Mir-X™ MicroRNA Quantification

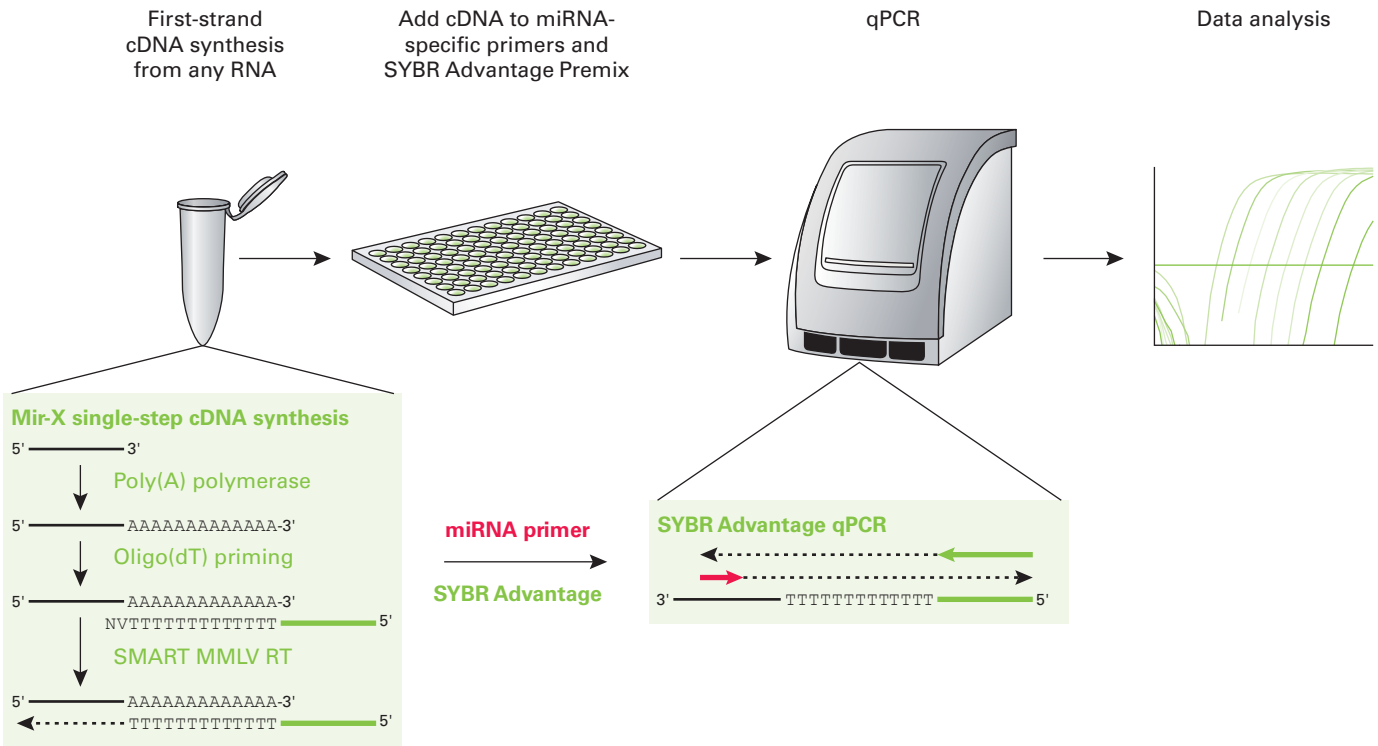
Quickly and accurately quantify your miRNAs and their mRNA targets with this complete qRT-PCR kit.

- **Quantify any miRNA and its target from the same RNA sample**
- **2-kits-in-1: cDNA synthesis and qPCR**
- **Simple, single-step cDNA synthesis reaction**

For the challenging task of unraveling microRNA (miRNA) expression profiles in tissues and cell lines, Clontech scientists have developed a sensitive, reliable, and easy-to-use miRNA quantification system. The **Mir-X miRNA qRT-PCR SYBR® Kits** are complete, dual-function systems for performing first-strand cDNA synthesis and quantitative PCR (qPCR) to precisely measure the level of your favorite miRNAs. The kits are available in economical, large-sized formats that provide 200 or 600 qPCR reactions.

Simple and Sensitive

A simple, single-step reaction uses an optimized mix of poly(A) polymerase and **SMART™ MMLV Reverse Transcriptase** to synthesize first-strand cDNA from your RNA sample. The cDNA is then specifically amplified and quantified by qPCR using your miRNA-specific primer and our **SYBR Advantage® qPCR Premix.** Multiple miRNA species, as well as the mRNA targets of the miRNAs, can be amplified from a single cDNA sample. The system is extremely sensitive and able to detect miRNAs down to 50 copies.



Mir-X miRNA qRT-PCR SYBR Kits use a single-step, single-tube reaction to produce first-strand cDNA, which is then specifically and quantitatively amplified using a miRNA-specific primer and SYBR Advantage qPCR chemistry. In the Mir-X cDNA synthesis reaction, RNAs are poly(A)-tailed using poly(A) polymerase, and then copied using a modified oligo(dT) primer and SMART MMLV Reverse Transcriptase.

