GenTarget Inc

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Cas12a Endonuclease Expression Lentivirus for CRISPR

Cat#	Product Name	Amounts
LVP1819	Cas12a (CMV, Puro), Concentrated Lentivirus	200ul x (1 x 10^8 IFU/mL)
LVP1820	Cas12a (CMV, Bsd), Concentrated Lentivirus	
LVP1821	Cas12a (CMV, Neo), Concentrated Lentivirus	
LVP1822	Cas12a (CMV, Hygro), Concentrated Lentivirus	
LVP1823	Cas12a (CMV, Zeo), Concentrated Lentivirus	
LVP1824	Cas12a (CMV, RFP-Bsd), Concentrated Lentivirus	
LVP1825	Cas12a (CMV, RFP-Puro), Concentrated Lentivirus	
LVP1826	Cas12a (CMV, GFP-Bsd), Concentrated Lentivirus	
LVP1827	Cas12a (CMV, GFP-Puro), Concentrated Lentivirus	
LVP1828	Cas12a (CMV, No antibiotic selection), Concentrated Lentivirus	
LVP1829	Cas12a (EF1α, Puro), Concentrated Lentivirus	
LVP1830	Cas12a (EF1α, Bsd), Concentrated Lentivirus	
LVP1831	Cas12a (EF1α, Neo), Concentrated Lentivirus	
LVP1832	Cas12a (EF1α, Hygro), Concentrated Lentivirus	
LVP1833	Cas12a (EF1α, Zeo), Concentrated Lentivirus	
LVP1834	Cas12a (EF1α, RFP-Bsd), Concentrated Lentivirus	
LVP1835	Cas12a (EF1α, RFP-Puro), Concentrated Lentivirus	
LVP1836	Cas12a (EF1α, GFP-Bsd), Concentrated Lentivirus	
LVP1837	Cas12a (EF1α, GFP-Puro), Concentrated Lentivirus	
LVP1838	Cas12a (EF1α, No antibiotic selection), Concentrated Lentivirus	

Storage: -80 °C, avoid repeat freeze/thaw cycles, stable for 12 months when stored appropriately.

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*. Lentivirus stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

Targeted and precise genomic gene editing technologies are the tools for genomic correction, modification and gene therapy. The TALEN, ZFN and

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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/Cas12a 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.

For genomic modification application, a double strand repair DNA is required after the Cas 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.

gRNA designed to direct the CRISPR-Cas nuclease to a specific DNA sequence in the genome. The guide RNA consists of two parts: a 20–21 base pair (bp) sequence that is complementary to the target genomic DNA (which can be on either strand), and a scaffold sequence that forms a secondary structure recognized by the Cas enzyme.

There are a few **Cas enzymes** are widely used in CRISPR gene editing. SpCas9, SaCas9 and Cas12a, are the most frequently used cas endonucleases. Each cas enzyme requires specific PAM site and Scaffold sequence for assemble the full-length gRNA.

The **PAM** (Protospacer Adjacent Motif) sequence, which is required for Cas recognition.

3. GenTarget's AsCas12a expression lentivirus:

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AsCas12a from Acidaminococcus Sp. (also known as Cpf1) Cas12a, is another most frequently used cas endonuclease (other than SpCas9, and SaCas9). Gentarget engineered the wild-type AsCas12a to incorporate the published amino acid changes, listed in table below. This engineered cas enzyme provides more selection for the PAM site (TTTV and TTYN), and enhances CRISPR gene editing success rate.

