Certificate of Analysis

pmirGLO Dual-Luciferase miRNA Target Expression Vector:

Cat.#	Size
E1330	20µg

Part# 9PIE133
Revised 8/16

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,b] is designed to quantitatively evaluate microRNA (miRNA) activity by inserting 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 a 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: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Signed by:

R. Wheeler, Quality Assurance

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[b]U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

Promega

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

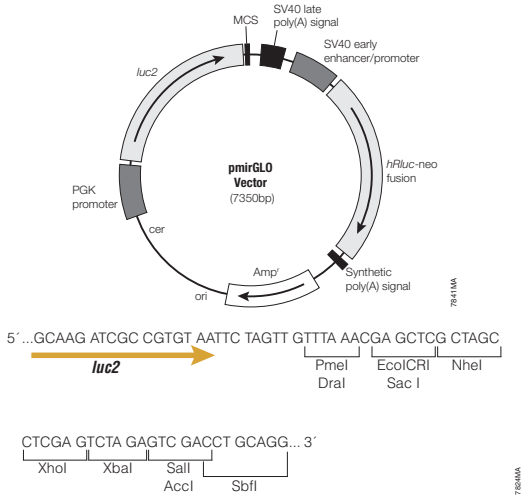

Figure 1. pmirGLO Vector multiple cloning site.

1. Sample Protocol

1.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. Ensure 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 ~140bp insert when digested with NotI due to 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, and then transfer to a 37°C water bath for 15 minutes. Use the annealed oligonucleotides immediately, or store at –20°C.

1.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, and 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/protocols/**

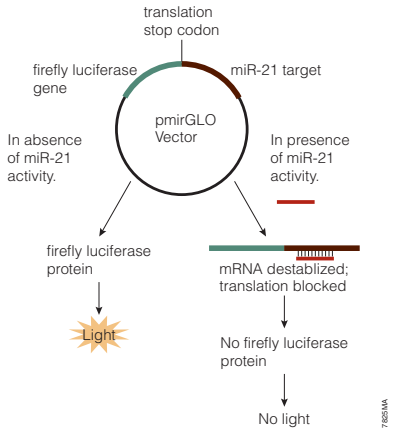

Figure 2. Mechanism of action of the pmirGLO Vector.

	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 antisense, PmeI and XbaI	3´ **TTTG** AT	CGCCGGCG	ATCA AGTTGTAGTC***AGA***CTATTCGAT	**AGATC** 5´

	PmeI	NotI internal site	mi-R21 target sequence	XbaI
mi-R21 mismatch sense, PmeI and XbaI	5´ **AAAC** TA	GCGGCCGC	TAGT TCAACATCAG***AA***GATAAGCTA	**T** 3´
mi-R21 mismatch antisense, PmeI and XbaI	3´ **TTTG** AT	CGCCGGCG	ATCA AGTTGTAGTC***TT***CTATTCGAT	**AGATC** 5´

7826MB

Figure 3. Sample oligonucleotides for mi-R21.

1.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 bold 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.

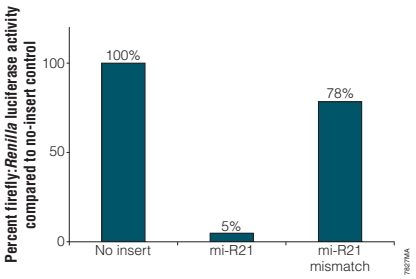

Figure 4. Normalized luciferase activity using the pmirGLO Vector with an mi-R21 target sequence.

2. 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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