

# 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 -20°C.

- Certificate of Analysis
- Application Guide

*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

HuSH™ shRNA Plasmids

Transfection Reagents

TissueScan

cat#: PCVMIR

<http://www.origene.com/cdna>

<http://www.origene.com/shRNA>

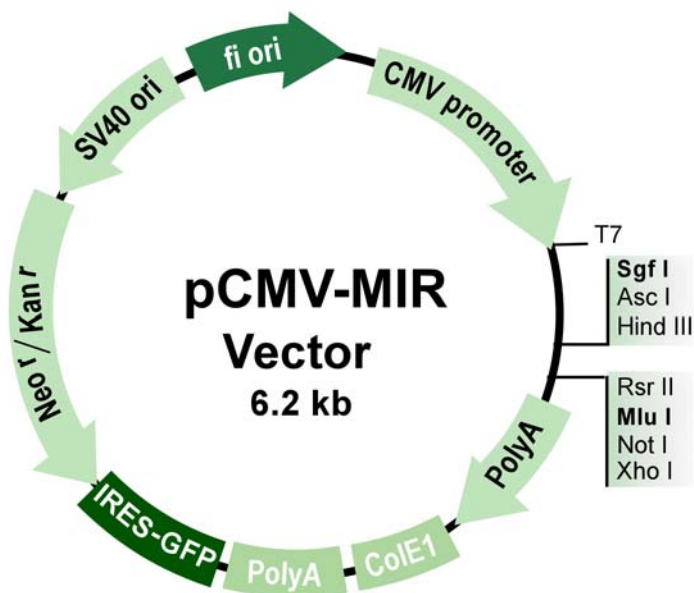
<http://www.origene.com/cdna/transfection.msp>

<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# PCMVIR) is the perfect control for the miRNA expression plasmids.

### Multiple cloning sites of the pCMV6-MIR vector

```

          EcoR I          BamH I Kpn I          Sgf I   Asc I   Bgl II
CTATAGGGCGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCCGCCGATCGCCGGCGGCCAGATCT

Hind III          Rsr II   Mlu I          Not I   Xho I
CAAGCTTAAGCTAGCTAGCGGACCG ACG CGT ACG CGG CCG CTC GAG CAG AAA CTC ATC TCA GAA GAG

          EcoR V          Pme I   Fse 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 precursors contain pre-miRNA (60-70nt) with 250-300 nts up- and down-stream flanking sequence. Each sequence was amplified from human genomic DNA and cloned into OriGene's pCMV6-Mir Vector. The expression of MicroRNA precursor is driven by the 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 300 nts of flanking 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**CNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 3'

Reverse primer with Mlu I

5' GCG**ACGCGT**NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 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  $\sim 5 \times 10^4$  adherent cells or  $\sim 5 \times 10^5$  suspension cells per well 24 hrs prior to transfection.
2. Preparation of the TurboFectin 8.0/DNA Complexes:
  - a. (Prepare immediately prior to transfection)
  - b. Dilute 1  $\mu$ g of DNA in 100  $\mu$ L of Opti-MEM I (Gibco 51985). Vortex gently.
  - c. Add 3  $\mu$ L of TurboFectin 8.0 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 TurboFectin 8.0 and DNA listed in Table 2; however, subsequent optimization may further increase the transfection efficiency.

3. Transfection
  - a. Gently add the TurboFectin 8.0/DNA mixture from step 2 to each well (already containing about 900  $\mu$ L culture medium). Generally, the volume of the TurboFectin 8.0/ 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 TurboFectin 8.0**

Tissue Culture Vessel	Growth area, cm <sup>2</sup> /well	μg of DNA	Ratio of TurboFectin: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](mailto:techsupport@origene.com) are also invited.

### ***Frequently Asked Questions***

**Q: What is the nature of the miRNA insert sequence?**

**A:** OriGene's miRNA inserts are composed of pri-mirs (60- 70nts) and the 250-300nts of flanking genomic sequence on both sides. In general, the flanking sequence is required for correct pri-mir expression and miRNA processing.

**Q: How do I know the miRNA is being expressed?**

**A:** To test the miRNA expression, isolate the total RNA fraction, including small RNAs, from the transfected cells. Then perform RT-PCR with the appropriate linkers to permit miRNA amplification. qPCR from the RT product using miRNA primers specific to the miRNA. OriGene randomly selected three miRNA expression plasmids and validated the expression of the correct miRNA using this method and qPCR-based primers.

**Q: Are the miRNA clones fully sequenced? Where can I find the sequence information?**

**A:** Yes, they are. All miRNA clones were sequenced to ensure that the pre-mir sequences match the reference sequences in miRBase. <http://microrna.sanger.ac.uk>. Please note that the exact flanking sequence of a miRNA clone may differ from the reference due to biological polymorphisms. This should not affect the function of the mature miRNA.

**Q: What sequencing primers should I use?**

**A:** VP1.5, 5' GGACTTTCCAAAATGTCG 3' (Tm=51C) and XL39, 5' ATTAGGACAAGGCTG-GTGGG 3' (Tm=60C) can be used to sequence from the 5' and 3'-end of the insert, respectively. Both plasmids are provided with each miRNA expression clone.

**Q: How can I monitor the transfection efficiency?**

**A:** We recommend using tGFP to monitor the transfection efficiency. The pCMV-mir contains a tGFP reporter that is expressed via an IRES in conjunction with the NEO resistance marker. Expression of tGFP and the pre-mir from different promoters is designed to minimize interference between the two expression cassettes and therefore tGFP can truly reflect the transfection efficiency.

**Q: Can I create stable cell lines with the miRNA plasmids? What is the selection marker?**

**A:** Yes. You can select stable lines using G418 selection or sort the transfected cells using GFP as a marker.

**Q: Is it true that only one miRNA is expressed in each plasmid?**

**A:** Not necessarily. About 10% of miRNA genes are tightly clustered on the chromosome and they naturally express together. Some of OriGene's miRNA clones contain miRNA cluster sequences and are predicted to express multiple miRNAs. In cases where more than one miRNA is within our insert sequence, the information is displayed on our website.

**Q: What is the quality control for the miRNA constructs?**

**A:** All OriGene's miRNAs are sequence-confirmed match the reference sequence in miRBase. <http://microrna.sanger.ac.uk>. Please note that the exact flanking sequence of a miRNA clone may differ from this reference due to naturally occurring polymorphisms.

**Q: What is the negative control plasmid for miRNA clones?**

**A:** The empty vector, pCMV-MIR, is the recommended control for our miRNA clones.

**Q: Do the miRNA plasmids come with any controls?**

**A:** Each miRNA plasmid is provided with two sequencing primers to allow verification after DNA amplification. We do not include the pCMV-MIR vector but this can be purchased separately and the cost is \$280/10ug.

**Q: How do I cite this product?**

**A:** 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.