mSMRT-qPCR : Robust, Sensitive, Scalable microRNA Quantification

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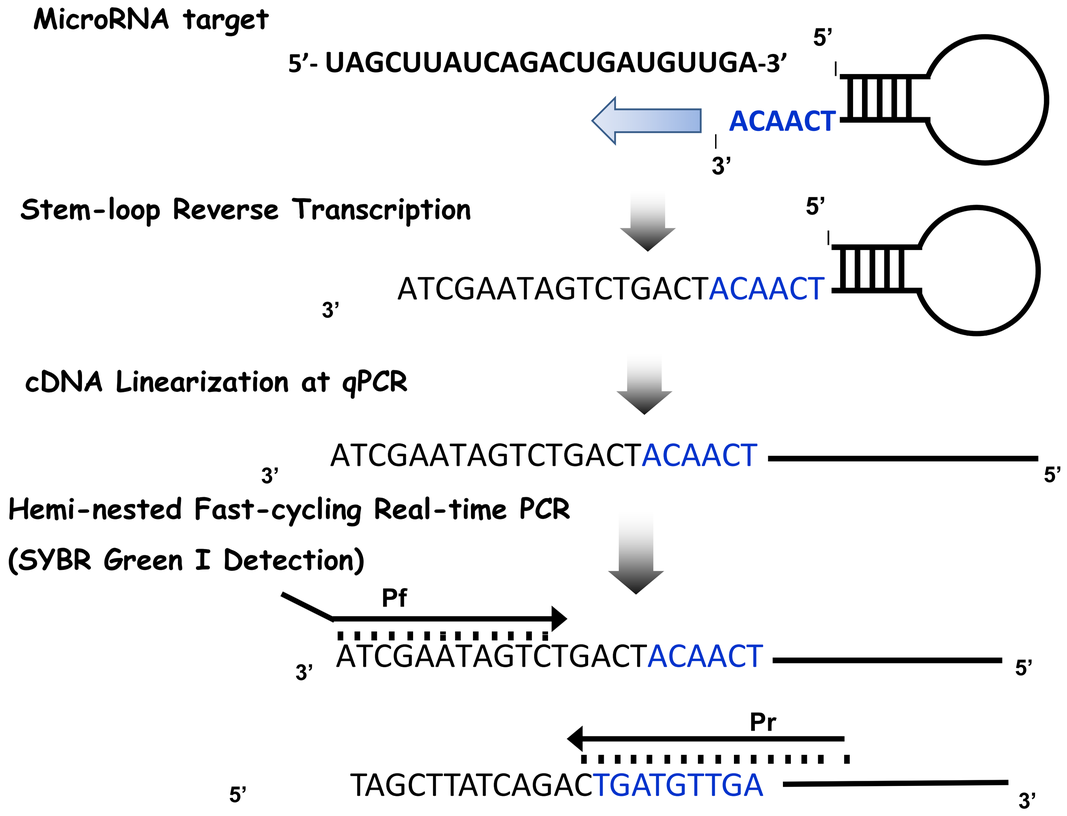
mSMRT-qPCR Overview



