Product Specification Sheet

Product Name	**pLV-U6-gRNA scaffold-EF1-NEO sgRNA Lentiviral Vector**
Description	CRISPR (clustered regularly interspaced short palindromic repeats) and CRISPR-associated protein 9 (Cas9) are components of the leading genome or gene editing system, CRISPR-Cas9. Since it was developed in 2012, this gene editing tool has revolutionized biology research, making it easier to study disease and enabling faster drug discovery. The detection of catalytically inactive Cas9 (dCas9) protein has greatly expanded this gene editing system to foster the study of gene expression and regulation in a wide range of organisms. This dCas9 protein lacks the ability to create DNA breaks in the target and serves as a DNA binding protein. When it fuses with a transcriptional repressor such as the Krüppel-associated box (KRAB) repressor or a transcriptional activator such as the tripartite fusion of three transcription activation domains: VP64, p65 and Rta (VPR), it can effectively regress or activate gene expression. To do this, a single guide RNA (sgRNA) of the gene of interest must be present. The Cas9-sgRNA complex binds to DNA sequences that are complementary to the sgRNA and causes a steric block that halts transcript elongation by RNA polymerase in CRISPRi systems. While in CRISPRa systems, the Cas9-sgRNA complex recruits transcription factors to increase desired gene expression. Gene specific gRNA that usually targets the promoter region is cloned into one of the sgRNA expression vectors in all the sgRNA vectors, human U6 promoter drives the sgRNA expression. pLV-U6-gRNA scaffold-EF1-NEO sgRNA Lentiviral Vector expresses gRNA with the human U6 promoter and NEO with the EF1 promoter to allow for NEO selection of transduced cells.
Catalog Number	CG103
Size	10 µg at 0.5 µg/µL in TE
Shipping	Room temperature
Storage and Stability	Store at -20°C immediately upon receipt. This product is stable for 6 months when stored as directed.
Quality Control	This plasmid is sequence verified.
Safety Precaution	Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. The ALSTEM Lentiviral Expression System is designed to minimize the chance of generating replication-competent lentivirus, but precautions should still be taken to avoid direct contact with viral supernatants.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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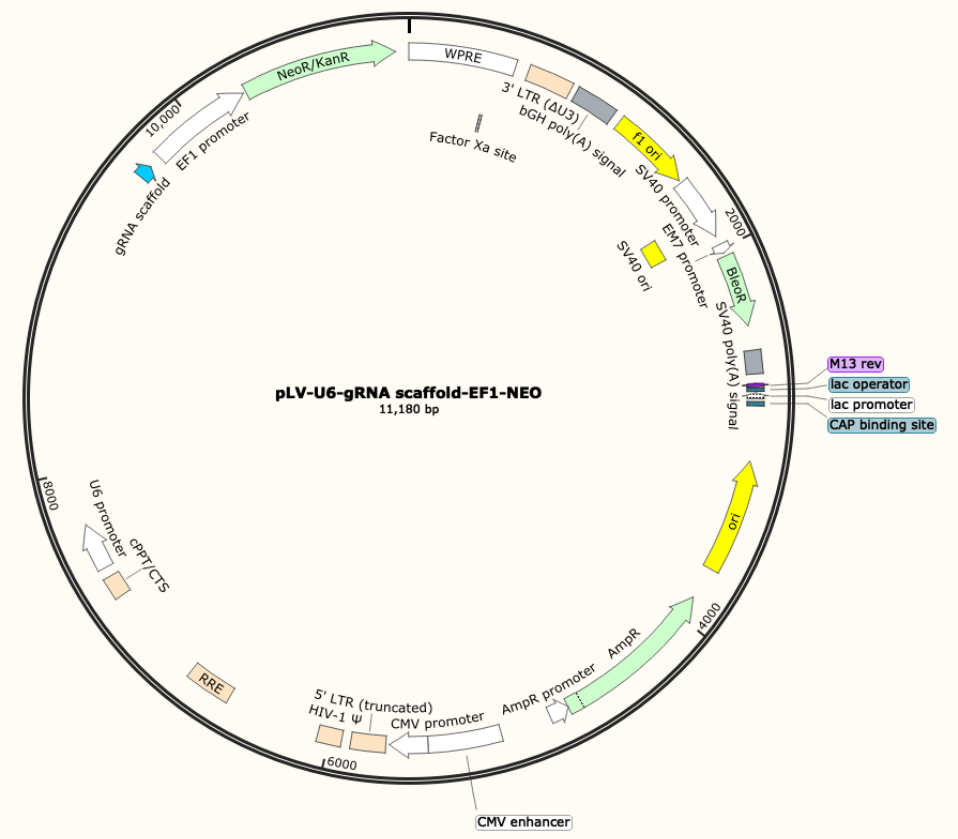
Vector Information

This is a lentiviral expression vector that contains all the elements for efficient and high yield viral production. A ubiquitous EF1 promoter drives the expression of NEO to allow for the selection of transduced cells. This vector is used in combination with the Cas9-KRAB or the Cas9-VPR to repress or activate the target gene expression, respectively.



