ORIGENE
Your Gene Company

MicroRNA Expression Plasmids

Application Guide

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Package Contents and Related Products

The following components are included:

- One (1) vial containing the cDNA clone as 10 ug lyophilized miRNA plasmid DNA.
- Forward (VP1.5) and reverse (XL39) DNA vector sequencing primers; dried onto the bottom of screw cap tubes.

Store at room temperature. Once DNA is re-suspended in water, store at -20ºC.

The microRNA expression clone is shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, clones are guaranteed to be stable for 12 months.

Related, Optional Reagents

Competent *E. coli* cells
LB agar plates with kanamycin, 25 μg/ml
LB broth (10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl. Adjust pH to 7.0 with 1 N NaOH)
DNA purification reagents

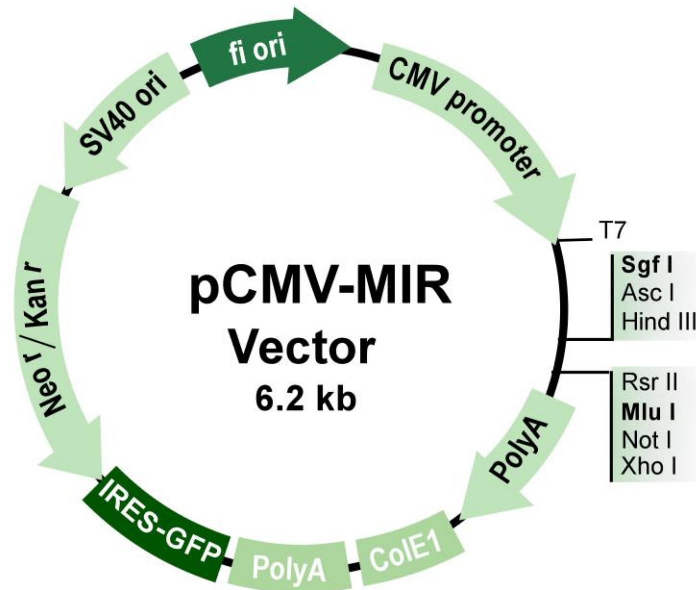
Related OriGene Products

PCMV-MIR plasmid	
TrueClone™ full length cDNA clones	http://www.origene.com/cdna
HuSH™ shRNA Plasmids	http://www.origene.com/shRNA
Transfection Reagents	http://www.origene.com/cdna/transfection.mspx
TissueScan	http://www.origene.com/tissuescan

Cloning vector:

All miRNA precursors are cloned into pCMV-MIR vector via SgfI and MluI site.

Vector map for pCMV-MIR



The empty vector (Cat# PCMVMIR) is the perfect control for the miRNA expression plasmids.

Multiple cloning sites of the pCMV6-MIR vector

pCMV-MIR

BamH I · *Sgf I* · *Asc I*

CTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCGCCGCGATCGCCGGCGCGCCAGATCT

Rsr II · *Mlu I* · *Not I* · *Xho I*

CAAGCTTAACTAGCTAGCGGACCG ACG CGT ACG CGG CCG CTC GAG CAG AAA CTC ATC TCA GAA GAG

EcoR V · *Pme I*

GAT CTG GCA GCA AAT GAT ATC CTG GAT TAC AAG GAT GAC GAC GAT AAG GTT TAA ACGGCCGGCC

Introduction

MicroRNAs (miRNA) are a class of small non-coding RNA genes, which are at the size of 19-23nt after maturation. MicroRNAs have been found in all species and play important roles in regulating translation and degradation of their target messenger RNA by binding to their complementary region in the mRNA. MiRNAs are processed from stem-loop sequences (pre-mir), which in turn are excised from much longer precursors (pri-mir). It is believed that pri-mirs of miRNAs are transcribed by RNA polymerase II, and the transcripts are capped and polyadenylated.
OriGene MicroRNA precursor contains pre-micoRNA (60-70nt) with 250-300 nts up- and down-stream flanking sequence. It was amplified from human genomic DNA and cloned into OriGene's pCMV6-Mir Vector. The expression of MicroRNA precursor is driven by CMV promoter and with human growth factor I poly(A) tailing signal.

Experimental protocols

Primer Design and PCR Amplification

The pre-microRNA (60-70nts) and their flanking 300nts sequence were PCR amplified from human genomic DNA using primers with SgfI and MluI adaptors. Then confirmed PCR products were cloned into pCMV-mir vector.

Forward primer with Sgf I
5' GAG**GCGATCGC**CNNNNNNNNNNNNNNNNNNNNNNN 3'

Reverse primer with Mlu I
5' GCG**ACGCGT**NNNNNNNNNNNNNNNNNNNNNNNNN 3'

Protocol for Transient Transfection

A sample protocol is listed here for experiments performed in 24-well plates. If performing experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate. See Table 2 for more details.

1. Preparation of cells
    a. Plate ~5x10^4 adherent cells or ~5x10^5 suspension cells per well 24 hrs prior to transfection.
2. Preparation of the MegaTran 1.0/DNA Complexes:
    a. (Prepare immediately prior to transfection)
    b. Dilute 1 µg of DNA in 100 uL of Opti-MEM I (Gibco 51985). Vortex gently.
    c. Add 3 uL of MegaTran 1.0 to the diluted DNA (not the reverse order) and vortex the solution immediately for 10 seconds.
    d. Incubate for 10 minutes at room temperature.