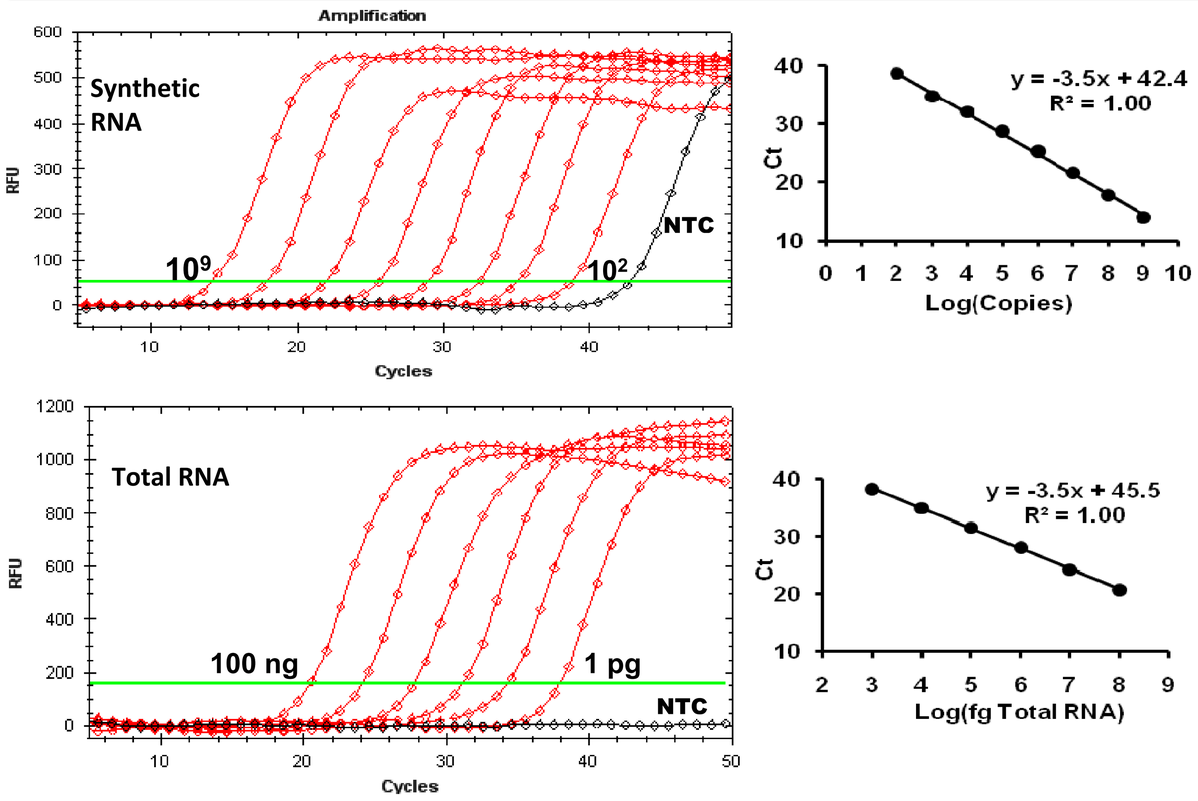
Abstract

Existing methods for miRNA quantification rely on sequence-dependent probes or chemically modified primers for optimal specificity and often require RNA isolation that is time-consuming, labour-intensive and which increase sample variability. We have developed a high performance approach for multiplexed detection of mature miRNAs termed modified stem-loop mediated reverse transcription quantitative PCR (mSMRT-qPCR).

- ✓ Original assay adapted from stand-specific detection of RNA-viruses (Anwar, 2006).
- ✓ Unmodified DNA oligos, common reagents and SYBR Green I qPCR detection.
- ✓ Specific for mature but not precursor miRNA; hemi-nested primers confer excellent discrimination against highly similar sequences.
- ✓ Robust assay is compatible with lysis using off-the-shelf surfactants such as Triton X-100.
- ✓ Capable of detecting novel miRNAs with no commercially available assays.

We have successfully used mSMRT-qPCR to investigate a hypothesis that GDNF specifically regulates a set of miRNAs in GFRα1 bearing glioma cells. In summary, mSMRT-qPCR provides a useful tool for rapid, robust, cost-effective and highly versatile quantification of existing and novel miRNAs.

Performance : Sensitivity



100 copies of miR21 could be detected from synthetic RNA standard or 1 pg of Total RNA.