***Note**: Bacterial culture of pLV vectors should be done in medium containing **10 µg/mL** Ampicillin. For maximal plasmid yield and quality, we recommend Stbl3 competent cells (Invitrogen).*

*To clone your gRNA into the sgRNA vector, be sure to include additional nucleotides into your two complementary oligonucleotides for the gRNA (Forward: 5' CACCg**Target(20bp)**-3' and Reverse: 3'-c**Target(20bp)**AAAC-5'). Phosphorylated and annealed gRNA is then cloned into the BsmB1 sites of the sgRNA vector. The BsmB1 digested sgRNA vector (8896 bp) should be gel purified and used for the gRNA cloning.*

Instructions for gRNA Cloning

1. Synthesize two complementary oligonucleotides for the gRNA
    a. Forward: 5' CACCg**Target(20bp)**-3'
    b. Reverse: 3'-c**Target(20bp)**AAAC-5'

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2. Digest 1µg of the sgRNA Lentivirus Expression Vector with *BsmBI*, prepare the reactions as in the following table and incubate for 30 min at 37°C

Component	Amount
Plasmid	1 µg
FastDigest *BsmBI*	1 µl
FastAP	1 µl
10x FastDigest Buffer	2 µl
ddH_2O	Supplement to 20 µl
Total	20 µl

3. Gel purify the digested plasmid (8896 bp) using QIAquick® Gel Extraction Kit and elute with elution buffer following the instructions of the manufacturer.

4. Phosphorylate and anneal each pair of oligos, prepare the reactions as in the following table.

Component	Amount
Oligo 1 (gRNA F, 100 µM)	1 µl
Oligo 2 (gRNA R, 100 µM)	1 µl
10x T4 Ligation Buffer	1 µl
T4 PNK	0.5 µl
ddH_2O	6.5 µl
Total	10 µl

Anneal in a thermocycler using the following parameters:
37°C 30 min
95°C 5 min and then ramp down to 25°C at 5°C/min

5. Set up ligation reaction, prepare the reactions as in the following table and incubate at room temperature for 10 min.

Component	Amount
BmsBI digested plasmid from step 2	50 ng
Phosphorylated and annealed oligo duplex from step 3 (1:200 dilution)	1 µl
2x Quick Ligation Buffer	5 µl
Quick Ligase	1 µl
ddH_2O	Supplement to 10 µl
Total	10 µl

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6. (optional) Treat ligation reaction with Plasmid Safe exonuclease to prevent unwanted recombination products. Prepare the reactions as in the following table and incubate reaction at 37°C for 30 min.

Component	Amount
Ligation reaction from step 4	10 µl
10x Plasmid Safe Buffer	1.5 µl
10mM ATP	1.5 µl
Exonuclease	1 µl
ddH_2O	1 µl
Total	15 µl

7. Transformation:

   a. Thaw 100 µl aliquots of DH5α competent cells from -80°C freezer on ice.
   b. Add 10 µl of the products from last step into 50 µl of ice-cold DH5α competent cells.
   c. Incubate the mixture on ice for 30 min.
   d. Heat shock the mixture at 42°C for 45 sec and return immediately to ice for 2 min.
   e. Add 600 µl SOC medium (without antibiotics) and incubate in 37°C shaker for 30-40 min.
   f. Plate 200 µl of the mixture onto an LB plate containing 100 µg/ml ampicillin and spread around.
   g. Incubate the plate overnight at 37°C.

8. Pick 2-4 colonies per construct. Grow colonies in LB/100 µg/mL ampicillin. Shake overnight at 37°C.

9. Miniprep each culture, extract genomic DNA using the Zymo genomic extraction kit following manufacturer's instructions.

10. Submit the extracted plasmids for Sanger sequencing to verify successful cloning of the candidate gRNAs using U6 forward primer (ACGATACAAGGCTG TTAGAGAG).

11. Choose a sequence-verified colony and inoculate into a maxiprep culture. Store the plasmids at -20°C.

IMPORTANT NOTICE

Store the vial at -20°C immediately upon receipt.

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