TaqMan® MicroRNA Assays

The Gold Standard

Applied Biosystems

Validation of microRNA (miRNA) expression is a critical component in the workflow for any miRNA research. Here we evaluate miRNA quantification products from various vendors.

Currently available PCR-based methods require a basic two-step RT-PCR process.

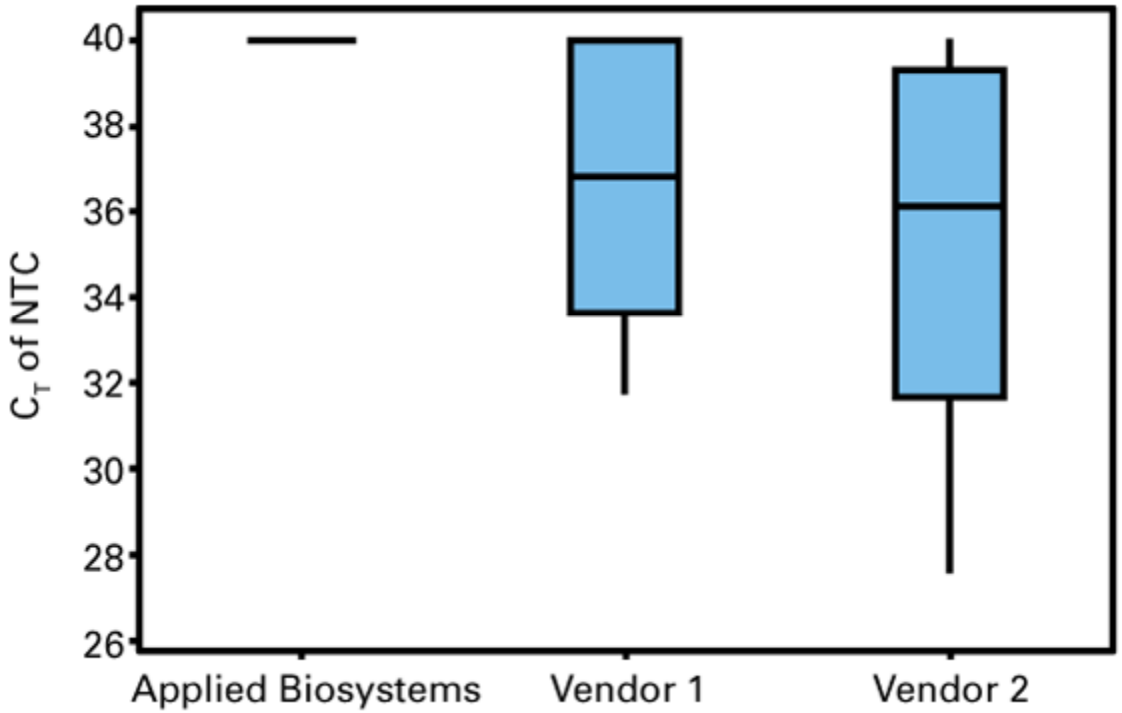
In this study, vendors employing one of each of the two general RT methods are chosen for comparison:

- Specific RT (Vendor 1)
- Nonspecific RT (Vendor 2)

The following parameters were evaluated:

