pMXs-miR-GFP/Puro Retroviral Expression Vector

CATALOG NUMBER: RTV-017 **STORAGE:** -20ºC

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.

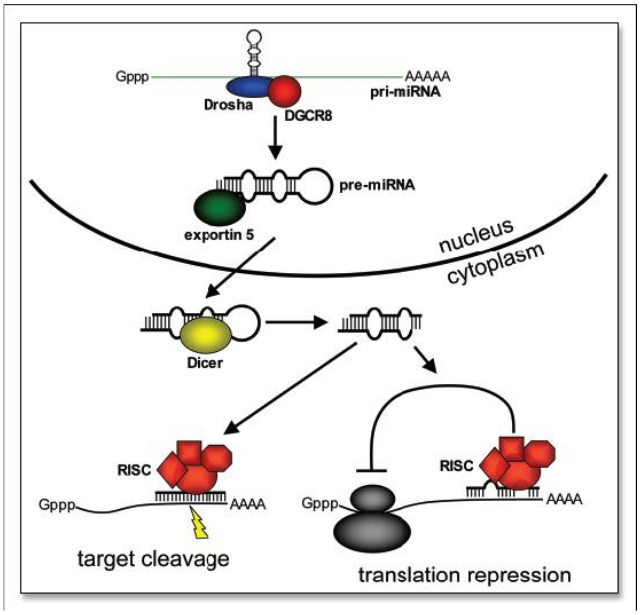


Figure 1. miRNA Biogenesis and function

Retroviruses are efficient tools for delivering heritable genes into the genome of dividing cells. Cell Biolabs' pMXs retroviral vector is based on Moloney murine leukemia virus (MMLV). The vector provides the viral package signal, transcription and processing elements, and MCS for cloning of a target gene. The viral *env* gene, produced by the package cell line, encodes the envelope protein, which determines the viral infectivity range. Transfection into a package cell line produces high-titer, replication-incompetent viruses. In addition to transfer and expression of exogenous genes in mammalian cells, recently, retroviruses have been used to express silencing RNAs (siRNA) to decrease the expression of target genes both *in vitro* and *in vivo*.

Cell Biolabs' pMXs-miR-GFP/Puro Retroviral 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 between Xho I sites (Figure 2).

The pMXs-miR-GFP/Puro retroviral expression vector contains the following features:

- **miRNA Processing** – miRNA stem loop precursor in its native context is cloned between Xho I sites. To preserve the putative hairpin structure and proper endogenous processing, miRNA stem loop sequence is flanked by its native intron sequence.
- **EF-1α 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*
- **Ampicillin Resistance Gene -** for selection in *E. coli*

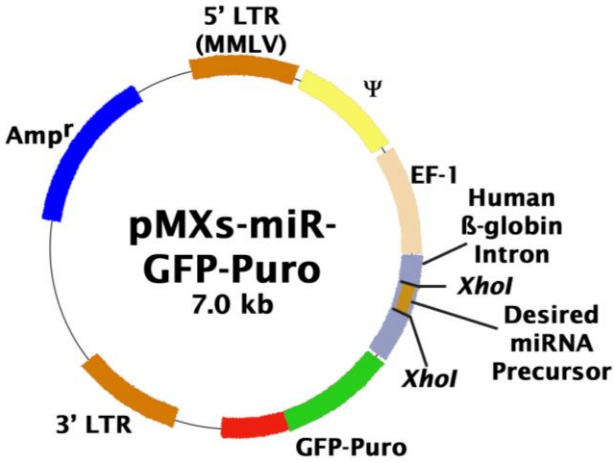
Related Products

1. RV-101: Platinum-E Retroviral Packaging Cell Line, Ecotropic
2. RV-102: Platinum-A Retroviral Packaging Cell Line, Amphotropic
3. RV-103: Platinum-GP Retroviral Packaging Cell Line, Pantropic
4. VPK-120: QuickTiter™ Retrovirus Quantitation Kit
5. RV-200: ViraDuctin™ Retrovirus Transduction Kit

