

## pSMPUW-miR-GFP/Puro Lentiviral Expression Vector

CATALOG NUMBER: VPK-220

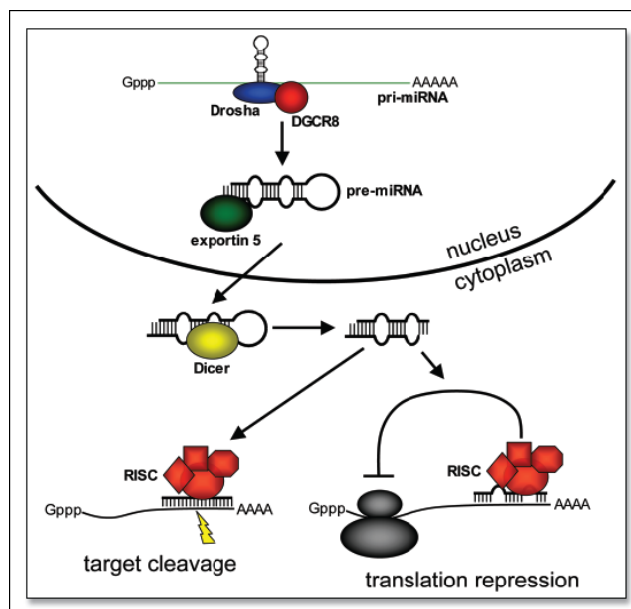
STORAGE: -20°C

QUANTITY AND CONCENTRATION: 10 µg at 0.25 µg/µL in TE

### Background

MicroRNAs (miRNAs) are 18–24 nucleotide RNA molecules that regulate the stability or translational efficiency of target mRNAs. These regulatory RNAs function by acting as sequence-specific guides which recruit a large protein complex known as the RNA-induced silencing complex (RISC) to target mRNAs which are subsequently silenced. Diverse functions have been attributed to miRNAs including the regulation of cellular differentiation, proliferation, and apoptosis. Moreover, significant evidence has accumulated implicating a fundamental role for miRNAs in the development of cancer.

miRNAs are initially transcribed as long precursor transcripts known as primary microRNAs (pri-miRNAs). Within these transcripts, the mature miRNA sequences are found in ~60–80 nucleotide hairpin structures. Mature miRNAs are generated from pri-miRNAs by sequential processing (Figure 1). Pri-miRNAs are initially recognized in the nucleus by the microprocessor complex which includes as core components the RNase-III enzyme Drosha and its obligate partner DGCR8. This complex excises the hairpin structure containing the mature miRNA sequence. The liberated hairpins, referred to as precursor miRNAs (pre-miRNAs), are recognized by the nuclear export factor exportin 5 which transports them to the cytoplasm. There, the RNase-III enzyme Dicer performs a second cleavage to generate a double-stranded 18–24 nucleotide RNA molecule. The RISC then associates with this RNA duplex and unwinds it. Generally, only one strand is stably incorporated into the RISC; the other is discarded and rapidly degraded. miRNAs guide the RISC to target messages that are subsequently cleaved or translationally silenced.



**Figure 1.** miRNA Biogenesis and function

Lentivirus vector based on the human immunodeficiency virus-1 (HIV-1) has become a promising vector for gene transfer studies. The advantageous feature of lentivirus vector is the ability of gene transfer and integration into dividing and non-dividing cells. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens the target cell range. Lentiviral vectors have been shown to deliver genes to neurons, lymphocytes and macrophages, cell types that previous retrovirus vectors could not be used. Lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses.

Cell Biolabs' pSMPUW-miR-GFP/Puro Lentiviral Expression Vector is designed to clone and express an individual miRNA precursor in its native context while preserving putative hairpin structures to ensure biologically relevant interactions with endogenous processing machinery and regulatory partners, leading to properly cleaved microRNAs. Individual miRNA precursor from any species can be cloned into PshAI site (Figure 2).

