concentrated Lentivirus	
LVP1796	SaCas9 (CMV, RFP-Puro), Concentrated Lentivirus	
LVP1797	SaCas9 (CMV, GFP-Bsd), Concentrated Lentivirus	
LVP1798	SaCas9 (CMV, GFP-Puro), Concentrated Lentivirus	
LVP1799	SaCas9 (CMV, No selection), Concentrated Lentivirus	
LVP1800	SaCas9 (EF1α, Puro), Concentrated Lentivirus	
LVP1801	SaCas9 (EF1α, Bsd), Concentrated Lentivirus	
LVP1802	SaCas9 (EF1α, Neo), Concentrated Lentivirus	
LVP1803	SaCas9 (EF1α, Hygro), Concentrated Lentivirus	
LVP1804	SaCas9 (EF1α, Zeo), Concentrated Lentivirus	
LVP1805	SaCas9 (EF1α, RFP-Bsd), Concentrated Lentivirus	
LVP1806	SaCas9 (EF1α, RFP-Puro), Concentrated Lentivirus	
LVP1807	SaCas9 (EF1α, GFP-Bsd), Concentrated Lentivirus	
LVP1808	SaCas9 (EF1α, GFP-Puro), Concentrated Lentivirus	
LVP1809	SaCas9 (EF1α, No selection), Concentrated Lentivirus	

Storage: -80 °C, avoid repeat freeze/thaw cycles, stable for > 6 months.

1. Product Description:

GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

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Targeted and precise genomic gene editing technologies are the tools for genomic correction, modification and gene therapy. The TALEN, ZFN and CRISPR/Cas are the three main genome editing technologies. The lately discovered, so called the third generation of gene editing technology, the **CRISPR** (Clustered Regularly Interspaced Short Palindromic Repeats) technology has (1) higher targeting accuracy; (2) much more target sequence selection; (3) much less complexity; and (4) much less off-target cell toxicity than the previous genome editing technologies: TALEN (transcription activator-like effector nuclease) and ZEN (Zinc-finger nuclease).

2. Mechanism of CRISPR/Cas systems:

In CRISPR, A target sequence-specific guide RNA molecule (gRNA) directs a cas endonuclease to the genomic DNA target sequence. Then, the Cas enzyme creates a double-strand break at the target sequence that can be repaired by either Non-Homologous End-Joining (NHEJ), which can result in insertion or deletions (InDels), or correction / Homology Directed Repair (HDR). InDels can disrupt expression of the target gene while repair by HDR, which requires the presence of a repair template, allows modification of the gene. Ther are a few Cas enzymes are widely used in CRISPR gene editing. **SpCas9**, **SaCas9** and **Cas12a (Cpf1)** are the most frequently used cas endonucleases.

CRISPR/Cas based genomic knock in/out editing requires three components:

1) **Target specific guild RNA (gRNA)**: gRNA comprises two segments, a targeting sequence (crRNA) containing the target complementary RNA, and an auxiliary trans-activating non-coding RNA sequence (tracrRNA). To make the gRNA, you first select a suitable target sequence, the crRNA region (see online tools for target selection below), and then synthesize and anneal the crRNA oligos, and clone it into the guild vector which transcripts the target specific "crRNA-tracrRNA" sequence.

   Each Cas9 has its own specific tracrRNA that is required for proper folding and function of the gRNA-Cas9 complex. The tracrRNA interacts with the crRNA to form a functional gRNA. For **SaCas9** (from Staphylococcus aureus), the tracrRNA sequence is:

   5'-
   GTTTTAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTAT
   CAACTTGAAAAAGTGGCACCGAGTCGGTGC-TTTTTTT -3'

Insert your 21-nt target-guild sequence at the 5' end of this scaffold sequence above to make the full sgRNA.

Each Cas9 has its own specific **PAM** (Protospacer Adjacent Motif) sequence. The PAM site of SaCa9 is listed below, which is different than that of SpCas9. SpCas9 and SaCas9 cut 3 base pairs upstream of the PAM on both DNA strands, producing a blunt-ended double-strand break (DSB).

NNGRRT
N = any nucleotide (A, T, C, or G)
R = purine (A or G)

2) **Cas9 endonuclease**: The co-existence of the gRNA sequence with a Cas9 enzyme leads to the formation of a gRNA-Cas9 complex that will bind to and cleave the corresponding genomic DNA target sequence. In some cases, the Cas9 and the gRNA is made in one vector (So call "One vector system" or "All in one vector". However, the separating Cas9 expression and guild gRNA into two vectors, provides more flexibility in genomic editing. You can use the pre-made cas9 expression lentivirus (like GenTargt's **SaCa9** expression lentivirus) and only construct your desired gRNA vectors.

3) **The donor DNA sequence ("knock In")**: For genomic modification application, a double strand repair DNA is required after the Cas9 creates the double stranded breaks at desired genomic loci. The donor DNA provides the desired sequence insertion that flanked by the gene loci's homology sequences: left homologous arm (LHA) and right homologous arm (RHA), for the genomic editing via HDR mechanism. The double stranded donor DNA cassette can be provided from DNA fragment synthesized, or use a linearized donor vector.

