## Certificate of Analysis

# pmirGLO Dual-Luciferase miRNA Target Expression Vector:

| Cat.# | Size |
|---|---|
| E1330 | 20µg |

Part# 9PIE133
Revised 10/09

**Cat.# E1330 contains:**

| Part No. | Name | |
|---|---|---|
| E133A | pmirGLO Vector | 20µg |
| C838A | Oligo Annealing Buffer | 1ml |

**Description:** The pmirGLO Dual-Luciferase miRNA Target Expression Vector[a–e] is designed to quantitatively evaluate microRNA (miRNA) activity by the insertion of miRNA target sites 3´ of the firefly luciferase gene (*luc2*). These target sites can be introduced by cloning putative miRNA binding sites alone, or the 3´ untranslated region (UTR) of a gene of interest, to study the influence of these sites on transcript stability and activity. Firefly luciferase is the primary reporter gene; reduced firefly luciferase expression indicates the binding of endogenous or introduced miRNAs to the cloned miRNA target sequence. This vector is based on Promega dual-luciferase technology, with firefly luciferase (*luc2*) used as the primary reporter to monitor mRNA regulation and *Renilla* luciferase (*hRluc-neo*) acting as a control reporter for normalization and selection. This vector contains the following features:

- Human phosphoglycerate kinase (PGK) promoter provides low translational expression, which is advantageous when reduction of signal is the desired response. The PGK promoter is a nonviral universal promoter, which functions across cell lines (yeast, rat, mouse and human).
- Firefly luciferase reporter gene (*luc2*) inversely reports miRNA activity in mammalian cells.
- Multiple cloning site (MCS) is located 3´ of the firefly luciferase reporter gene (*luc2*).
- Humanized *Renilla* luciferase-neomycin resistance cassette (*hRluc*-neo) is used as a control reporter for normalization of gene expression and stable cell line selection.
- Amp$^r$ gene allows bacterial selection for vector amplification.
- SV40 late poly(A) signal sequence is positioned downstream of *luc2* to provide efficient transcription termination and mRNA polyadenylation.
- Synthetic poly(A) signal/transcription stop site.

**Concentration:** 1µg/µl in 10mM Tris-HCl, 1mM EDTA; final pH 7.4.

**GenBank® Accession Number:** FJ376737.

**Storage Conditions:** See the storage temperature and expiration date on the Product Information Label.

## Quality Control Assays

### Functional Assays

**Identity Assay:** The vector has been sequenced completely and has 100% identity with the published sequence available at: **www.promega.com/vectors/**

**Restriction Digestion:** The functional purity of this vector DNA is verified by complete digestion with restriction enzymes at the optimal temperature for 1 hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

### Contaminant Assays

**Contaminating Nucleic Acids:** RNA, single-stranded DNA and chromosomal DNA are not evident in specified quantities of this vector as determined by agarose gel electrophoresis.

**Nuclease Assay:** Following incubation of 1µg of this vector in Restriction Enzyme Buffer at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

**Physical Purity:** $A_{260}/A_{280}$ ≥1.80, $A_{260}/A_{250}$ ≥1.05.

Signed by: J. Stevens

J. Stevens, Quality Assurance

[a]READ THIS FIRST BEFORE OPENING PRODUCT
The sale of this product and its use by the purchaser are subject to the terms of a limited use label license, the full text of which is shipped with this product and also available at: **www.promega.com\LULL**. That text must be read by the purchaser prior to opening this product to determine whether the purchaser agrees that all use of the product shall be in accordance with the license terms. If the purchaser is not willing to accept the terms of the limited use label license, Promega is willing to accept the return of the unopened product and provide the purchaser with a full refund. However, if the product is opened for any reason, then the purchaser agrees to be bound by the terms of the limited use label license.
[b]U.S. Pat. No. 5,670,356.
[c]Australian Pat. No. 2001 285278 and other patents pending.
[d]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.
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### Features List and Map for the pmirGLO Vector

| | |
|---|---|
| SV40 late poly(A) signal | 106–327 |
| SV40 early enhancer/promotor | 426–844 |
| *hRluc*-neo fusion protein coding region | 889–2664 |
| Synthetic polyadenylation signal | 2728–2776 |
| β-lactamase (Amp$^r$) coding region | 3037–3897 |
| *Col*E1-derived plasmid origin of replication | 4052–4088 |
| Human phosphoglycerate kinase promoter | 5094–5609 |
| *luc2* reporter gene | 5645–7297 |
| Multiple cloning site (MCS, Figure 1) | 7306–7350 |

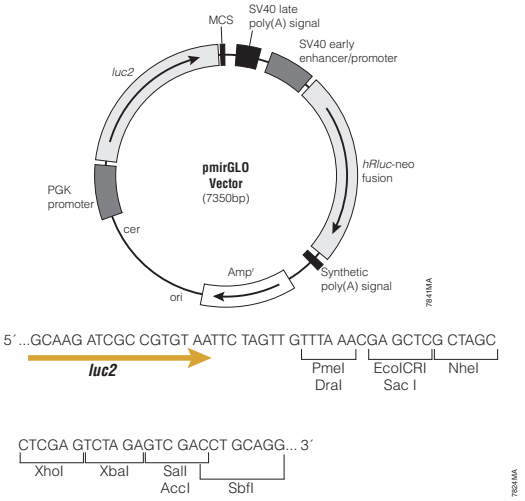


Figure 1. pmirGLO Vector multiple cloning site.

