TaqMan Pri-miRNA Assays

Detect the origins of microRNA expression

- **Highly specific**—quantitate microRNA transcription from a single genomic locus
- **Fast, simple, and scalable**—two-step RT-qPCR provides high-quality results
- **A new perspective**—measure microRNA expression at the gene level

Introduction

Primary microRNAs (pri-miRNAs) are long noncoding RNA transcripts with at least one (more commonly, multiple) ~65 nt stem-loop precursor microRNA (pre-miRNA) containing mature microRNA (miRNA) sequences (Figure 1). Cleavage of the pri-miRNA transcript eventually results in the release of the biologically active mature miRNA and ultimately the degradation, or more commonly translational repression, of messenger RNA targets.

Assay design and selection

All Applied Biosystems™ TaqMan™ Pri-miRNA Assays are designed using our state-of-the-art Applied Biosystems™ TaqMan™ Assay design algorithms, delivering gold-standard assay performance and data quality. Since pri-miRNA transcripts have not been exhaustively mapped, assays are designed within close proximity to each and every stem-loop sequence represented in the Sanger miRBase data repository, enabling accurate measurement of the primary transcript containing the mature miRNA of interest. As an additional benefit to this approach, each publicly available stem-loop has a matched TaqMan Pri-miRNA Assay and Applied Biosystems™ TaqMan MicroRNA Assay available, enabling RNA sequences produced at either end of the miRNA maturation pathway to be quantified independently.

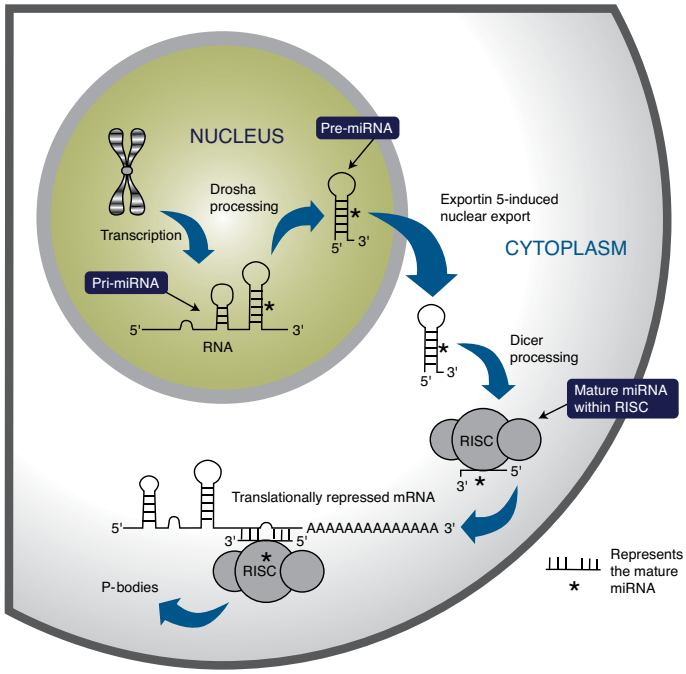

Figure 1. MicroRNA biogenesis. MicroRNA genes are transcribed in the nucleus, giving rise to long primary miRNA transcripts (pri-miRNAs). A pri-miRNA sequence contains at least one (more commonly, multiple) stem-loop sequence containing mature miRNA sequences. The pri-miRNA transcript is processed in the nucleus by cleavage at the base of each stem-loop by Drosha. These precursor stem-loop molecules (pre-miRNAs) are then exported to the cytoplasm where they are processed by Dicer prior to the mature sequence being loaded into the RNA-induced silencing complex (RISC). Each of these maturation steps is potentially a target for regulation.

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The design process starts with mapping the stem-loop sequences known to belong to a pri-miRNA on miRBase to the most current version of the genome for human, mouse, and rat species. Next, 500 bp regions immediately flanking either side of the stem-loop structure are considered for design, and the best-scoring assay is selected (Figure 2). Assays that do not map to a single chromosomal coordinate are heavily penalized, helping to ensure that the final assay selected measures transcription from a single genomic position.

