Product Data Sheet

miRNASelect™ pEGP-mmu-mir-546 Expression Vector

CATALOG NUMBER: MMU-MIR-546 **STORAGE:** -80ºC

QUANTITY: 100 µL of bacterial glycerol stock

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 (Fig. 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.

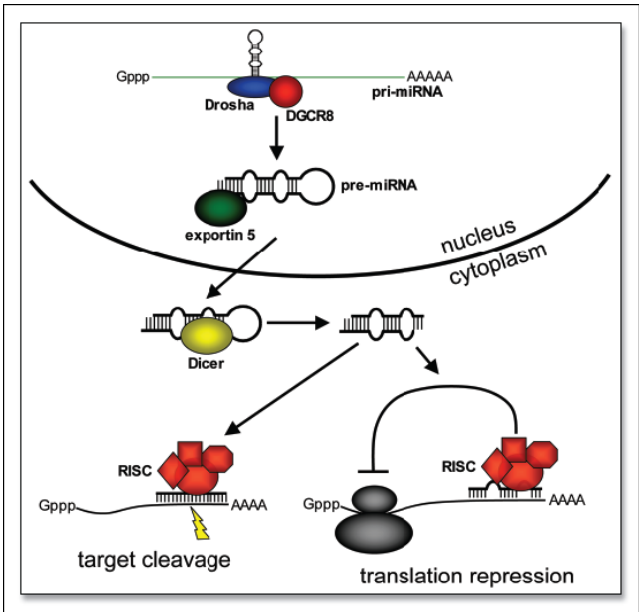

Figure 1. miRNA Biogenesis and function

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' microRNA precursor vectors express each individual mouse miRNA precursor in its native context while preserving putative hairpin structures to ensure biologically relevant interactions with endogenous processing machinery and regulatory partners, and that leads to properly cleaved microRNAs.
Each individual miRNA precursor is cloned between BamHI and Nhe I sites (Figure 2).

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*

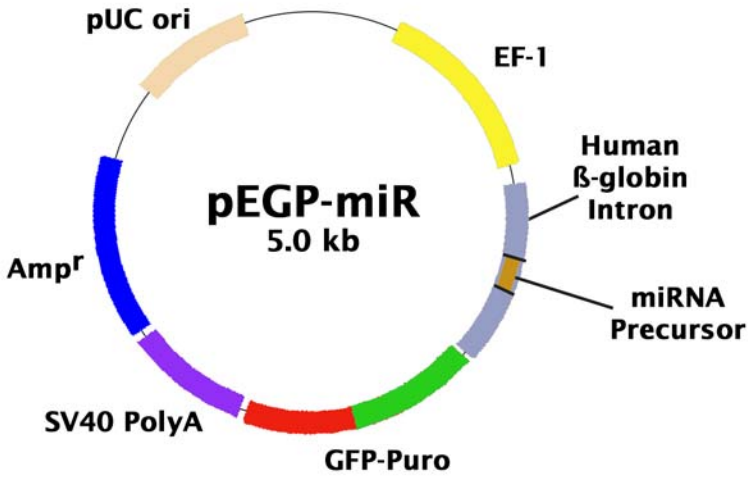

Figure 2. Schematic representation of pEGP-miR expression vector.

miRNA precursor sequence:

CGATTAGTTCTCGAGGATCCGACTGGAGCCCTGTTACTTCTCCATGAAACCCCAGCTCTCTAGG
CCTGACTGCCACCAGAGTCCTCTTACCACCTGCAGTCTGCCACAAAGATGCCATATCAACTCCT
CTCTTCCCAAAACTAAACAGGATCTATCATGGTGGCACGGAGTCAGCTAGATGTTGTCTCTTGC

TATCCCTGTGCTAGTTCCATGTTTTAGAAGCAAAGTGATACTGGGGAGGAGAGGAGGGCAAGTG
AGAGGAGCCAGTGCCTCCTGAAGCCCTTGCTCCACTGCCACCAGCCCGCCTGCCCCAGTGCAGA
CTGGTCTTCAGGAGTCAGTAGACCACAAGTCGCTAGCTCGAGCTTTTGGAG

Method

1) Bacterial culture: the microRNA precursor construct 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..

Warranty

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

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