## I. Sample Protocol

### A. Vector Cloning

1. Design oligonucleotides: Order oligonucleotide pairs that contain the desired miRNA target region and, when annealed and ligated into the pmirGLO Vector, result in the miRNA target region in the correct 5´ to 3´ orientation. Insure that the overhangs created by oligonucleotide annealing are complementary to those generated by restriction enzyme digestion of the pmirGLO Vector in Step 2. Add an internal restriction site to your oligonucleotides for clone confirmation (e.g., NotI in Figure 3 creates a ~125bp insert when digested with NotI because of a NotI site at position 93 in the vector).
2. Digest vector: Linearize the pmirGLO Vector with the appropriate restriction enzymes to generate overhangs that are complementary to the annealed oligonucleotide overhangs.
3. Anneal oligonucleotides: Dilute both oligonucleotides (supplied by user) to 1µg/µl. Combine 2µl of each oligonucleotide with 46µl of Oligo Annealing Buffer. Heat at 90°C for 3 minutes, then transfer to a 37°C water bath for 15 minutes. Use the annealed oligonucleotides immediately, or store at –20°C.

### B. Ligation and Transformation

1. Dilute annealed oligonucleotides 1:10 in nuclease-free water to a final concentration of 4ng/µl per oligonucleotide. Ligate 4ng of annealed oligonucleotides and 50ng of linearized vector using a standard ligation protocol. Transform ligated pmirGLO Vector using high-efficiency JM109 competent cells (e.g., Cat.# L2001).
2. Select clones on ampicillin-containing plates, then select clones containing the oligonucleotides by digesting miniprep-purified DNA (e.g., purified using the PureYield™ Plasmid Miniprep System, Cat.# A1221) using the unique restriction site in the oligonucleotide pair. The purified plasmid DNA can be transfected directly or expanded to generate more DNA.

Additional information about annealing, ligation, transformation and oligonucleotide design can be found in the *GeneClip™ U1 Hairpin Cloning Systems Technical Manual*, #TM256, which is available at: **www.promega.com/tbs/**

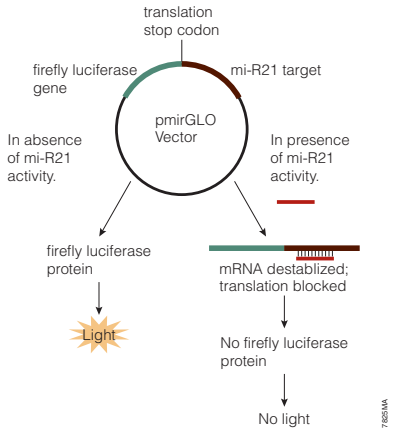


Figure 2. Mechanism of action of the pmirGLO Vector.

| | PmeI | NotI internal site | mi-R21 target sequence | XbaI |
|---|---|---|---|---|
| **mi-R21 sense, PmeI and XbaI** | 5´ **AAAC** TA | GCGGCCGC | TAGT TCAACATCAG*TCT*GATAAGCTA | **T** 3´ |
| **mi-R21 mismatch sense, PmeI and XbaI** | 5´ **AAAC** TA | GCGGCCGC | TAGT TCAACATCAG*AA*GATAAGCTA | **T** 3´ |

| | XbaI | mi-R21 target sequence | NotI internal site | PmeI |
|---|---|---|---|---|
| **mi-R21 antisense, PmeI and XbaI** | 5´ **CTAGA** | TAGCTTATC*AGA*CTGATGTTGA ACTA | GCGGCCGC | TA **GTTT** 3´ |
| **mi-R21 mismatch antisense, PmeI and XbaI** | 5´ **CTAGA** | TAGCTTATC*TT*CTGATGTTGA ACTA | GCGGCCGC | TA **GTTT** 3´ |

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Figure 3. Sample oligonucleotides for mi-R21.

### C. An Example of Detecting mi-R21 Activity Using the pmirGLO Vector:miR-21 Construct

An overview describing the use of the pmirGLO Vector to interrogate endogenous mi-R21 microRNA is shown in Figure 2.

The presence of broadly endogenous microRNA mi-R21 was monitored in HeLa cells. Constructs contained either an exact match to the 21bp mi-R21 target sequence or a mismatched version of that target site (1) as well as PmeI, XbaI and NotI restriction sites (Figure 3; mismatched sequence is in italics). Twenty-four hours after transfection with the mi-R21 pmirGLO Vector constructs, cells were analyzed for luciferase activity using the Dual-Glo® Luciferase Assay System (Cat.# E2920) and a MicroLumatPlus LB96V luminometer (Berthold). Normalized firefly luciferase activity (firefly luciferase activity/*Renilla* luciferase activity) for each construct was compared to that of the pmirGLO Vector no-insert control. For each transfection, luciferase activity was averaged from six replicates.

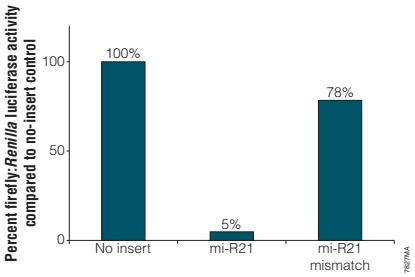


Figure 4. Normalized luciferase activity using the pmirGLO Vector with an mi-R21 target sequence.

## II. Reference

1. Zeng, Y. and Cullen, B.R. (2003) Sequence requirements for micro RNA processing and function in human cells. *RNA* **9**, 112–23.

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