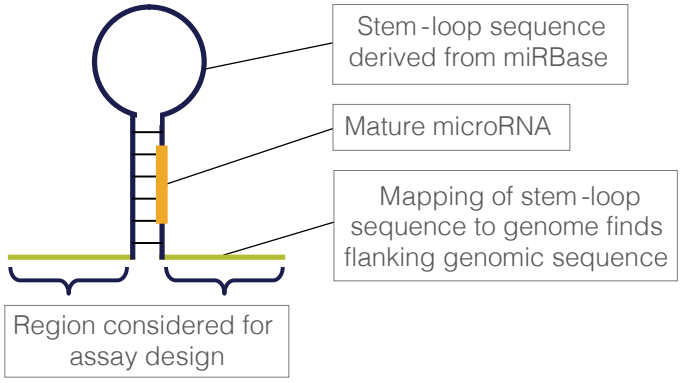

Figure 2. Design approach for TaqMan Pri-miRNA Assays. Stem-loop sequences derived from Sanger miRBase are mapped to the genome. The flanking sequences extending 500 bp on either side of the stem-loop are considered for assay design.

For convenience, both TaqMan Pri-miRNA Assays and TaqMan MicroRNA Assays can be identified, selected, and purchased in parallel through the same online interface. Understanding the spatial relationship between the two assay types is simplified using an alignment map viewer (Figure 3).

Applications

TaqMan Pri-miRNA Assays enable transcription of this gene class to be easily and conveniently quantified with the sensitivity and specificity necessary to discriminate among the many miRNA loci. This is ideal for addressing the following key questions in miRNA expression and functionality:

- **Regulation of miRNA gene transcription**
  11% of mature miRNA sequences can arise from transcription at multiple genomic loci. That is, mature miRNA sequences can arise from multiple stem-loops and thus, multiple pri-miRNA sequences. By using TaqMan Pri-miRNA Assays specific to respective pri-miRNA transcripts, transcriptional changes can be directly correlated with changes detected at the mature level for an miRNA of interest.