**The pSMPUW-miR-GFP/Puro lentiviral expression vector contains the following features:**

- **miRNA Processing** – miRNA stem loop precursor in its native context is cloned in PshAI site. To preserve the putative hairpin structure and proper endogenous processing, miRNA stem loop sequence is flanked by its native intron sequence.
- **EF-1 $\alpha$  Promoter** - ensures a high level of expression in mammalian cells
- **GFP-Puro Fusion Marker** - to monitor cells positive for expression and stable selection with either GFP or puromycin resistance.
- **pUC Origin** - for high copy replication and maintenance of the plasmid in *E. coli*
- **Kanamycin Resistance Gene** - for selection in *E. coli*

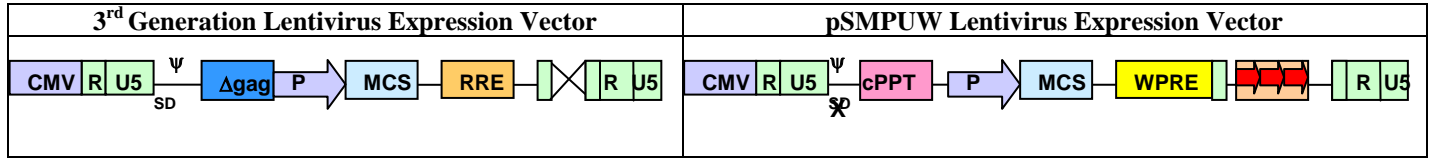
**Related Products**

1. VPK-205: ViraSafe™ Lentiviral Packaging System, Ecotropic
2. VPK-206: ViraSafe™ Lentiviral Packaging System, Pantropic
3. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
4. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
5. LTV-200: ViraDuctin™ Lentivirus Transduction Kit

**Safety Considerations**

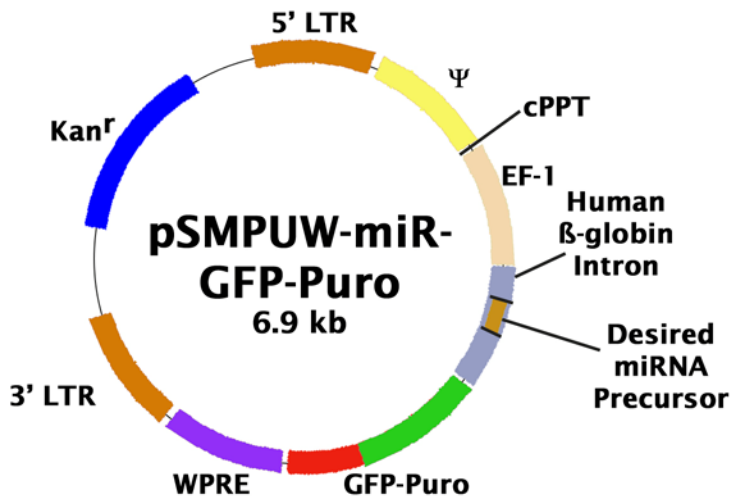
Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. The ViraSafe™ Lentiviral Expression System is designed to minimize the chance of generating replication-competent lentivirus, but precautions should still be taken to avoid direct contact with viral supernatants.

## Unique Elements of the pSMPUW Lentivirus Expression Vector



| Element                 | Name                     | Benefits compared to other 3 <sup>rd</sup> Generation Systems  |
|-------------------------|--------------------------|--|
| <i>ELEMENTS ADDED</i>   |                          |  |
|                         | Central Polypurine Tract | <ul style="list-style-type: none"> <li>Increased gene expression levels</li> </ul>   |
|                         | Hybrid 3' LTR Poly(A)    | <ul style="list-style-type: none"> <li>Increased safety: prevents read-through transcription</li> <li>Increased viral titer: vector transcript more stable in packaging cells</li> </ul> |
|                         | WPRE                     | <ul style="list-style-type: none"> <li>Increased viral titer</li> </ul>  |
| <i>ELEMENTS DELETED</i> |                          |  |
|                         | Gag sequence             | <ul style="list-style-type: none"> <li>Increased safety: reduces sequence homology</li> </ul>  |
|                         | Rev-Responsive Element   | <ul style="list-style-type: none"> <li>Increased safety: reduces sequence homology</li> </ul>  |

## pSMPUW-miR-GFP/Puro Lentiviral Expression Vector



**Figure 2.** pSMPUW-miR-GFP/Puro Lentiviral Cloning and Expression Vector (6891 bp, **Kanamycin**-resistant). Hind III Digestion: 1331 bp, 1982 bp, 3578 bp.