Safety Consideration

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

pMXs-miR-GFP/Puro Retroviral Expression Vector



MCS: GATTAGTTCTCGAGGATCCGACTGAAGTCGCTAGCTCGAGCTTTTGGA
XhoI XhoI

Figure 2: pMXs-miR-GFP/Puro Retroviral Expression Vector (7001 bp, **Ampicillin**-resistant).

miRNA Precursor Cloning

All of our premade human amd 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

```
   uu   g   u             uagaa ua a
agg  gag uag agguuguauaguu     u  c u
|||  ||| ||| |||||||||||||     |  | c
ucc  uuc auc uccgacaugucaa     a  g a
   -u   g   c             --uag gg a
```

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

```
AGGUUGAGGUAGUAGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUCCUAGCUUUCCU
```

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
   CATCAAGGGAGATAACTGTACAGCCTCCTAGCTTTCCTTGGGTCTTGCACTAAACAACATGGTGAGA
   ACGATCATGATTCCTCCAGGCCTTTTCTCCCTATGAAAGGTAAGATTGGGTACGATTATTTTATGGT
   ATTT

2) Design PCR primers including Xho I site with four extra bases.

   Forward PCR Primer: `tcga-ctcgag (XhoI)-21 nt`
   Reverse PCR Primer: `tcga-ctcgag (XhoI)-21 nt`

   For human let-7a-2 miRNA precursor:
   Forward PCR Primer: `tcga-ctcgag-gcccaaataggtgacagcacg`
   Reverse PCR Primer: `tcga-ctcgag-aaataccataaaataatcgta`

3) PCR the miRNA precursor from genomic DNA and clone into the XhoI sites of the expression vector.

   PCR Product of let-7a-2 precursor: let-7a-2 stem-loop sequence is underlined.

```
  1 tcga<b>ctcgag</b> gcccaaatag gtgacagcac gatgaatcat tataagacta acttgtaatt
 61 tccctgctta agaaatggta gttttccagc cattgtgact gcatgctccc aggttgaggt
121 agtaggttgt atagtttaga attacatcaa gggagataac tgtacagcct cctagctttc
181 cttgggtctt gcactaaaca acatggtgag aacgatcatg attcctccag gccttttctc
241 cctatgaaag gtaagattgg gtacgattat tttatggtat tt<b>ctcgag</b>tc ga
```

4) Validate the insert by DNA sequencing.

   Forward Sequencing Primer: `TTTGCACCATTCTAAAGAAT`
   Reverse Sequencing Primer: `AAACCTCTTACATCAGTTAC`

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. Kitamura T., *et al*., (2003) *Exp. Hematol.* **31**, 1007-1014.

Recent Product Citations

1. Bergamini, C. et al. (2023). MiR-494 induces metabolic changes through G6pc targeting and modulates sorafenib response in hepatocellular carcinoma. *J Exp Clin Cancer Res*. **42**(1):145. doi: 10.1186/s13046-023-02718-w.
2. Kariba, Y. et al. (2020). Brown adipocyte-derived exosomal miR-132-3p suppress hepatic Srebf1 expression and thereby attenuate expression of lipogenic genes. *Biochem Biophys Res Commun*. S0006-291X(20)31011-1. doi: 10.1016/j.bbrc.2020.05.090.
3. Pollutri, D. et al. (2018). The epigenetically regulated miR-494 associates with stem-cell phenotype and induces sorafenib resistance in hepatocellular carcinoma. *Cell Death Dis*. **9**(1):4. doi: 10.1038/s41419-017-0076-6.

4. Ribecco-Lutkiewicz, M. et al. (2016). MicroRNA expression in amniotic fluid cells. *Fetal Stem Cells in Regenerative Medicine*. doi:10.1007/978-1-4939-3483-6_11.
5. Mansour, M. et al. (2013). The TAL1 complex targets the FBXW7 tumor suppressor by activating miR-223 in human T cell acute lymphoblastic leukemia. *J. Exp. Med*. **210**:1545-1557.

License Information

pMXs vector is licensed from the University of Tokyo.

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