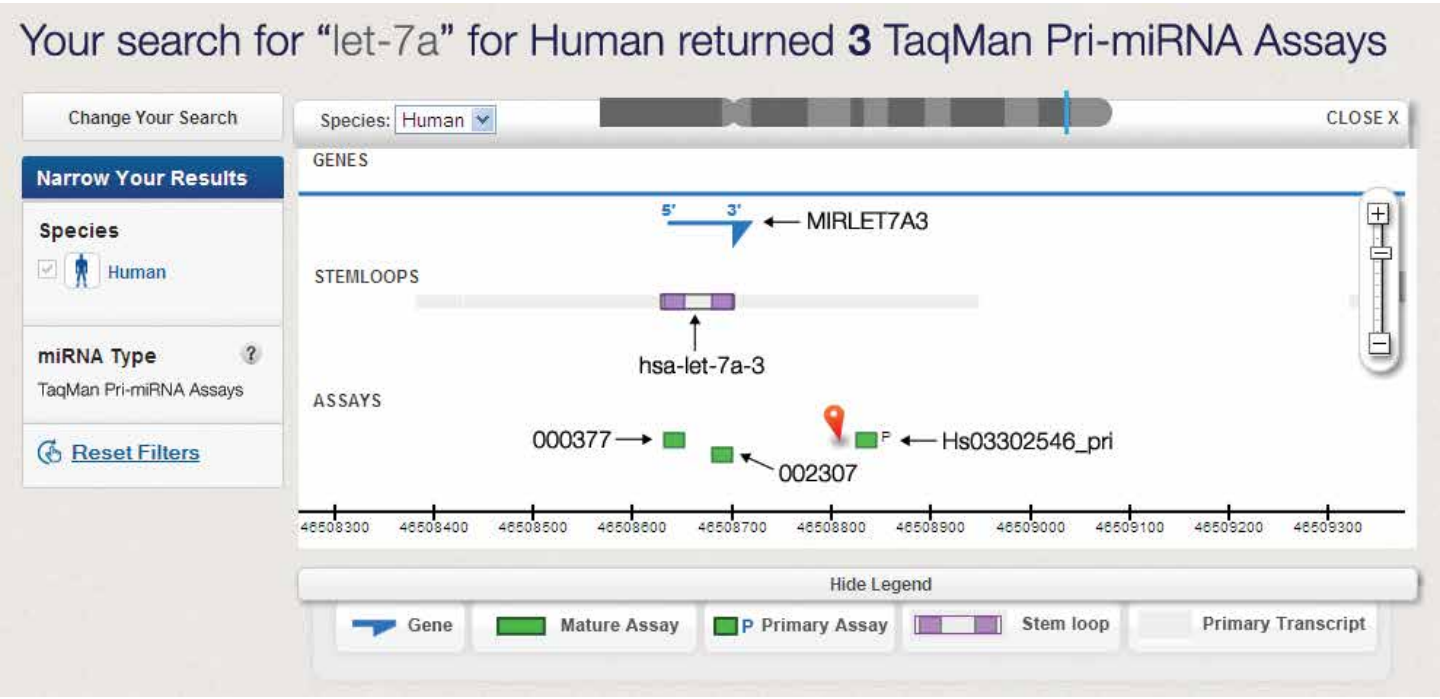

Figure 3. Alignment map viewer. The position of each TaqMan Pri-miRNA Assay is displayed in the context of its stem-loop sequences and mature miRNA sequence, enabling the spatial relationship of each assay within the primary transcript to be easily understood. Gray areas represent regions considered for pri-miRNA assay design. NCBI gene name (MIRLET7A3), miRBase stem-loop name (hsa-let-7a-3), and pri- and mature miRNA assay IDs (000377, 002307, Hs03302546_pri) are shown by mouse-over. The red pin locator indicates the assay that was selected.

- **Regulation of miRNA biogenesis**
  Recent observations indicate that many of the steps in the maturation pathway may be subject to regulation [1,2]. Critical to elucidate, when such regulatory events are responsible for detected changes to mature miRNA levels, transcriptional changes of the primary transcript at the gene level must be ruled out. TaqMan Pri-miRNA Assays are the ideal tool for quantifying the pool of primary transcripts available to the maturation pathway, and when used in conjunction with TaqMan MicroRNA Assays that are specific to their corresponding mature miRNAs, they enable regulatory events occurring at the level of the maturation pathway to be identified and characterized.

- **Mapping of pri-miRNA transcripts**
  MicroRNA gene loci are typically polycistronic, wherein many miRNA sequences are clustered within the same primary transcript. However, the primary structures of many pri-miRNA transcripts are not yet well characterized. Taking advantage of the unique design approach used, the degree of correlation between multiple TaqMan Pri-miRNA Assays enables a unique and rapid approach to mapping miRNA stem-loop clusters.

Assay performance

TaqMan Pri-miRNA Assays deliver the high sensitivity, superior specificity, and wide linear dynamic range for which TaqMan Assays are renowned. Because of the high assay sensitivity, TaqMan Pri-miRNA Assays require minimal sample input: as little as 1 ng of total RNA is sufficient for quantifying expression from moderate- to high-expression miRNA loci.

Working with leading researchers in the field of miRNA research, we have demonstrated the ability of the assays to discriminate between different genomic loci containing closely related miRNA stem-loop sequences (Figure 4). This is critical to their use in studying this important aspect of gene regulation.

Workflow

TaqMan Pri-miRNA Assays follow the same simple and convenient workflows established for all Applied Biosystems™ TaqMan™ Gene Expression Assays. Whether it is the robustness of a two-step real-time quantitative PCR (RT-qPCR) protocol, the convenience of a one-step RT-qPCR protocol, or the speed of Fast cycling, Thermo Fisher Scientific provides validated reagent kits supporting these various assay workflows. As it is important to ensure that samples are DNA-free prior to quantification, the assays have additionally been validated using the Invitrogen™ TURBO DNA-*free*™ Kit for up-front sample clean-up.

Because TaqMan Gene Expression Assays and TaqMan Pri-miRNA Assays share the same design approach and experimental workflow, endogenous control assays available for use with TaqMan Gene Expression Assays are also recommended for use with TaqMan Pri-miRNA Assays.