*Note: Bacterial culture of pSMPUW vectors should be done in medium containing **10 µg/mL** Kanamycin. For maximal plasmid yield and quality, we recommend Stbl3 endoA1+ competent cells (Invitrogen) and*

treatment with alkaline proteinase (Promega #A1441 or Sigma #P8038) for 4-5 min using 10 units of proteinase per mL of bacterial lysate before adding neutralization solution.

miRNA precursor cloning site (PshAI): GATTAGTTCTCGAGGATCCGACTG/AAGTCGCTAGCTCGAGCTTTTGGG  
PshAI

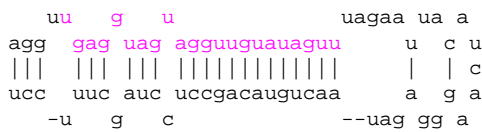
**miRNA Precursor Cloning**

All of our premade human and mouse miRNA precursor clones in pEP-miR and pEGP-miR vectors are based on the following design, and the resulting overexpression of the mature miRNA is confirmed by Northern blot or Real Time PCR. Here we use human let-7a-2 miRNA as an example:

**1. Download desired miRNA stem loop sequence from Sanger’s miRNA database:**

<http://microrna.sanger.ac.uk/sequences/>



Homo sapiens let-7a-2 stem-loop structure



Homo sapiens let-7a-2 stem-loop sequence

AGGUUGAGGUAGUAGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAAACUGUACAGCCUCCUAGCUUUCCU

**2. Blast search miRNA stem loop sequence: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>**

>  [ref|NT\\_033899.7|Hs11\\_34054](#)  Homo sapiens chromosome 11 genomic contig, reference assembly  
Length=38509590

```

Query 1          AGGTTGAGGTAGTAGGTTGTATAGTTTAGAATTACATCAAGGGAGATAACTGTACAGCCT 60
          |||
Sbjct 25579717    AGGTTGAGGTAGTAGGTTGTATAGTTTAGAATTACATCAAGGGAGATAACTGTACAGCCT
25579658
Query 61          CCTAGCTTTCCT 72
          |||
Sbjct 25579657    CCTAGCTTTCCT 25579646
```

**3. PCR and Cloning:**

- 1) Add 100 base native flank sequence to both upstream and downstream of the miRNA stem loop.

Human let-7a-2 miRNA precursor sequence including the 100 base flank sequences on both ends of the stem loop: let-7a-2 stem-loop sequence is underlined.

GCCCAAATAGGTGACAGCACGATGAATCATTATAAGACTAACTTGTAATTTCCCTGCTTAAGAAATG  
GTAGTTTTCCAGCCATTGTGACTGCATGCTCCCAGGTTGAGGTAGTAGGTTGTATAGTTTAGAATTA  
CATCAAGGGAGATAACTGTACAGCCTCCTAGCTTTCCTTGGGTCTTGCCTAAACAACATGGTGAGA  
ACGATCATGATTCCTCCAGGCCTTTTCTCCCTATGAAAGGTAAGATTGGGTACGATTATTTTATGGT  
ATTT



2) Design PCR primers.

For human let-7a-2 miRNA precursor:

Forward PCR Primer: GCCCAAATAGGTGACAGCACG

Reverse PCR Primer: AAATACCATAAAAATAATCGTA

3) PCR the miRNA precursor from genomic DNA and clone the blunt-end PCR fragment into the PshAI site of the expression vector.

4) Validate the insert by DNA sequencing.

