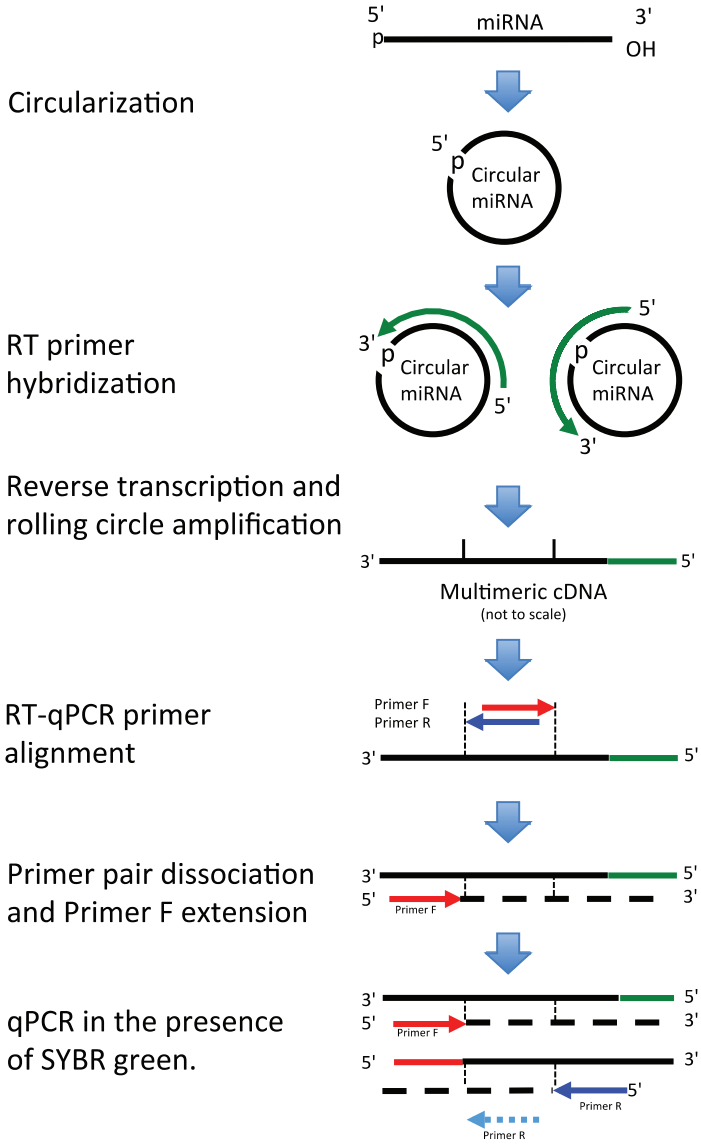
somagenics

RNA Detection

miR-ID®: Sensitive and Specific microRNA Quantification from total RNA

miR-ID® is a novel platform for quantifying microRNAs (miRNAs) and related isomiRs and isoforms using a circularization-based real-time quantitative PCR (qPCR) method. miRNAs from total RNA preparations are circularized and reverse transcribed by rolling circle amplification. The resulting cDNAs are quantified using SYBR-Green based qPCR. All steps up to RT-qPCR are performed in multiplex for all miRNAs of interest.

Overview



- RT primer can be positioned anywhere along the circularized miRNA, enabling discrimination of miRNA isoforms and isomiRs with single nucleotide differences at any position along the molecule
- miR-ID® can distinguish 2'O-methyl modifications at the 3'-terminus of miRNAs

- Largely complementary qPCR primers increase hybridization specificity to target cDNA sequence

