

Coexpress MicroRNAs with Fluorescent Proteins

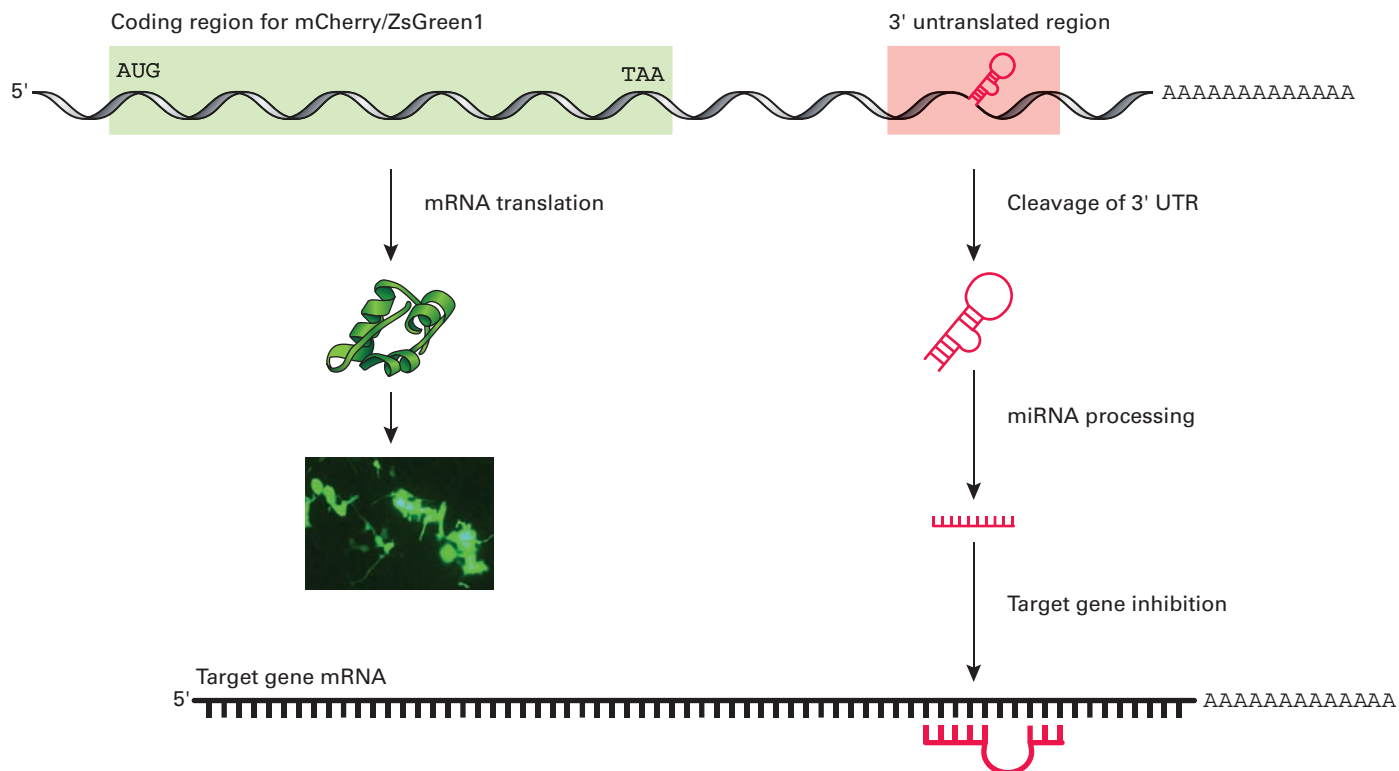
These vectors coexpress your miRNA with either the mCherry or ZsGreen1 fluorescent protein marker

- **High-level miRNA and fluorescent protein coexpression**
- **Simultaneously verify miRNA expression and transfection efficiency**
- **Very bright fluorescent proteins**

Clontech offers two new vectors that provide high-level miRNA expression and allow you to verify it with fluorescent protein expression. The **pmR-mCherry** and **pmR-ZsGreen1 Vectors** couple your miRNA expression cassette to a bright red or green fluorescent reporter, for miRNA expression you can see and select.

How Does It work?

Clone your miRNA sequence into the vector's multiple cloning site (MCS) located in the 3' untranslated region of the fluorescent protein's mRNA transcript. This enables both molecules to be expressed simultaneously from the vector's strong CMV promoter. Each vector is equipped with the high-level CMV promoter, a selectable marker, and a fluorescent protein-miRNA expression cassette containing either mCherry or ZsGreen1—our two brightest Living Colors® Fluorescent Proteins (Figure 1). In short, you can clone and express your favorite miRNA, and then select, sort and/or visualize the cells in which it is expressed.



The pmR-mCherry and pmR-ZsGreen1 vectors will coexpress a fluorescent protein and an miRNA sequence that is embedded in the 3' UTR of the vector's mRNA transcript. miRNA expression can be selected for and/or verified in transfected cells by monitoring red or green fluorescence.

miRNA Expression

Once an miRNA sequence is cloned in the multiple cloning site of these vectors, miRNA expression can be delivered into any transfectable cell line. We used the pmR-ZsGreen1 and pmR-mCherry vectors to express several different miRNAs in 293T cells (Figure 2). The miRNA sequences were amplified from human genomic DNA and then cloned into the vectors. The sequences included the indicated miRNA stem-loop along with ~300 bp of flanking DNA. Following transfection into separate cultures, samples of total RNA were prepared and treated with DNase prior to quantifying the expressed miRNAs using Clontech's **Mir-X™ miRNA qRT-PCR SYBR® Kit**. Both vectors produced similarly high levels of expression for each miRNA, which were elevated to a range of values between 75- and >3000-fold over the vector-only controls.