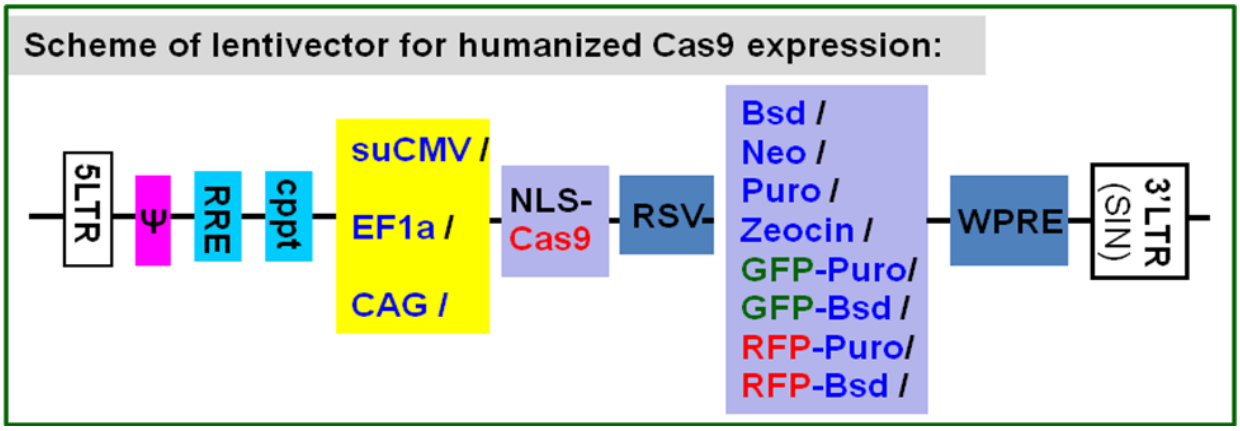
2. GenTarget's SaCas9 expression lentivirus:

GenTarget offers the standalone SaCas9 expression lentivirus products. The ready-to-use SaCas9 lentivirus are produced from our proprietary high-titer lentivectors that express the nuclear penetrating, human codon optimized, wild-type SaCas9 gene (originated from *Staphylococcus Aureus*). The SaCas9 enzyme is driven by different promoters with a variety of antibiotic selection markers (see the core expression vector map scheme below), providing you an easy delivery for cas9 expression in almost all cell types, included the hard-to-

transfected cell types, primary cells and non-dividing cells, which makes the gene editing possible in all cell types.

With using the ready-to-use SaCas9 lentivirus, you can simply synthesize the "targeting expression cassette "(U6/H1-crRNA-tracrRNA)" or construct the guild vector (gRNA) by clone the target specific "crRNA-tracrRNA" into a desired gRNA vector (without cas9 cassette).

nuclear penetrating Cas 9 expression lentivector core structure:



Gentarget's SaCas9 expression lentivirus has the following Key advantages:

1) **High efficient SaCas9 expression delivery with markers:** High titer lentivirus providing most efficient SaCas9 delivery in almost all cell types including primary cells and non-dividing cells. When desired, it includes a fluorescent-antibiotic dual marker allowing the real-time check the lentivirus transduction efficiency.
2) **Different promoter selection** (**CMV, EF1a**) for SaCas9 expression for different promoter strength in cell types
3) **Different selection marker:** Ca9 expression lentivirus carry different antibiotic selection, or Fluorescent-antibiotic dual selection for enrich the transduced cells or generate the Cas9 expression stable cells when desired. You can also pick the Ca9 expression lentivirus without any selection.
4) **Best nuclear penetrating for SaCas9 enzyme**: the SaCas9 is expressed with an optimized, proprietary Nuclear Localization Signal (NLS), providing the efficient cas9 delivery into the nuclear region where the gene editing occurs.

5) **No need for tedious cloning work or vector construction**: you can simply synthesize the gRNA (and donor cassette when desired) and used together with the SaCas9 lentivirus for the gene editing.
6) **Allow multiple gene editing at the same time:** no need to construct each targeting vector for different gene. Instead, you just select the target sequence and synthesize the gRNA (each single strand RNA or double stranded DNA cassette) that to used with the standalone SaCas9 expression particles.

3. CRISPR target sequence selection:

Selection of the target sequence within the gene of interest is critical to the efficacy and specificity of genetic editing with CRISPR/SaCas9.