Note: We recommend starting with the ratios of MegaTran 1.0 and DNA listed in Table 2; however, subsequent optimization may further increase the transfection efficiency.

3. Transfection
    a. Gently add the MegaTran 1.0/DNA mixture from step 2 to each well (already containing about 900 uL culture medium). Generally, the volume of the MegaTran 1.0/ DNA mixture represents 1/10 of the total volume of the culture medium. Gently rock the plate to achieve even distribution of the complexes. Incubate at 37°C for 24-48 hrs.

Note: The above incubation is designed for transfection without a media change. If a media change is preferred, incubate for 30 minutes (if centrifugation is possible) or for 3-4 hrs (if centrifugation is not possible). Replace the media with the fresh complete growth media. Incubate for 24-48 hrs. Expression of the transgene can often be detected in as little as 24-48 hrs post-transfection.

Table 1. Recommended starting transfection conditions for MegaTran 1.0

Tissue Culture Vessel	Growth area, cm^2/well	µg of DNA	Ratio of MegaTran:DNA
96-well plate	0.3	0.05-0.25	3:1
24-well plate	2	0.25-1.25	3:1
12-well plate	4	0.5-2.5	3:1
6-well plate	9.5	1-5	3:1
35 mm plate	8	1-5	3:1
60 mm plate	20	2-10	3:1
100 mm plate	60	5-25	3:1

Protocol for Stable Transfection:

Perform a transfection as described above (protocol for transient transfection). Twenty-four hrs post-transfection, passage the cells (at 1:10 or higher dilution) into fresh growth medium containing selective agent. A mock transfection should be performed in parallel as a control. Grow and passage the cells as necessary, maintaining selection pressure by keeping the selective agent in the growth medium. After 1-2 weeks, a large number of the cells will be killed; the cells that remain growing in the selective medium have retained the expression plasmid, which stably integrates into the genome of the targeted cells. Monitor the mock control to ensure the cells are dying.

Troubleshooting and Frequently Asked Questions

For questions not addressed here, please contact OriGene's Technical Support professionals. You may dial 888-267-4436 from any US location, or 301-340-3188 outside the US. E-mail inquiries to techsupport@origene.com are also invited.

Frequently Asked Questions

1. What is cloned into the miRNA clones?
   OriGene's miRNA clone inserts is composed of pri-mir (60- 70nts) and its 250-300nts flanking genomic sequence on both sides. In general, the flanking sequence is required for correct pri-mir expression and mature microRNA processing.

2. How do I know the miRNA is being expressed?
   To test the miRNA expression, isolate the small RNAs from transfected cells. Then perform RT-PCR and qPCR using specific miRNA primers to measure the expression of miRNA expression. OriGene randomly selected three over-expression plasmids and demonstrated the over-expression of intended miRNA in all cases using qPCR.

3. Are the miRNA clones fully sequenced? Where can I find the sequence information?
   Yes, they are. All miRNA clones were sequenced to ensure that the pre-mir sequences match reference sequence in miRBase. http://microrna.sanger.ac.uk. Please note that the exact flanking sequence of a miRNA clone may differ from the NCBI reference with respect to biological polymorphisms. This should not affect the function of the mature miRNA.

4. What sequencing primers should I use?
   VP1.5, 5' GGACTTTCCAAAATGTCG 3' (Tm=51C) and XL39, 5' ATTAGGACAAGGCTGGTGGG 3' (Tm=60C) can be used to sequence from the 5' and 3'-end of the insert, respectively. Both plasmids are provided with miRNA expression clone.

5. How can I monitor the transfection efficiency?
   In OriGene we monitor tGFP signal for transfection. PCMV-mir vector contains an IRES-tGFP reporter gene, which is driven by SV40 promoter and is down-stream of neomycin gene. Independent tGFP and pre-mir expression from different promoters is designed to minimize the interference between the two expression cassettes and therefore tGFP can truly reflect the transfection efficiency.

6. Can I create stable cell line with the miRNA plasmids? What is the selection marker?
   Yes. You can create the stable line using G418 selection or sort the transfected cells using GFP expression as a marker.

7. Is it true that only one miRNA is expressed in each plasmid?
   No. About 10% of the miRNA genes are clustered in close region in chromosome and they may be naturally express together. Some of OriGene's miRNA clones cover a region that contains a cluster of miRNA. In the case where more than one miRNA is present, the co-expressing miRNA information is displayed on the website.

8. How is the miRNA vector validated?
   All OriGene's miRNAs match reference sequence in miRBase. http://microrna.sanger.ac.uk. Always note that the exact flanking sequence of a miRNA clone may differ from the NCBI reference with respect to biological polymorphisms.

9. What is the control plasmid for miRNA expression plasmid?
   The empty vector, pCMV-MIR, is the perfect control for miRNA clones.

10. Do the miRNA plasmids come with any controls?
    No. pCMV-MIR vector can be purchased and the cost is $280/10ug.

11. How do I cite this product?
    We recommend that you refer to the product by its specific catalog number and refer to us as OriGene Technologies (Rockville, MD). Furthermore, we'd love to hear from you when your paper is published. Inform us and we will send a gift.