miRNASelect™ pEP-miR Cloning and Expression Vector

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

QUANTITY: 2 vectors; each contains 100 µL of bacterial glycerol stock

Components

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

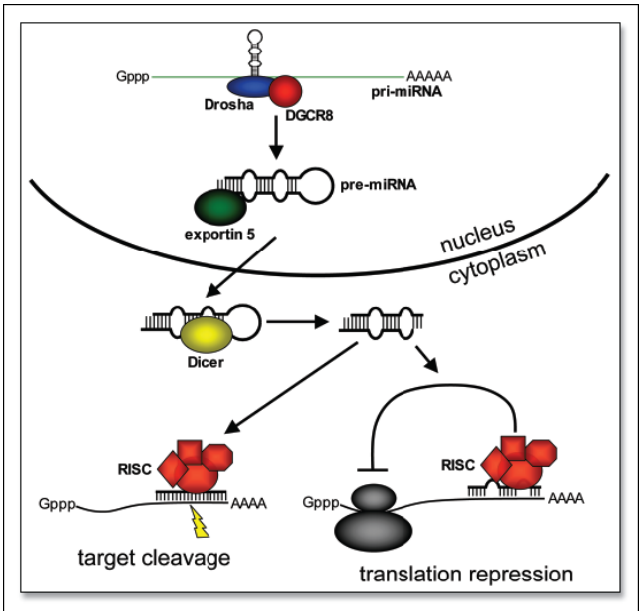

Figure 1. miRNA Biogenesis and function

The miRNASelect™ pEP-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
- **Puromycin resistance marker -** to monitor cells positive for expression and stable selection
- **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*

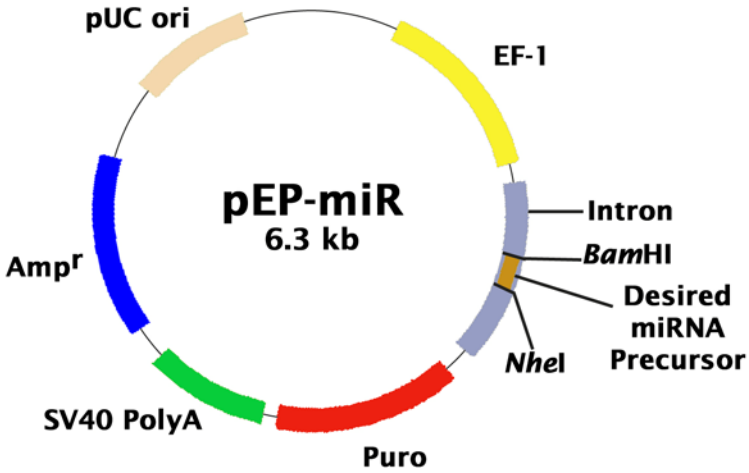

Figure 2. Schematic representation of pEP-miR cloning and expression vector.

Cell Biolabs' miRNASelect™ pEP-miR Null Control Vector is similar to the pEP-miR vector except it does not contain any miRNA precursor or the BamHI and NheI cloning sites (Figure 3).

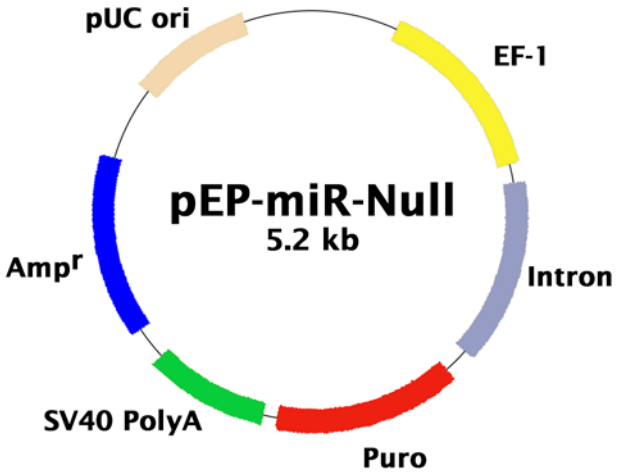

Figure 3. Schematic representation of pEP-miR null control vector.

miRNA precursor cloning sites: BamHI-1.1 kb -NheI

```
   1 tcgaggatcc ctttaagtct tctgaggagc atggacctgc tagactgcaa gctgctgcat
  61 tctgaggagc agggacctgc taaactgcaa gctgccgcag gcggagattt ttgcctgttt
 121 ggtttatttt gttaccactg agtccccagc tctgagaaca gagcgaggca tgtgatcgat
 181 gcatcgtaac tgttgagcaa atgaatgctg agctgtgatg gcaccgacgt gtccggggag
 241 aggacgcacc cggctgtgtg gacatgtgcc cagggcccag gacagcgcca cggaagagga
 301 cacacccggc tgtgtggaca tgtgcccagg gcccgggaca gcgccacgga agaggacgca
 361 cccggctgtg tgcacatgtg cccagggccc gggacagcgc cacggaagag gacgcacccg
 421 gctgtgtgca catgtgccca gggcccggga cagcgccacg gaagaggacg cacccggctg
 481 tgtgcacatg tgcccagggc ccgggacagc gccacggaag aggacgcacc cggctgtgtg
 541 gacatgtgcc cagggcccgg gacagcgcca cggaagagga cgcacaggac agcgccacgg
 601 aagaggacgc acccggctgt gtgcacatgt gcccagggcc cgggacagcg ccacggaaga
 661 ggacgcaccc ggctgtgtgc acatgtgccc agggcccggg acagcgccat ggaagaggac
 721 gcacccggct gtgtgcacat gtgcccaggg cccgggacag cgccacggaa gaggacgcac
 781 ccggctgtgt gcacatgtgc ccagggcccg ggacagcgcc acggaagagg atgcacccgg
 841 ctgtgtggac atgtgcccag ggcccgggac agcgccacgg aagaggacgc acccggctgt
 901 gtggacatgt gcccagggcc cgggacagcg ccacggaaga ggacgcaccc ggctgtgtgg
 961 acatgtgccc agggcccggg acagcgccac ggaagaggac gcacaggaca gcgccacgga
1021 agaggacgca cccggctgtg tggcagggga ggttcccaag agcaggctca gggctctggg
1081 gtctgagtga gggctctcca gccaggctag ctcga
```

Note: pEP-miR Cloning and Expression Vector should NOT be used as a transfection control vector, because the vector itself contains 1.1 kb of mir-941 precursor sequence between the BamHI and NheI sites. For transfection control, we recommend pEP-miR Null Control Vector.

miRNA Precursor Cloning

All of our premade human 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: `tcctcagccg tcgcttcatg`
Reverse Sequencing Primer: `gtgtggggaa actccatcgc`

Methods

1) Bacterial culture: the miRNA cloning and expression vector and the null control vector are provided as bacterial glycerol stocks. 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 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..

Recent Product Citation

Mazzacurati, L. et al. (2015). Use of miRNA response sequences to block off-target replication and increase the safety of an unattenuated, glioblastoma-targeted oncolytic HSV. *Mol Ther.* **23**:99-107.

Warranty

this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

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