Forward Sequencing Primer: TTTGCACCATTCTAAAGAAT

Reverse Sequencing Primer: AAACCTCTTACATCAGTTAC

### **Lentivirus Production**

1. One day before transfection, plate sufficient 293T cells or 293LTV cells (Cat. #LTV-100) to achieve 70-80% confluence on the day of transfection.
2. Transfect cells by Calcium Phosphate or other transfection reagents.

*Note: We suggest transfecting cells with FuGENE® Transfection Reagent (Roche Applied Science) or Lipofectamine™ Plus (Invitrogen). We recommend the ratio of vectors at 3:1:1:1 (pSMPUW: pCMV-VSV-G:pRSV-REV:pCgpV).*

3. Harvest lentiviral supernatant 36-72 hours after transfection. Supernatant can be harvested 2 or 3 times, every 12 hours. Keep it at 4°C over the collecting period.
4. Pool the collected supernatants, centrifuge 5 minutes at 1500 rpm to remove cell debris and filtrate on 0.22 µm.
5. Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80°C in aliquots.

### **Post-Packaging Considerations**

Packaging your lentivirus is only the first step to ensuring successful expression of your gene. The following steps should be considered prior to infection of your host cell:

1. **Concentration and purification of your lentivirus:** Because of the latent nature of lentivirus, it is imperative that your virus be highly concentrated before infecting your host cell. Also, impurities from your viral supernatant can decrease the efficiency of infection. We recommend using Cell Biolabs' ViraBind™ Lentivirus Concentration and Purification Kit (Catalog # VPK-090).
2. **Measure the titer of your lentivirus:** This is an important step to ensure consistent viral transduction into your host cell. However, QPCR or stable clone counting can take as much as 1-2 weeks to

perform. Traditional p24 ELISA kits can greatly overestimate your lentiviral titer. Our advanced p24 ELISA, QuickTiter™ Lentivirus Titer Kit (Catalog # VPK-107), uses exclusive technology that eliminates free p24 from your supernatant, giving you much more accurate lentiviral titers. Results are obtained in 6-18 hours.

3. **Use transduction reagents to increase infection efficiency:** Many cells are difficult to infect with lentivirus, and without supplemental reagents transduction efficiencies can be low. Reagents such as Polybrene® can help, but are often insufficient. Cell Biolabs' proprietary reagents in our ViraDuctin™ Lentivirus Transduction Kit (Catalog # LTV-200) form a super-complex with your virus to increase transduction efficiencies by promoting virus and cell interaction.

## References

1. microRNA sequences listed in Sanger's miRBase (<http://microrna.sanger.ac.uk/sequences/>).
2. John, B., C. Sander and D. S. Marks (2006) *Methods Mol Biol* **342**: 101-13.
3. Chen, M. et al. (2002). *Nature Genetics* **32(4)**: 670-675.
4. Naldini, L., U. Blomer, P. Gally, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma, and D. Trono (1996) *Science* **272**:263-267.
5. Verma, I. M., and N. Somia (1997) *Nature* **389**:239-242
6. Kahl C. A., Marsh J., Fyffe J., Sanders D. A., and K. Cornetta (2004) *J Virol.* **78**:1421-30.
7. White S. M., Renda M., Nam N. Y., Klimatcheva E., Zhu Y., Fisk J., Halterman M., Rimel B. J., Federoff H., Pandya S., Rosenblatt J. D., and V. Planelles (1999) *J Virol.* **73**:2832-40.
8. Kafri T., van Praag H., Ouyang L., Gage F. H., and I. M. Verma (1999) *J Virol.* **73**:576-84.

## Appendix

### Vector Features:

1084 ~ 1479: EF-1 $\alpha$  Promoter

1512 ~ 2002: human  $\beta$ -globin intron

1734 ~ 1743: PshAI

2030 ~ 3352: GFP-Puro Fusion (GFP: 2307 ~ 3023; Puro: 3030 ~ 3629)

5941 ~ 6756: Kanamycin Resistance Gene (complementary strand)

### Plasmid Sequence:

```
ACTAGTCGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCATTGAC
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