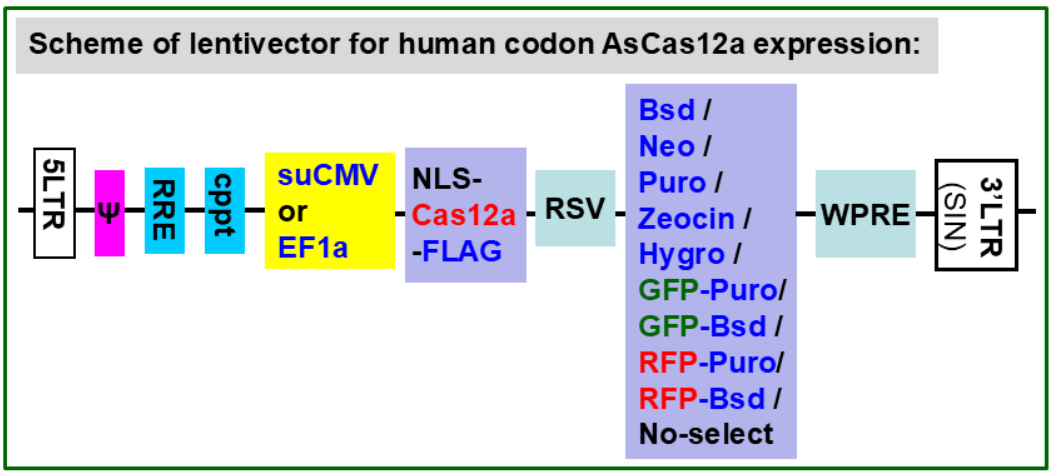
Mutation	Effect
M537R	Improved DNA binding; increased on-target activity
F870L	Increased editing efficiency and expression stability
K548V	Expands PAM recognition (from TTTV to TTYN)
E174R	Enhances enzyme solubility and stability
K607R	Linked to increased editing efficiency

This engineered AsCas12a contains the NLS (Nuclear Localization Signal) for efficient nuclear penetration for genomic editing, and a C-terminal FLAG tag which provides a convenient tool to detect or purify the expressed AsCas12a enzyme.

The co-existence of the gRNA sequence with a AsCas12a enzyme leads to the formation of a gRNA-Cas complex that will bind to and cleave the corresponding genomic DNA target sequence. In some cases, the Cas and the gRNA is made in one vector (So call "One vector system" or "All in one vector". However, the separating Cas expression and guild gRNA into two vectors, provides more flexibility in genomic editing. You can use the pre-made cas expression lentivirus and only construct your desired gRNA vectors.

GenTarget offers the standalone enhanced AsCas12a expression lentivirus products. The ready-to-use AsCas12a lentivirus are produced from our proprietary high-titer lentivectors that express the nuclear penetrating, human codon optimized, enhanced AsCas12a enzyme. This AsCas12a 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 cas12a 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. The AsCas12a lentivirus are

provided as the **200ul** of concentrated virus in PBS with titer at 1x10^8 IFU/ml.



To use the ready-to-use AsCas12a lentivirus, you can simply synthesize the gRNA-expression-cassette as "(**U6/H1 Promoter-crRNA-target-specific-sequence**-TTTTTT)" or construct the guild vector (gRNA) by sub-clone the "crRNA-targeting-sequence" into a desired gRNA vector.

4. Key Advantage of AsCas12a expression lentivirus:

1) **High efficient AsCas12a expression delivery with selection markers:** High titer lentivirus providing most efficient AsCas12a 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 AsCas12a expression for different promoter strength in cell types
3) **Different selection marker:** The expression lentivirus carry different antibiotic selection, or Fluorescent-antibiotic dual selection for enrich the transduced cells or generate the Cas12a expression stable cells when desired. You can also pick the Cas12a expression lentivirus without any selection.
4) **Best nuclear penetrating for AsCas12a enzyme**: the AsCas12a is expressed with an optimized, proprietary Nuclear Localization Signal (NLS), providing the efficient cas12a delivery into the nuclear region where the gene editing occurs.

5) **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 that to be used with the standalone AsCas12a expression lentivirus.

