miRNASelect™ pEGP-miR Cloning and Expression Vector

CATALOG NUMBER: MIR-EXP-GP-C **STORAGE:** -80ºC

QUANTITY: 100 µL of bacterial glycerol stock

Components

1. miRNASelect™ pEGP-miR Cloning and Expression Vector (Part No. MIR-EXP-GP): One tube
2. miRNASelect™ pEGP-miR Null Control Vector (Part No. MIR-NULL-GP): One tube

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.

Synthetic miRNA molecules based on predicted mature miRNA sequence are sometimes used. Despite their optimized design criteria, synthetic miRNAs underscore the importance of primary miRNA in its native expressed form. The primary miRNA contains critical biological components involved in mature miRNA expression and cellular processing, and is often processed into several mature miRNA molecules.

Cell Biolabs' miRNASelect™ Cloning and 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 BamHI and Nhe I sites (Figure 2).

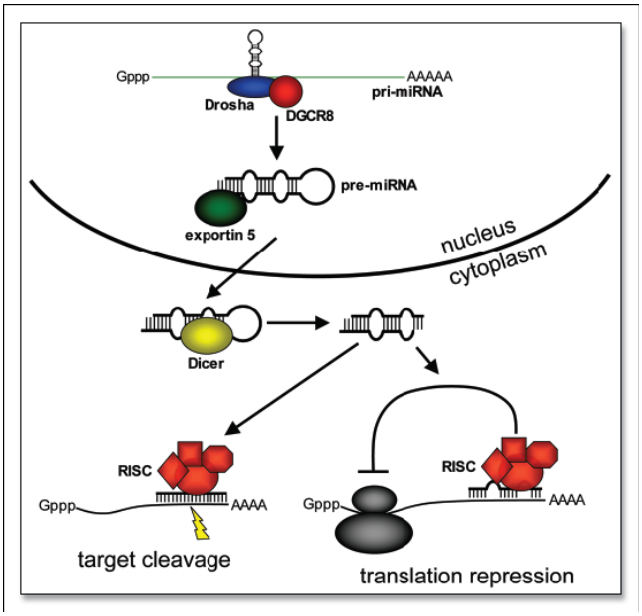

Figure 1. miRNA Biogenesis and function

The miRNASelect™ pEGP-miR cloning and expression vector contains the following features:

- **miRNA Processing** – miRNA stem loop precursor in its native context is cloned between BamHI and Nhe 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.
- **SV40 Polyadenylation Signal -** enables efficient termination of transcription
- **pUC Origin -** for high copy replication and maintenance of the plasmid in *E. coli*
- **Ampicillin Resistance Gene -** for selection in *E. coli*

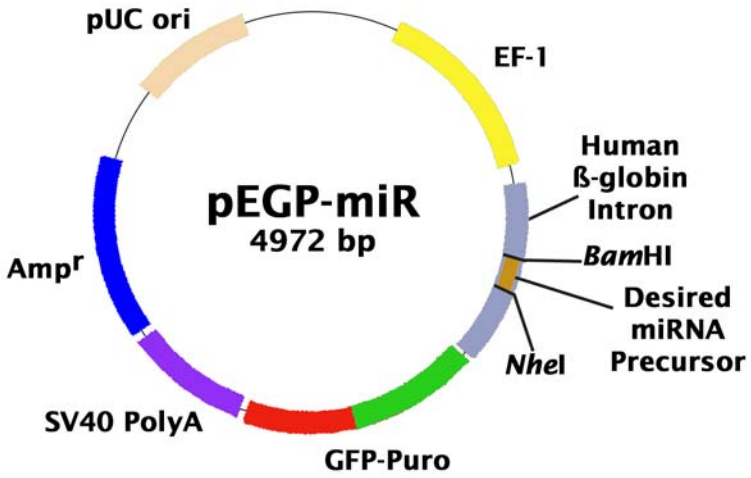

Figure 2. Schematic representation of pEGP-miR cloning and expression vector.

miRNA precursor cloning sites: BamHI - mmu- 304 bp - NheI

CGATTAGTTCTCGA**GGATCC**GACTGTTCTTTTCCCTCATTACACAGGAAACCGGAATTACAAAG
GAGAACGGCTTCCTGTGATGCTCAGCTGTGATTACTTTCAACATTCACCCTGGATGTTCTCTTC
ACTGTGGGATGAGGTAGTAGGTTGTATAGTTTTAGGGTCACACCCACCACTGGGAGATAACTAT
ACAATCTACTGTCTTTCCTAAGGTGATGGAAAAGTCTGCATTCATGGGGTCTCATAGGAAACCA
AGAACAAACTGCAGTGTTTTAAAGTATATCTTGCCTTAAAAGCATTTGCTTATGCTATGCATGA
AGTC**GCTAGC**TCGAGCTTTTGGAG

Note: pEGP-miR Cloning and Expression Vector should NOT be used as a transfection control vector, because the vector itself contains 304 bp of mmu-let7a-1 precursor sequence between the BamHI and NheI sites. For transfection control, we recommend pEGP-miR Null Control Vector.