The crRNA segment of the gRNA will only bind to DNA targets that are immediately upstream of the proper Protospacer Adjacent Motif (PAM) sequence, which for CRISPR/SaCas9 is **NNGRRT**. The target sequence (**21bp ~ 24bp**) can be in either the sense or anti-sense orientation with respect to the target gene. It is a good idea to create several target sequences for your gene of interest and to select sequences with minimal homology to other genes, in order to find a sequence with good cleavage efficiency and minimal off-target effects. Many online bioinformatics tools assist in selecting a gRNA sequence with minimal off-target effects.

target sequence selection: 5'- "(20-nt target sequence) + PAM (NGG)"

(Note: the selected sequences are in front of the NGG in genomic sequence, but NGG should not be included in the synthesized gRNA)

4. CFISPR Protocol (as general reference only):

1) select or design the 20bp target specific sequence (crRNA) using an online CRISPR designer tool;
2) generate the gRNA that can be carried out by one of the methods listed below:
   - **method 1**: or construct the gRNA transcription vector by cloning the 20nt crRNA into a gRNA vector (that containing the tracrRNA already); (GenTarget provides services to construct your desired gRNA lentivector and ready-to-use gRNA lentivirus).

- **method 2:** synthesize the linear double stranded DNA cassette that transcripts the gRNA ("crRNA-tracrRNA"), driven by either human U6 or H1 promoter:

  "U6 promoter==(crRNA-tracrRNA)-terminator (ttttttctag)" (~369bp)
  "H1 promoter==(crRNA-tracrRNA)-terminator (ttttttctag)" (~210bp)

- **method 3:** By synthesize the single stranded RNA: "20nt crRNA + 80nt tracrRNA" (100 bases);
  "crRNA/(20nt)---tracrRNA /(~80nt)"

3) generate the Donor by the one of the methods listed below (optional for knock-in genomic editing:
   - **method 1**: synthesize the double stranded DNA cassette for sequence modification as:
     " LHA (500bp target specific left homologues arm) + (marker / insert +poly A terminator) + (RHA (500bp target specific right homologues arm)"
   - **method 2:** construct the donor vector clone by cloning the target specific "LHA-(marker / insert +poly A terminator)-RHA" into a donor vector;
4) Add SaCas9 expression lentivirus and gRNA lentivirus to target cells;
   (Note: if gRNA is double stranded DNA or not lentivirus, then the gRNA has to be delivered via DNA transfection, such as lipid-based delivery.)
5) (Optional for knock-In, apply Donor cassettes into target cells by lipid based transfection or other delivery mothed);
6) select the sequence modified colonies;

Note: If you want GenTarget to prepare the target specific gene editing reagents for you, please *contact GenTarget* for a service quote.

The human codon, nuclear penetrating SaCas9 lentivirus are provided as the **200ul** of concentrated virus in PBS with titer at $1x10^8$ IFU/ml.

5. SaCas9 Lentivirus Transduction Protocols:

Note: Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50 µl of virus into each well of 24-well-plate

where cell density is 50% to 75%. After 24 ~ 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight.

Note: at the time of transduction, cells should be 50%-75% confluent. For example, seed HeLa cells at 0.5 x 10^5/ml x 0.5ml in a well of a 24-well plate.

Day 1:

- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI.
- Return cells to 37°C, CO_2 incubator.
  Note: Try to avoid freezing and thawing. If you do not use all of the virus at one time, you may re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

Day 3:

At 48~72hr after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava) (only for the products containing a fluorescent marker)

Day 3 + (optional):

Sort transduced cells by FACS, and select for antibiotic resistance. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines).

Note: Filter wavelength settings:
GFP filter: ~Ex450-490; ~Em525;
RFP filter: ~Ex558; ~Em583;

6. Safety Precaution:

Gentarget lentiviral particles adapts must advanced lentiviral safety features (using the third-generation vectors with self-inactivation SIN-3UTR), and the premade lentivirus is replication incompetent. However, please use extra caution when using lentiviral particles. Use the lentiviral particles in Bio-safety II cabinet. Wear glove all the time when handling Lentiviral particles!

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Please refer CDC and NIH's guidelines for more details regarding to safety issues.

7. Warranty:

This product is for research use only. It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.

8. **Attachment:** GenTarget's pre-made lentivirus product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Repoter Lentivirus for all kinds of pathway screening assays
Cell Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
ImmunoOncology Research	Lentivirus products for immuno therapy research: CAR and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
CAR-T, TCR Lentivirus	**CARs** Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; **TCRs**: MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied wild-type **SaCas9** endonuclease, the **dSaCas9**, gRNAs, **CRISPR** gene editing research
Epigenomic: CRISPRi and CRISPRa	"**dSaCas9-Protein**" fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
Cell-Specific Reporter	a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter

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Infectious Antigens	Llentivirus that express all kinds of infectious antigens with C-term 6His-tag.
Virus Like Particles (VLP)	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
shRNA Knockdown	Knockdown verifeid and customized shRNA lentivirus for target knockdown,
microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Anti-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla, Red-Luc and more,** with different antibiotic selection markers.
Fluorescent Markers	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fusioned to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
Cytoskeleton Imaging	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
Unstable GFP	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection

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near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
CRE, Flp ColorSwtich	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
TetR Repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Negative control lentiviruses	Premade **negative control lentivirus with different markers**: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.