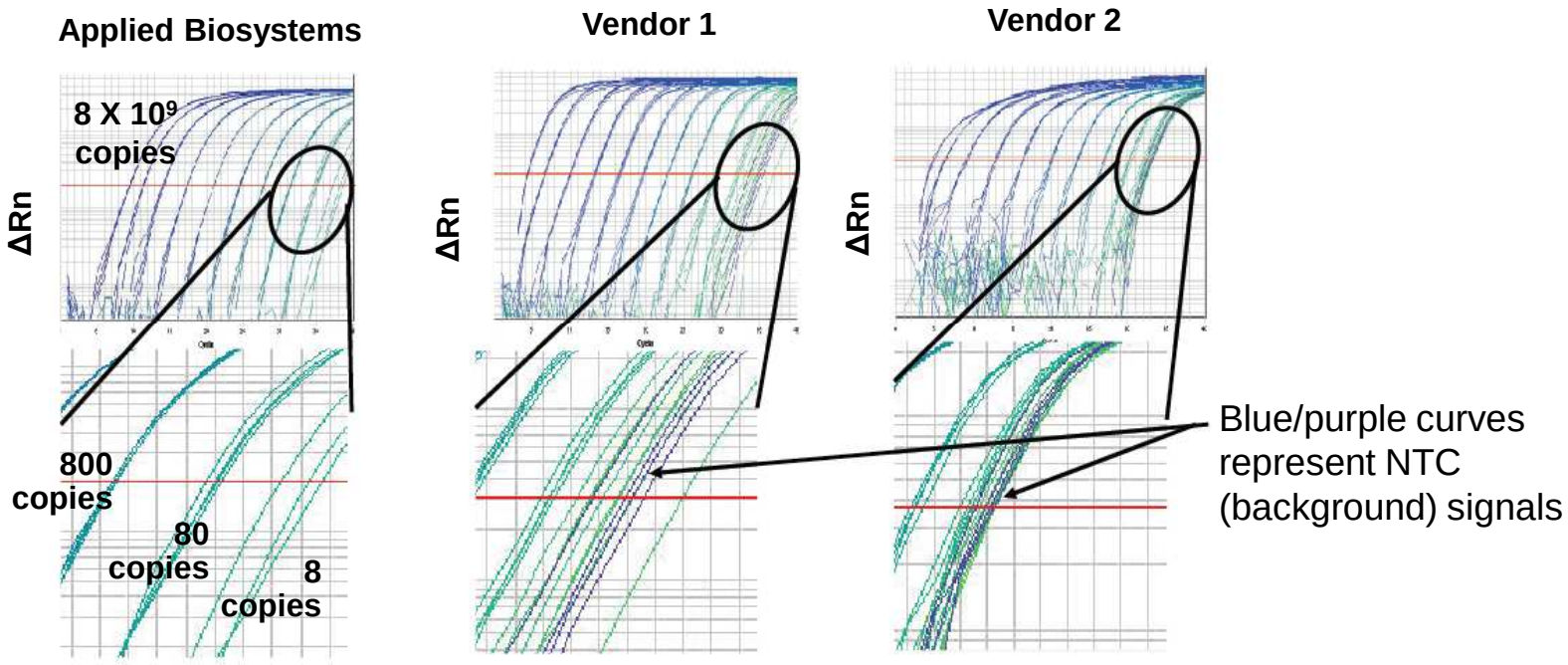
- No-template controls (NTC—background signal)
- Linear dynamic range
- Sensitivity
- Cross-specificity between closely related miRNAs
- Specificity between mature and precursor sequences
- Two-fold expression change

The gold standard begins with providing minimal background and variation, and a C_T of 40 for no-template controls.



Capturing significant noise down to a C_T of 32 is detrimental to validation. Values at that level may fall within the same range as miRNAs expressed at lower levels and abundant miRNAs measured at low sample input.

The essence of TaqMan® MicroRNA Assays as a gold standard lies in their broad dynamic range and high sensitivity.



Applied Biosystems TaqMan® MicroRNA Assays have a linear dynamic range of 9 log units and can reliably detect as few as 10 copies of synthetic miRNAs. Other methods show high variation and background signal with the same amount of RNA. Compromising sensitivity increases the risk of inefficient detection of miRNAs at various expression levels.

TaqMan® MicroRNA Assays use four oligonucleotides (TaqMan® probe, RT primer, and forward and reverse PCR primers) to help achieve high specificity.

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	Synthetic Template			
Assay	let-7a	let-7b	let-7c	let-7d
let-7a	1.00	0.00	0.03	0.01
let-7b	0.00	1.00	0.07	0.00
let-7c	0.00	0.02	1.00	0.00
let-7d	0.03	0.00	0.01	1.00

Vendor 1

	Synthetic Template			
Assay	let-7a	let-7b	let-7c	let-7d
let-7a	1.00	0.04	0.51	0.03
let-7b	0.00	1.00	0.23	0.00
let-7c	0.02	0.83	1.00	0.00
let-7d	Assay not available			

Vendor 2

	Synthetic Template			
Assay	let-7a	let-7b	let-7c	let-7d
let-7a	1.00	0.00	0.00	0.01
let-7b	0.00	1.00	0.00	0.00
let-7c	0.00	0.02	1.00	0.00
let-7d	0.24	0.00	0.00	1.00

```
hsa-let-7a    UGAGGUAGUAGGUUGUAUAGUU
hsa-let-7b    UGAGGUAGUAGGUUGUGUGGUU
hsa-let-7c    UGAGGUAGUAGGUUGUAUGGUU
hsa-let-7d    AGAGGUAGUAGGUUGCAUAGUU
```