- miR-ID can be used for absolute or relative miRNA quantification

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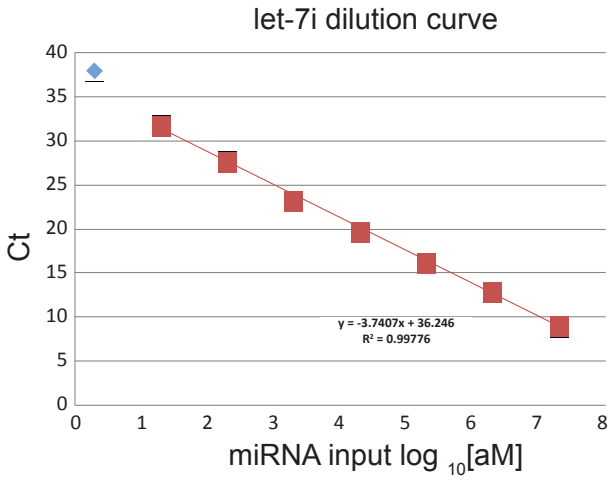
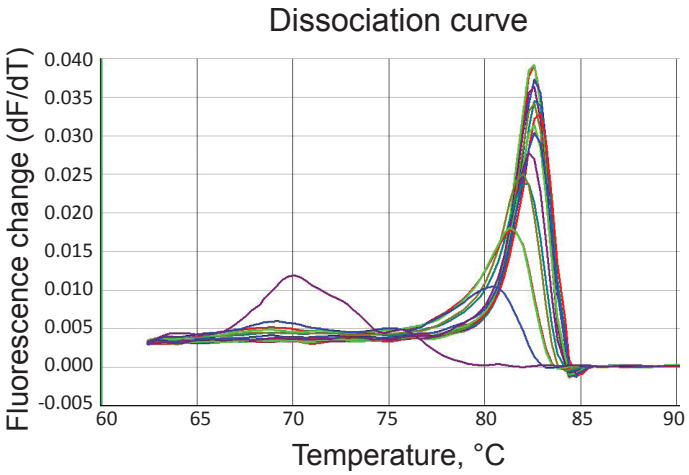
miR-ID® sensitivity range and dissociation curve





Example of a miR-ID® - analysis. The left panel shows Ct values for 10-fold dilutions of a synthetic sample miRNA (let-7i, red symbols) and the no-input control (blue symbol). Peaks of the dissociation curve (right panel) decrease with decreasing input and show a background peak (purple) for the no-input control.

miR-ID® discriminates isomiRs derived from a common pre-miRNA

pre-miRNA 5'-....NNNNNC ——— ANNNNNN.....3'

miRBase 0/0	----------	
isomiR 0/+1	----------A	3'-addition (templated)
isomiR -1/0	C----------	5'-addition (templated)
isomiR -1/+1	C----------A	5'- and 3'-addition (templated)

	miR-ID® assays			
	0/0	0/+1	-1/0	-1/+1
0/0 (mature)	100%	0.09%	1.72%	0.00%
0/+1 isomiR	0.09%	100%	0.20%	0.17%
-1/0 isomiR	0.75%	0.72%	100%	0.00%
-1/+1 isomiR	0.01%	0.05%	0.13%	100%

	AT assay
	0/0
0/0 (mature)	100%
0/+1 isomiR	37.89%
-1/0 isomiR	8.89%
-1/+1 isomiR	21.53%

miR-ID® allows optimal RT placement due to miRNA circularization and uses overlapping, highly specific PCR primers. These features permit miR-ID® to provide exceptional discrimination between isomiRs with single nucleotide polymorphisms. The competitor assay for the canonical miRNA detects significant amounts of the related isomiRs.

miR-ID® discriminates 3' A additions/deletions in isomiRs

...AGGAAACCGUUACCAUUACUGAGUUUAGUA...

miR-451a 0/0	AAACCGUUACCAUUACUGAGUU	(miRBase)
miR-451a +3'A	AAACCGUUACCAUUACUGAGUUA	(3'-A addition, NGS)

	miR-ID®	AT assay
miR-451a	100%	100%
miR-451a +3'A	0.2%	87.7%

...CUGUAGCAGCACAUCAUGGUUUACAUGC...

mir-15b-5p 0/0	UAGCAGCACAUCAUGGUUUACA	(miRBase)
miR-15b-5p 0/-1	UAGCAGCACAUCAUGGUUUAC	(3'-trim, NGS)

	miR-ID®	AT assay
miR-15b	100%	100%
miR-15b 0/-1	0.1%	144.8%

miR-ID® discriminates the canonical miRNA from isomiRs with 3' A modifications. RT-qPCR technologies that employ 3' poly-A tailing cannot discriminate these modifications.