Single Molecule *in situ* Detection and Direct Quantification of miRNAs in Cells and FFPE Tissues

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ABSTRACT #118

Background:

miRNAs have emerged as key regulators in diverse biological processes that underlie the development and physiology of complex organisms. Recently, there is also increasing evidence implicating abnormal miRNA expression in human cancers and neurological disorders [1-6]. Significant advances in the field of miRNA research have been made possible in part through the introduction of novel research tools, enabling investigators to discover, profile, quantify, validate, and functionally analyze miRNAs. This study reports the development of two powerful assays, based on proprietary nucleic acid chemistry, novel probe designs and second generation branched DNA nanostructure, that enable direct, specific, and quantitative detection of miRNAs without RNA isolation, reverse transcription, or PCR amplification. Performed in a 96-well plate and measured by chemiluminescence, the QuantiGene® 2.0 miRNA Assay allows direct quantification of miRNAs from lysates of cultured cells, plasma/blood, and fresh frozen or FFPE tissues down to single-base resolution, whereas the QuantiGene® View miRNA *in situ* assay permits visual detection and quantitative validation of miRNA expression down to a 2-nt difference at a single cell resolution. Here, supporting data demonstrating accurate determination of miRNA copy number in cells and tissues as well as detection of miRNA and mRNA in cells and FFPE tissues will be presented.

Conclusion:

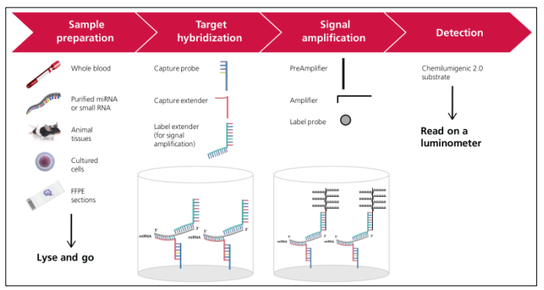
- QuantiGene ® 2.0 miRNA Assay allows direct and accurate miRNA quantification from a variety of sample types without RNA purification, cDNA synthesis or PCR amplification.
- Novel probe design and proprietary nucleic acid chemistry offer sensitive and highly specific detection of target sequences down to single-base resolution.
- Data obtained for 20 miRNAs demonstrated concordance between QuantiGene ® 2.0 miRNA Assay and GeneChip® miRNA Array.
- QuantiGene ® View *in situ* Assay enables visual detection and quantitative validation of miRNA and mRNA expression simultaneously at single cell resolution.

References:

1. Liu N. K., *et al.* MicroRNA in Central Nervous System Trauma and Degenerative Disorders. *Physiol Genomics* (2011). [Epub ahead of print]
2. He L., *et al.* A micro RNA component of the p53 tumor suppressor network. *Nature* **447**(7148):1130-4 (2007).
3. Gaur A., *et al.* Characterization of microRNA expression levels and their biological correlates in human cancer cell lines. *Cancer Research* **67**(6):2456-68 (2007).
4. Soifer H. S., *et al.* MicroRNAs in disease and potential therapeutic applications. *Molecular Therapy* **15**(12):2070–9 (2007).
5. Esquela-Kerscher A., *et al.* Oncomirs—miRNAs with a role in cancer. *Nature Reviews Cancer* **6**(4):259-69 (2006).
6. Volinia S., *et al.* A microRNA expression signature of human solid tumors defines cancer gene targets. *PNAS* **103**:2267–81 (2006).

1. QuantiGene® 2.0 miRNA Assay Protocol

● No miRNA purification ● No cDNA synthesis ● No PCR amplification



Day 1: Release and capture target miRNA

Step 1: Lyse cells to release miRNA.

Step 2: Overnight hybridization of miRNA to the 96-well plate and with target specific probe sets (CE and LE).

Day 2: Signal amplification and detection

Step 1: Signal amplification via sequential hybridization of PreAmp, Amp and LP.

Step 2: Detection by adding chemiluminescent substrate.

Figure 1: QuantiGene® 2.0 miRNA Assay Protocol. The simplified workflow of the QuantiGene 2.0 miRNA Assay provides quick and accurate miRNA quantification without the need for miRNA purification, cDNA synthesis, or PCR amplification.