Highly Specific Detection

To demonstrate the specificity of Mir-X miRNA quantification, we used a series of 8 highly similar synthetic Let7 miRNA variants (Figure 1). We first spiked each of the Let7 miRNAs into separate samples of yeast polyA+ RNA and generated cDNA using the Mir-X single-tube reaction. We then tested a panel of variant-specific primers with each cDNA sample to determine each primer's ability to specifically and individually quantify the Let7 subtypes in the cDNA sample. Despite the high degrees of similarity among the variants and the primers (Figure 1, Panel A), Mir-X qPCR was highly specific in detecting each Let7 variant (Figure 1, Panel B).

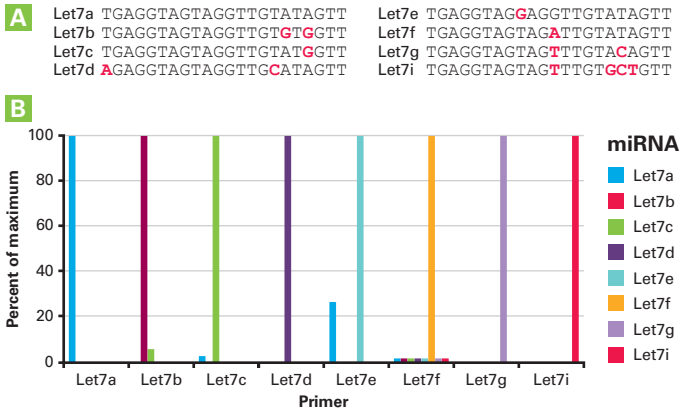


Figure 1. Specific quantification of Let7 miRNA variants. Using miRNA-specific primers (**Panel A**), Mir-X qRT-PCR was able to specifically detect and quantify each member of a series of 8 synthetic Let7 variants that had been spiked into a background of yeast polyA+ RNA (**Panel B**). The primers detected each of their corresponding Let7 miRNA cognates, but did not detect the off-target variants in 63 of 64 possible combinations.

Diverse Research Applications

Since the Mir-X system is able to detect multiple miRNAs, shRNAs, or mRNA targets in a single RNA sample, it can be used for a variety of applications. In principle, any RNA that is, or can be, polyadenylated may be quantified using the Mir-X method.
In mouse embryonic stem cells, we were able to monitor the alterations in expression for a panel of 12 miRNAs that respond to trichostatin A (TSA) treatment (Figure 2).

Exposing aggregated mouse P19 cell clusters to retinoic acid (RA) causes them to acquire neural cell phenotypes, which are accompanied by changes in the cellular miRNA pool. Using the Mir-X system, we tracked the abundance of one such miRNA, miR-9, which was induced by RA and continued to accumulate in these cells following a 5 day exposure to RA (Figure 3).

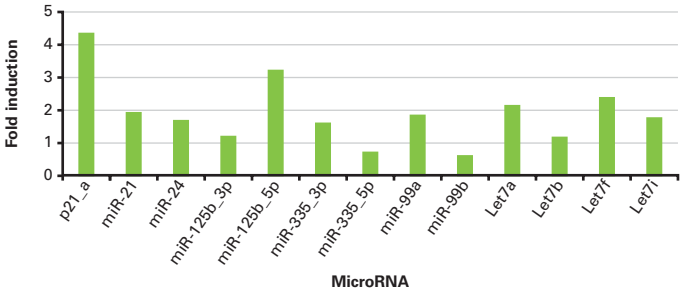


Figure 2. Trichostatin A treatment alters miRNA expression in mouse ES cells. Mouse embryonic stem cells were harvested either prior to or after being treated with trichostatin A (TSA) for 18 hr. RNA was prepared from the cells, and was then analyzed by Mir-X miRNA qRT-PCR using primers specific for the 12 indicated miRNAs and for a p21 control mRNA known to be induced by TSA.

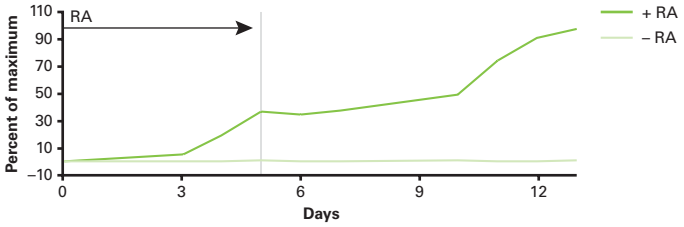


Figure 3. Induction of miR-9 in mouse P19 cells. P19 mouse embryonal carcinoma cells were plated on agarose-coated petri dishes and allowed to form embryoid bodies (EB) in the presence or absence of retinoic acid (RA). After five days of culture, EBs were dissociated with trypsin and replated on tissue culture dishes without RA. Cells were harvested on days 3–7 and 10–13, and the induction of miR-9 was followed and quantified using the Mir-X miRNA qRT-PCR SYBR Kit and primers specific for miR-9 and U6 (as a normalization control).

Summary

Mir-X miRNA qRT-PCR SYBR Kits are complete, dual-function qPCR systems that have the flexibility to monitor the level of your favorite miRNA or any other RNA species in your RNA sample. The single-tube cDNA synthesis is faster and far less complicated than other available methods, while the miRNA qPCR is very sensitive and extremely accurate.

Ordering Information

Product	Size	Cat. No.	
Mir-X miRNA qRT-PCR SYBR Kit*	200 rxns 600 rxns	638314 638316	NEW!
Mir-X miRNA First-Strand Synthesis Kit	20 rxns 60 rxns	638313 638315	NEW!

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

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