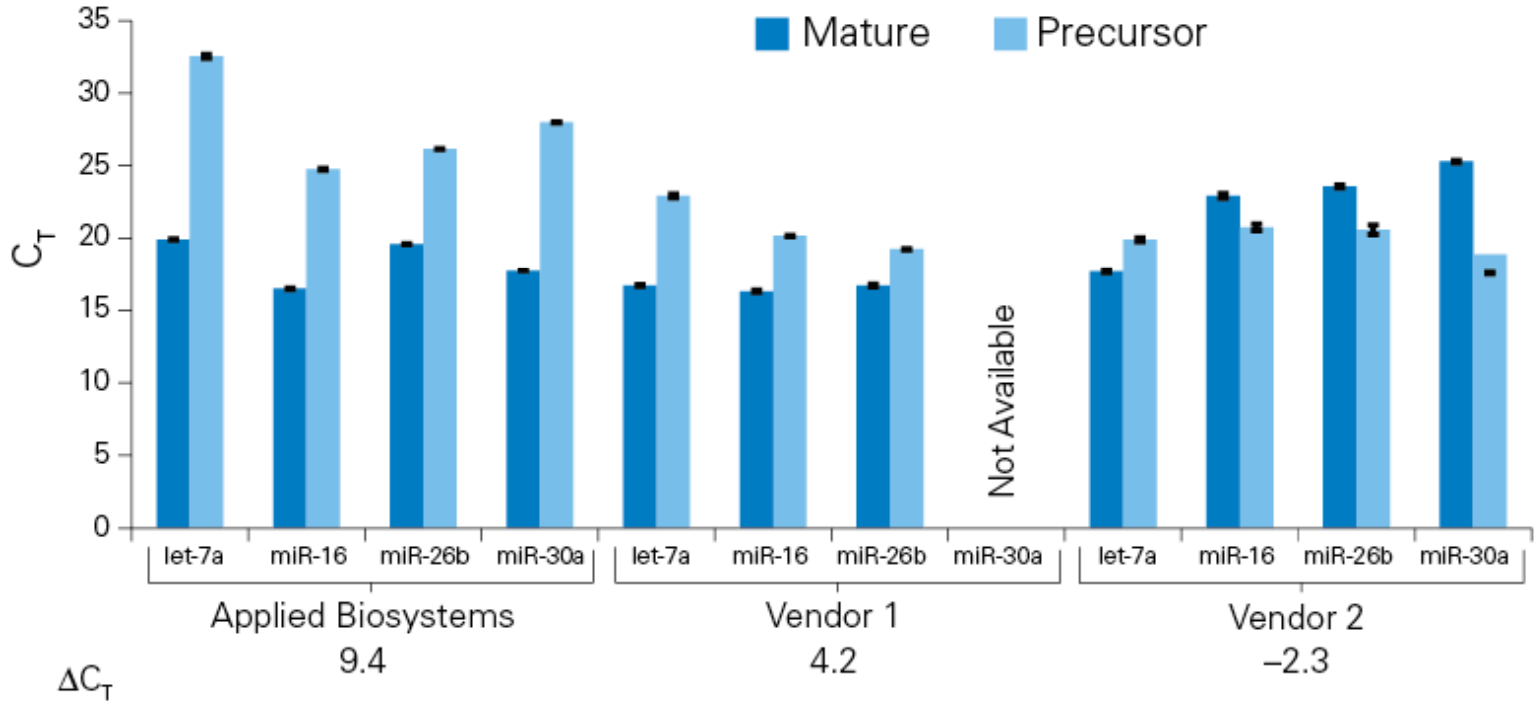
Indicates a perfect match

Indicates low cross-reactivity

Indicates high cross-reactivity

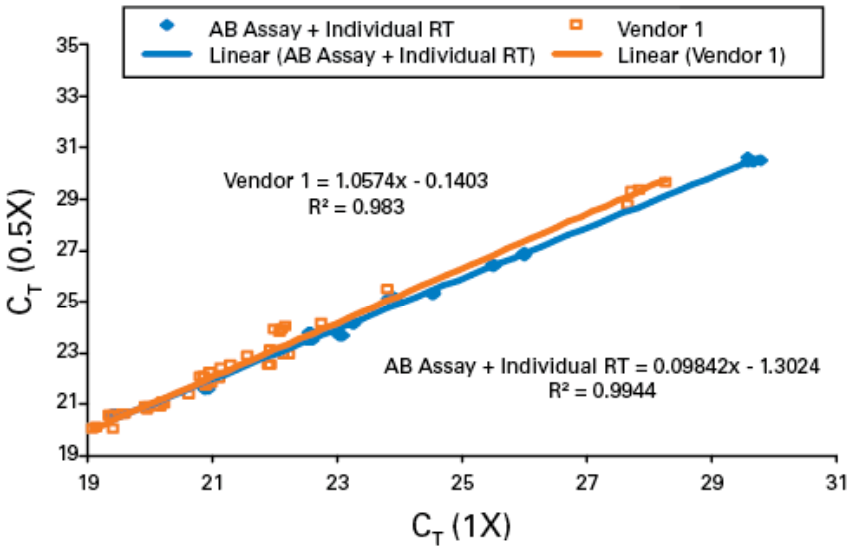
Applied Biosystems uses the same bioinformatic pipeline for all TaqMan® MicroRNA Assays, ensuring consistent specificity and sensitivity. Manually adjusting individual assays to increase specificity results in altered sensitivity.