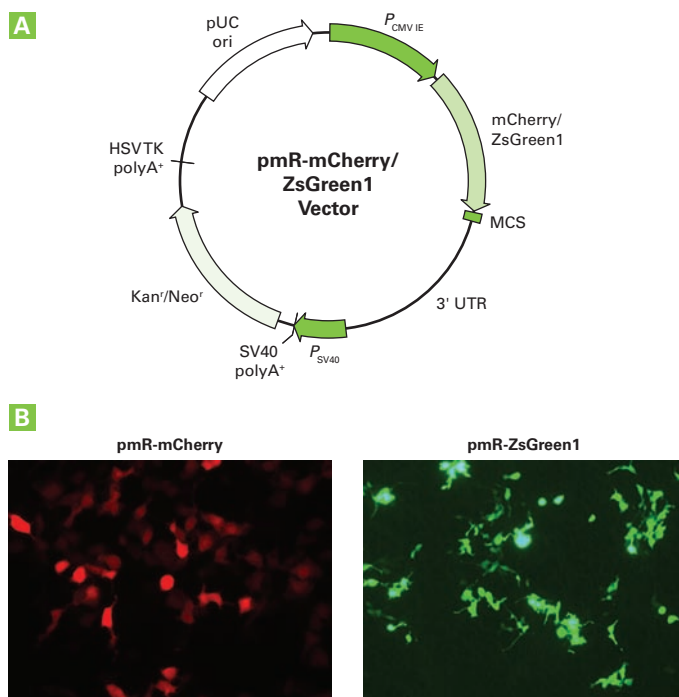


Figure 1. The pmR-mCherry and pmR-ZsGreen1 Vectors. Map of the vectors (**Panel A**). Cells transfected with the vectors exhibit red or green fluorescence (**Panel B**).

miRNA Quantification

Clontech's Mir-X miRNA qRT-PCR SYBR Kit has a diverse variety of applications, as it is able to detect and quantify multiple miRNAs, shRNAs, or mRNA targets in a single RNA sample. The complete, dual-function kit includes a fast and simple, one-step protocol for first-strand cDNA synthesis, as well as the reagents needed for the qPCR of your RNA target using SYBR Advantage® technology.

From verifiable expression to accurate miRNA analysis, Clontech has the highly effective, state-of-the-art tools you need for investigating any miRNA network.

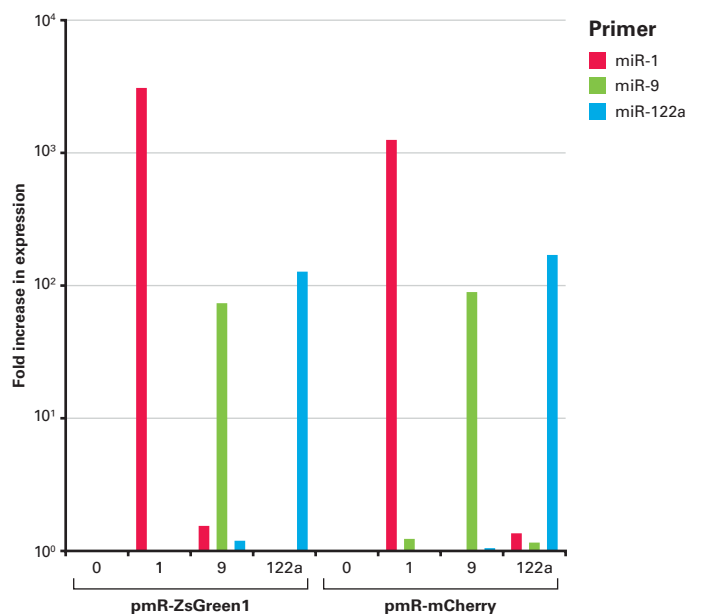


Figure 2. miRNA expression from pmR vectors. DNA sequences for the miR-1, miR-9, and miR-122 miRNAs were cloned into the pmR-ZsGreen1 and pmR-mCherry vectors, and the recombinant plasmids (1, 9, & 122a, respectively), as well as the parental vectors (0), were each transfected into separate cultures of 293T cells. After 48 hr, cells were harvested and the RNA was isolated for Mir-X miRNA qRT-PCR analysis using specific primers and the U6 snRNA as a normalization standard. Each primer was used with each RNA sample, but detected only the corresponding miRNA cognate.

Ordering Information			
Product	Size	Cat. No.	
pmR-ZsGreen1 Vector	20 µg	632541	NEW!
pmR-mCherry Vector	20 µg	632542	NEW!
Mir-X miRNA qRT-PCR SYBR Kit*	200 rxns	638314	NEW!
	600 rxns	638316	

* Includes a Mir-X miRNA First-Strand Synthesis Kit.

Notice to Purchaser

Please see the Advantage® and TITANIUM™ PCR Products, CMV Sequence, Fruit Fluorescent Protein Products, Hot Start Antibody, Living Colors® Fluorescent Protein Products, Molecular Probes, Inc., and PCR licensing statements at www.clontech.com/licensing