2. Differentiate between pre-miRNA and mature miRNA

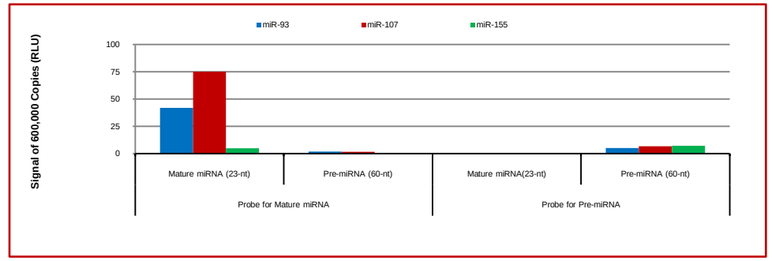


Figure 2: Differentiate between pre-miRNA and mature miRNA with the QuantiGene 2.0 miRNA Assay. Novel probe design and proprietary nucleic acid chemistry offer both sensitive and highly specific detection of target molecules whether the probe sets is designed against the mature or precursor miRNA molecules.

3. Discriminate between Let-7 miRNA family members

let-7 family cross		synthetic miRNA							
		let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i
Probeset	let-7a	100%	1%	3%	5%	13%	3%	2%	0%
	let-7b	2%	100%	0%	0%	0%	0%	0%	0%
	let-7c	8%	8%	100%	0%	0%	5%	0%	0%
	let-7d	9%	0%	1%	100%	6%	0%	0%	0%
	let-7e	18%	0%	0%	0%	100%	1%	1%	1%
	let-7f	31%	0%	1%	0%	33%	100%	0%	0%
	let-7g	0%	0%	0%	0%	0%	0%	100%	0%
	let-7i	0%	0%	0%	0%	0%	0%	0%	100%

Figure 3: Discrimination of let-7 miRNA family members. QuantiGene 2.0 miRNA Assay is able to distinguish highly homologous miRNA targets down to a single-base resolution. Percentage of cross signal for each probe set was calculated by dividing the signal obtained for the mismatched target by the signal yielded by its specific target. A total of 6 x 10^6 copies of synthetic RNA was added to each assay.

hsa-let-7a
UGAGGUAGUAGGUUGUAUAGUU
hsa-let-7b
UGAGGUAGUAGGUUGUGUGGUU
hsa-let-7c
UGAGGUAGUAGGUUGUAUGGUU
hsa-let-7d
AGAGGUAGUAGGUUGCAUAGUU
hsa-let-7e
UGAGGUAGGAGGUUGUAUAGUU
hsa-let-7f
UGAGGUAGUAGAUUGUAUAGUU
hsa-let-7g
UGAGGUAGUAGUUUGUACAGUU
hsa-let-7i
UGAGGUAGUAGUUUGUGCUGUU

4. miRNA detection by the QuantiGene® 2.0 miRNA Assay in various sample types

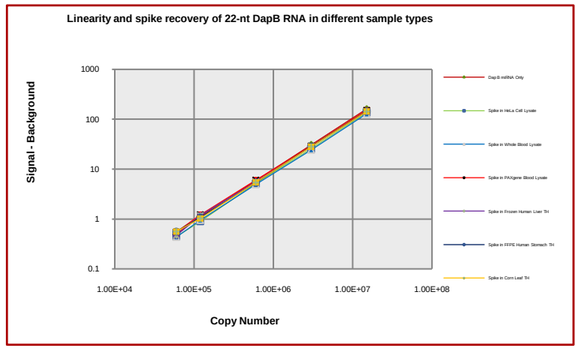


Figure 4: miRNA detection in various sample types. QuantiGene 2.0 miRNA Assay can be used with great results in a variety of samples such as cell lysates, whole or PAXgene blood, plasma, frozen or FFPE tissues, and plants. Various cell lysates were spiked with different amount of a 22-nt synthetic RNA (DapB) from *B. subtilis* genome and typical spike recovery rate ranging from 80-120% was obtained using QG 2.0 miRNA Assay. Three miRNAs (miR-93, miR-146a, and miR-155) were analyzed in different tissue types as well as in whole blood and showed variable expression (**Table I**).