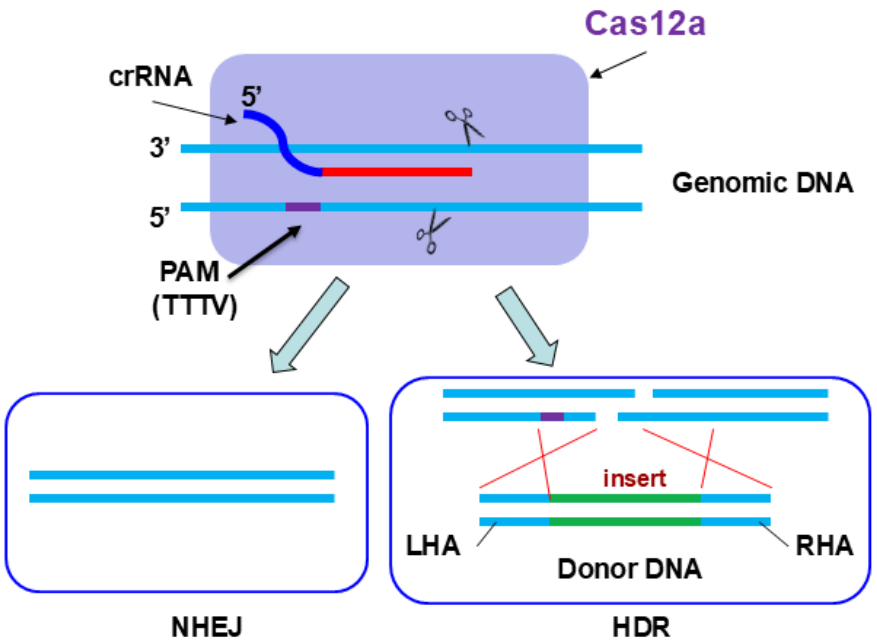
5. CRISPR target sequence selection using AsCas12a:

Selection of the target sequence within the gene of interest is critical to the efficacy and specificity of genetic editing with CRISPR/AsCas12a.

For this enhanced **Cas12a**, its PAM: 5'-TTTV (V=A/C/G) and TTYN (Y=C/T and N= A/T/C/G), is located **upstream** (5') of the target sequence. Cas12a cleaves downstream of the PAM, at positions:

- **18–23 nucleotides** downstream from the PAM on the **non-target strand**
- **22–24 nucleotides** downstream from the PAM on the **target strand**
  This results in a 5' overhang (sticky ends), typically 4 – 5 nucleotides long. See scheme below.

3'---------- (22-24 nt)----↓--5'
5'-TTTV-(target sequence: 20-24bp)-(18-23 nt)↑-----3'



gRNA sequence: AsCas12a use the Direct Repeat (DR) as Scaffold Sequence as: 5'- **TAATTTCTACTAAGTGTAGAT**

For example, If the target-specific sequence is: 5'GACGTGACCTGACATCGTGA (20nt), the full-length gRNA for Cas12a will be: "(**DR)** + (Your Target)", listed below:

5'- TAATTTCTACTAAGTGTAGATGACGTGACCTGACATCGTGA

The target sequence (**20bp ~ 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.

(**Note**: the selected targeting sequences is at the downstream of PAM site, but PAM sequence should not be included in the synthesized gRNA)

6. CRISPR 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
   - **method 2:** synthesize the linear double stranded DNA cassette that transcripts the gRNA, driven by either human U6 or H1 promoter:

     "U6-promoter==(TAATTTCTACTAAGTGTAGAT-(target-sequence)-terminator (ttttttctag)"
     "H1-promoter==(TAATTTCTACTAAGTGTAGAT-(target-sequence)-terminator (ttttttctag)"

   - **method 3:** By synthesize the single stranded **RNA**:
     " TAATTTCTACTAAGTGTAGAT-(target-sequence)"

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 AsCas12a expression lentivirus and gRNA lentivirus to target cells; (Note: if gRNA comes from the 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.

7. 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;

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

9. 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.

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

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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 **En-Cas12a** endonuclease, the **dEn-Cas12a**, gRNAs, **CRISPR** gene editing research
Epigenomic: CRISPRi and CRISPRa	"**dEn-Cas12a-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
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.

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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
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),

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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.