Performance : Specifcity

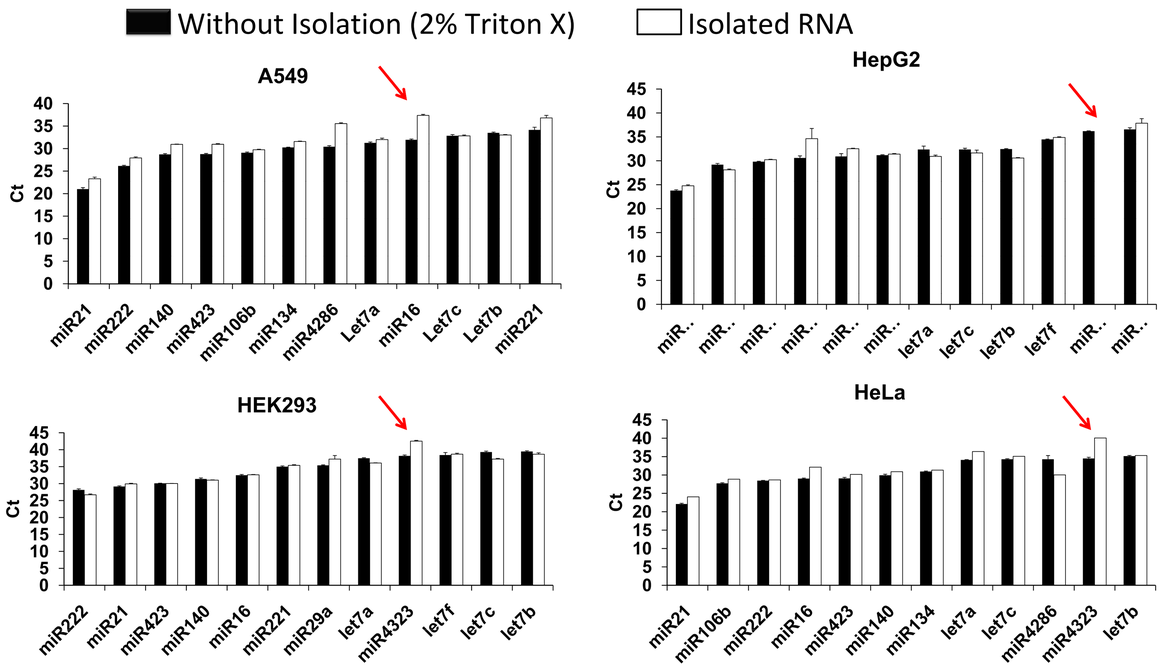
Relative Detection (%)		miRNA real-time RT-PCR assay							
		let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i
Synthetic miRNA target	let-7a	100.0	0.001	0.036	0.005	0.225	0.298	0.000	0.000
	let-7b	0.002	100.0	0.248	0.002	0.001	0.001	0.000	0.000
	let-7c	1.778	0.178	100.0	0.001	0.003	0.075	0.000	0.000
	let-7d	0.072	0.000	0.000	100.0	0.000	0.000	0.000	0.000
	let-7e	0.002	0.000	0.000	0.000	100.0	0.000	0.000	0.000
	let-7f	0.029	0.000	0.000	0.002	0.002	100.0	0.000	0.000
	let-7g	0.000	0.000	0.000	0.000	0.001	0.000	100.0	0.000
	let-7i	0.000	0.000	0.000	0.001	0.001	0.000	0.001	100.0

Relative detection when assays were challenged with 10^9 copies of mismatched targets from Let-7 family at least 50-fold specificity and no cross-reactions in most cases.

	Precursor (Ct)	Mature (Ct)	ΔCt
miR-21	28.0	18.9	9.1
miR-7-1	30.2	16.1	14.1
mir-7-2	26.0	16.1	9.9
let-7g	26.8	18.6	8.2

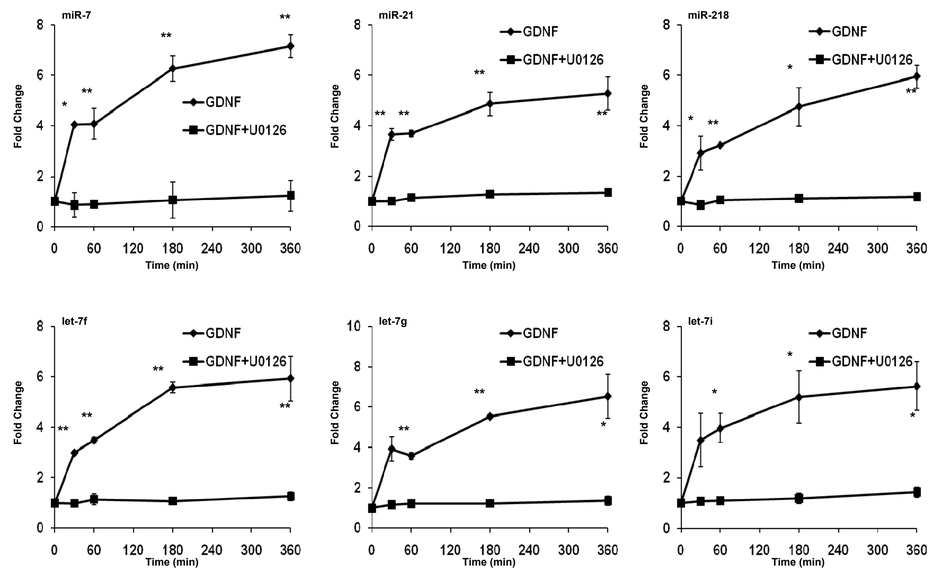
mSMRT-qPCR assay is specific for the mature but not precursor forms of miRNA.

Robust Detection using off-the-shelf Reagents



Detection from cell lysate was comparable if not superior to detection from isolated RNA of the same number of cells. Novel miRNAs with no available commercial assays could be detected.

Specific Regulation of Glioma miRNAs by GDNF



miRNAs specifically regulated by GDNF MEK-ERK dependent signalling in human glioma cell U251.

References :

Anwar, A., et al. (2006). Anal Biochem **352(1): 120-128.**

Wan, G., et al. (2010). Rna **16(7): 1436-1445.**