Table I. Expression profiles of three miRNAs in various samples

miRNA	miRNA Expression Level (attomoles)					
	Whole blood*	Frozen Normal Liver**	Frozen Normal Breast**	Frozen Tumor Breast**	FFPE Tumor Colon***	FFPE Tumor Breast***
hsa-miR-93	10.24	0.026	0.00	0.00	32.04	16.86
hsa-miR146a	1.93	0.073	0.01	0.01	76.10	33.06
hsa-miR-155	0.63	0.013	0.00	0.01	98.73	41.76

**Data from 1 µL of blood* ***Data from 1 µg of frozen tissue* ****Data from 1 slide of FFPE tissue sample*

5. Concordance with GeneChip® miRNA 2.0 Array

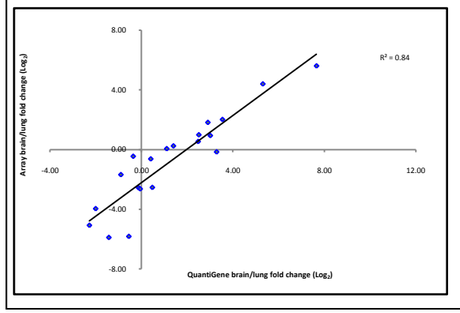


Figure 5: Concordance between QuantiGene ® miRNA Assay and GeneChip® miRNA Array for 20 miRNAs.
The QuantiGene® 2.0 miRNA Assay was used to validate GeneChip miRNA 2.0 Array results. To evaluate the concordance of fold changes in miRNA expression between Ambion FirstChoice® Human Brain Total RNA (AM7962) and Ambion FirstChoice Human Lung Total RNA (AM7968) as determined by the QuantiGene 2.0 miRNA Assays and GeneChip miRNA 2.0 Array, a regression analysis of fold differences was performed using the QuantiGene and GeneChip data for 20 miRNAs in common. The correlation coefficient (R2 value) between these two assays was 0.84, indicating good fold-change correlation and concordance between these two independent yet complementary methods.

6. QuantiGene® View miRNA *in situ* Assay

Visual detection and quantitative validation of miRNA expression in cells and FFPE

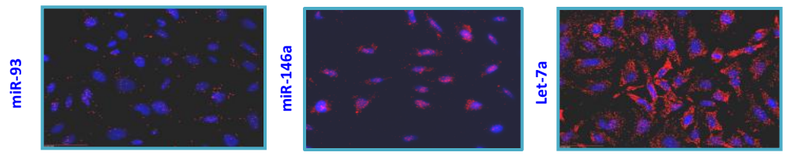
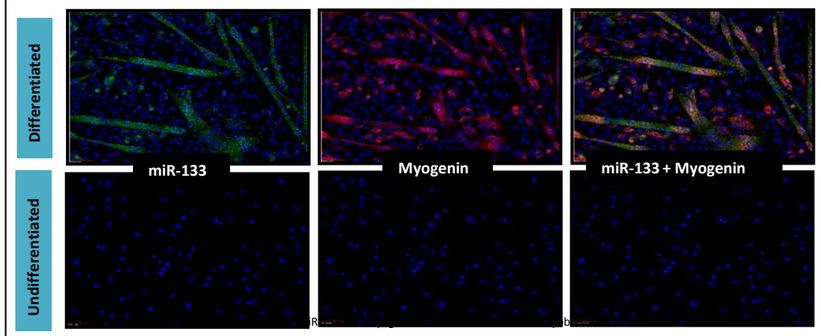


Figure 6A: QuantiGene View *in situ* detection of endogenous miR-93, miR-146a, and let-7a expression in HeLa cells.



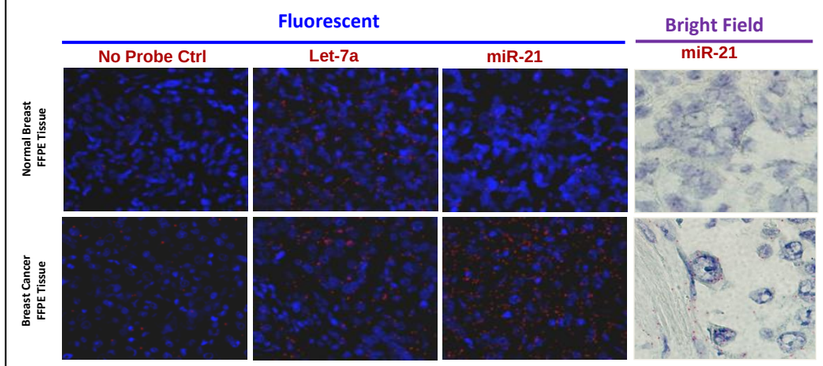


Figure 6C: QuantiGene View *in situ* detection of let-7a and miR-21 expression by in human normal and cancer breast FFPE tissues.