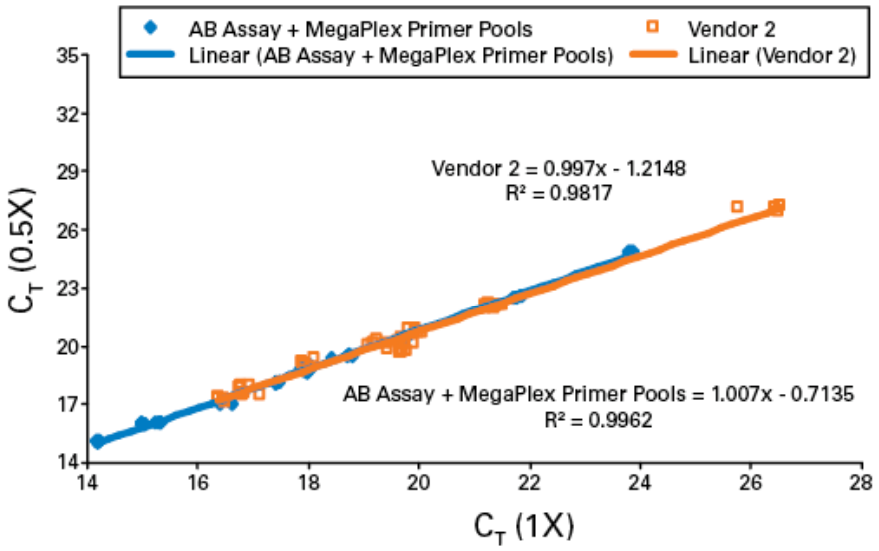
The greater biological significance of mature miRNAs[1] necessitates the high level of discrimination between precursor and mature forms available with TaqMan® MicroRNA Assays.



1. Du, T and Zamore, PD (2005) microPrimer: the biogenesis and function of microRNA *Development* **132:** 4645–4652

TaqMan® MicroRNA Assays enable reliable quantification of the miRNAs present in real tissue samples.





Lung samples were evaluated for expression of 12 miRNAs. TaqMan® assays demonstrated excellent discrimination of two-fold expression (Avg ΔC_T closest to 1) and the least variation (R^2 closest to 1). A touted benefit of nonspecific RT (Vendor 2) is the ability to simultaneously generate cDNAs for all miRNAs. For comparison to Vendor 2, Applied Biosystems Megaplex™ RT Primer Pools were used instead of the individual RT primers.

TaqMan® MicroRNA Assays hold true to their promise of being the gold standard.

With TaqMan® MicroRNA Assays:

- You can be confident that you have a clean assay with minimal background noise
- You can achieve unrivaled linear dynamic range and unsurpassed sensitivity, detecting down to 10 copies miRNA
- You can discriminate between closely related miRNAs, and be sure that only biologically active mature miRNAs are detected
- You get reliable detection and quantification of miRNAs present in your sample

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Materials and Methods

- NTC data were obtained for 15 assays chosen to represent a challenging set (below—Sanger 10.0).
- For dynamic range and sensitivity tests, data are displayed for the let-7a assay. These were performed at 10 different concentrations (green curves) in addition to the NTC (in blue/purple). Four replicate reactions were performed at each concentration (see Slide 4).
- For the mature/precursor specificity tests, 4 replicate assays were performed for each miRNA tested.
- For the 2-fold expression change, 12 miRNAs were evaluated (see below, without 7i, RNU44, and RNU48). Total RNA input was within the recommended range—1 ng/µL (1X) and 0.5 ng/µL (0.5X) for individual RT reactions, or 20 ng/µL (1X) and 10 ng/µL (0.5X) for multiplexed RT reactions.
- All other methods were performed according to the vendors' protocols and recommendations.

let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i	miR-16	miR-23a	miR-23b	miR-148b	miR-194	RNU44	RNU48

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