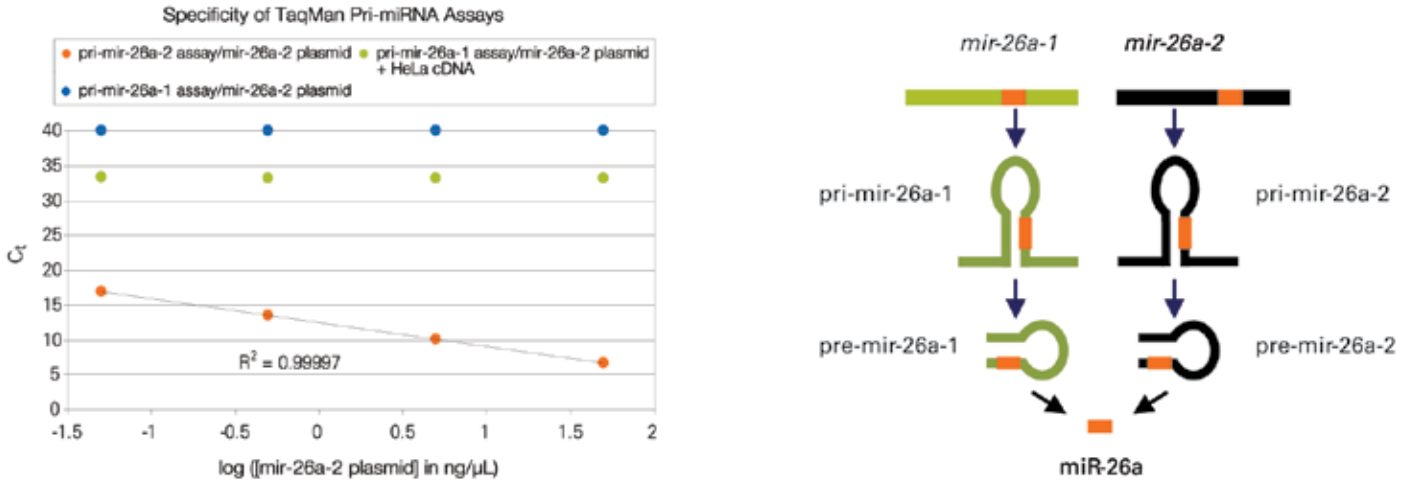

Figure 4. TaqMan Pri-miRNA Assays demonstrate high specificity. Right: The mature miRNA hsa-miR-26a **(orange)** is derived from two stem-loops, mir-26a-1 **(green)** and mir-26a-2 **(black)**. **Left:** A plasmid containing the stem-loop mir-26a-2 was run with the TaqMan Pri-miRNA Assay specific for stem-loop mir-26a-1 or mir-26a-2. Linear expression is seen across 3 logs with the pri-miRNA assay for mir-26a-2 **(orange)**; no expression above background is seen when the plasmid is run against the assay to hsa-mir-26a-1 with **(green)** or without **(blue)** HeLa cDNA. (Courtesy of Young-Kook Kim and Narry Kim, Seoul National University, South Korea.)

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Ordering information

Product description and contents (reaction volume)	Number of reactions (concentration)	Cat. No.
TaqMan Pri-miRNA Assays [Made-to-Order; single-tube (20 µL)]		
Small-scale	360 (20X)	4427012
Medium-scale	750 (20X)	4427013
Large-scale	2,900 (60X)	4427014
TaqMan Gene Expression Assays, Endogenous Controls [Inventoried, single-tube (20 µL)]		
Small-scale	250 (20X)	4331182
TaqMan MicroRNA Assays [1 RT primer tube (15 µL) + 1 TaqMan Assay tube (20 µL)]		
Inventoried assays		
Small-scale	50 RT (5X); 150 PCR (20X)	4427975
Made-to-order assays		
Extra small-scale	25 RT (5X); 75 PCR (20X)	4440885
Small-scale	50 RT (5X); 150 PCR (20X)	4440886
Medium-scale	750 RT (20X); 750 PCR (20X)	4440887
Large-scale	2,900 RT (60X); 2,900 PCR (60X)	4440888
Related products required for use with TaqMan Pri-miRNA Assays		
TaqMan Gene Expression Master Mix		
1-Pack (1 x 5 mL)	200 reactions	4369016
2-Pack (2 x 5 mL)	400 reactions	4369514
10-Pack (10 x 5 mL)	2,000 reactions	4369542
*mir*Vana miRNA Isolation Kit	40 purifications	AM1560
TURBO DNA-*free* Kit	50 reactions	AM1907
High Capacity RNA-to-cDNA Kit	50 reactions	4387406
TaqMan RNA-to-C_T *1-Step* Kit		
Mini-Pack	40 reactions	4392653
1-Pack	200 reactions	4392938
10-Pack	2,000 reactions	4392656

References

1. Heo I, Joo C, Cho J et al. (2008) Lin28 mediates the terminal uridylation of let-7 precursor microRNA. *Mol Cell* 32:276–284.
2. Viswanathan SR, Daley GQ, Gregory RI (2008) Selective blockade of microRNA processing by Lin28. *Science* 320:97–100.

For more information and full terms of the TaqMan Assays qPCR Guarantee, go to **thermofisher.com/taqmanguarantee**

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