Cell Biolabs' miRNASelect™ pEGP-miR Null Control Vector is similar to the pEGP-miR Cloning and Expression Vector except it does not contain any miRNA precursor (Figure 3).

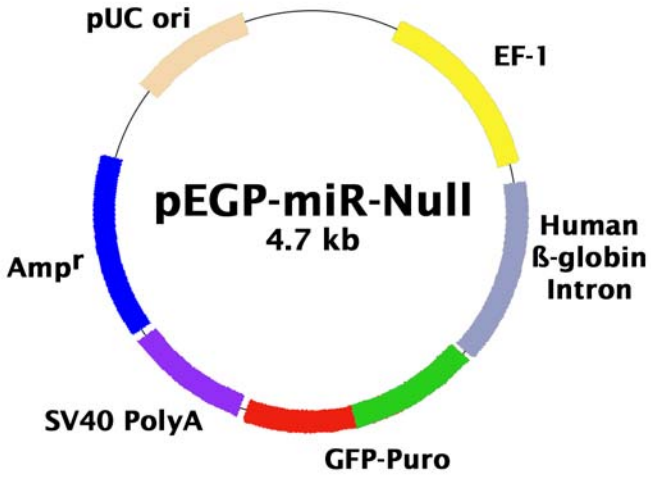

Figure 3. Schematic representation of the miRNASelect™ pEGP-miR Null control vector.

Note: pEGP-miR Null Control Vector should NOT be used as a cloning vector, because the vector will not be digested well with BamHI due to secondary structure.

miRNA Precursor Cloning

All of our premade human amd mouse miRNA precursor clones are based on the following design, and the resulting overexpression of the mature miRNA is confirmed by Northern blot. 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 primer including BamHI site at forward primer with four extra bases and NheI site at reverse primer.

   Forward PCR Primer: `tcga-ggatcc (BamHI)-21 nt`
   Reverse PCR Primer: `tcga-gctagc (NheI)-21 nt`

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

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

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

```
  1 tcgaggatcc 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 ttgctagctc ga
```

4) Validate the insert by DNA sequencing.
   Forward Sequencing Primer: TTTGCACCATTCTAAAGAAT
   Reverse Sequencing Primer: AAACCTCTTACATCAGTTAC

Methods

1) Bacterial culture: the miRNA cloning and expression vector is provided as bacterial glycerol stock. Individual colonies can be obtained by culturing in an LB-ampicillin plate.
2) Plasmid isolation: we recommend EndoFree Plasmid Kits (QIAGEN).
3) Transfection into target cells: we recommend Lipofectamine 2000 (Invitrogen).
4) Stable selection: 48 hrs post-transfection, select stable clones by green fluorescence sorting or Puromycin resistance in 1-10 µg/mL Puromycin-containing medium.

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. Johnson, S. M., H. Grosshans, J. Shingara, M. Byrom, R. Jarvis, A. Cheng, E. Labourier, K. L. Reinert, D. Brown and F. J. Slack (2005) *Cell* **120**: 635-47.
4. Kim, V. N. (2005) *Mol Cells* **19**: 1-15.
5. Lee, R. C., R. L. Feinbaum and V. Ambros (1993) *Cell* **75**: 843-54.
6. Lee, Y., K. Jeon, J. T. Lee, S. Kim and V. N. Kim (2002) *Embo J* **21**: 4663-70.
7. Yi, R., Y. Qin, I. G. Macara and B. R. Cullen (2003) *Genes Dev* **17**: 3011-6..

Appendix

Vector Features:

130 ~ 525: EF-1α Promoter
558 ~ 1342: human β-globin intron
774 ~ 779: BamHI
1084 ~ 1089: NheI
1370 ~ 2692: GFP-Puro Fusion (GFP: 1370 ~ 2086; Puro: 2093 ~ 2692)
2712 ~ 2842: SV40 PolyA
3051 ~ 3911: Ampicllin Resistance

Plasmid Sequence:

CCCAACTTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACT
AAAGAATTACAAAAACAAATTACAAAATTCAAAATTTTATCGATGCCTCCCCGTCACCACCCCCCCCAACCCGCCCCGACCGGAG
CTGAGAGTAATTCATACAAAAGGACTCGCCCCTGCCTTGGGGAATCCCAGGGACCGTCGTTAAACTCCCACTAACGTAGAACCCA
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CGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAG
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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com