

## Quantifying MicroRNA Using RainDrop<sup>®</sup> Digital PCR System

Unlike most other potential biomarkers, microRNA molecules appear to be cell type and disease specific, as well as stable and readily accessible. They promise to have an impact in medicine as new diagnostic and prognostic markers, as indicators of therapeutic response, and as targets of novel therapies. This application note demonstrates the use of RainDrop<sup>®</sup> Digital PCR System as an exciting new tool for microRNA analysis.

### MicroRNAs as Useful Biomarkers

MicroRNAs (miRNAs) are small non-coding RNA molecules which are processed into functional units ~22 nucleotides in length. There are estimated to be approximately 1900 miRNA genes in the human genome<sup>1</sup>. These molecules have become recognized as one of the major regulatory gatekeepers of coding genes in the human genome and have been shown to be up- or down-regulated in specific cell types and disease states<sup>2</sup>. They are expressed in a tissue-specific manner, and abnormal miRNA expression within a tissue type can be correlated with disease status<sup>3-4</sup>. A relevant feature of microRNA biology is their remarkable stability. MicroRNAs are preserved well in tissue samples even after formalin-fixation and paraffin-embedding and can be efficiently extracted and evaluated<sup>5</sup>. In addition, their stability in blood and other bodily fluids makes them sufficiently robust to serve as practicable clinical biomarkers<sup>6</sup>.

### Many Examples in Cancer

Aberrant miRNA expression has been observed in many cancers, for example in breast cancer. miR-155 is up-regulated in breast cancer, suggesting that it may act as an oncogene<sup>7-8</sup>. Up-regulation of miR-373 and miR-520c promote metastasis by inhibiting CD44 expression with increased expression of the CD44s isoform associated with overall survival in breast cancer patients<sup>9-10</sup>. Overproduction of miR-10b can promote tumor invasion *in vivo*<sup>7</sup> and miR-17-5p (also known as miR-91) has been found to be down-regulated in breast cancer<sup>11</sup>. The important role of miRNAs in lung, colorectal, pancreatic and prostate has also been investigated<sup>2</sup>.

### Novel Applications in Other Clinical Situations

In addition, miRNA expression has been shown to be highly correlated with various clinical outcomes. For example, three placental microRNAs (miR-526a, miR-527 and miR-520d-5p) have been used to accurately distinguish pregnant from non-pregnant women<sup>5</sup>, and distinctive expression of placental microRNAs are associated with preeclampsia<sup>12</sup>, highlighting that serum levels of particular microRNAs may serve as future diagnostic biomarkers during pregnancy. Alterations in miRNA expression in cardiovascular disease, neurological and autoimmune diseases, and >70 other diseases have also been reported<sup>13</sup> and may be candidates for biomarker development.

### Digital PCR as a Promising New Tool in MicroRNA Analysis

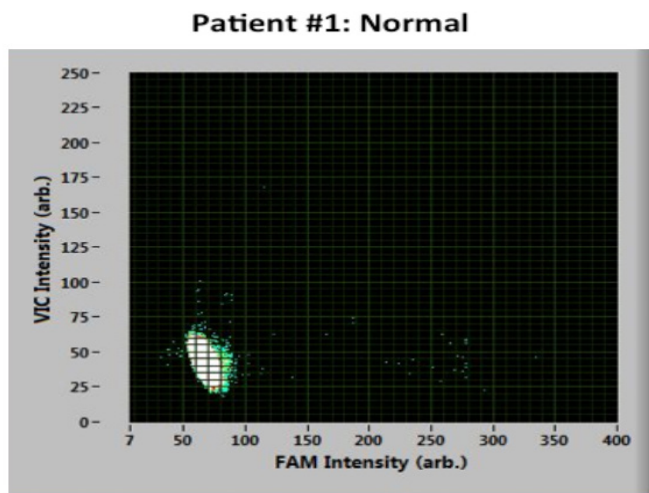
There are several methods for detecting miRNAs, including the use of microarrays, bead-based arrays, and quantitative real-time PCR. Digital PCR is a recently developed measurement technique that separates individual target molecules into compartments for 'digital' counting using qPCR primers and probes. Compared to other techniques, digital counting provides superior precision and absolute quantification. With up to 10 million droplet-based reactions per sample, the RainDance RainDrop Digital PCR System uniquely provides a simple closed-tube workflow, superior sensitivity and the capability for easy development of multiplexed single-molecule assays. (See Multiplex and Rare Variant Applications Notes for examples).

## Counting miRNA Transcripts with Digital PCR

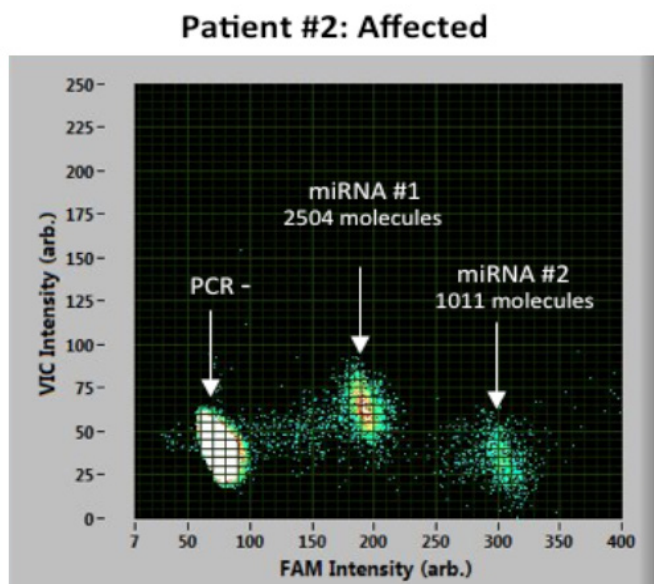
Assays to two miRNA biomarker candidates were developed using a commercially available hairpin loop RT primer/Taqman configuration. These were combined into a duplex assay based on FAM probe intensity with each target molecule identified and counted based on endpoint PCR using different FAM probe concentrations Zhong et. al.<sup>14</sup>

Two patient samples, one normal and one affected, were processed using Applied Biosystems® TaqMan® MicroRNA Reverse Transcription Kit to produce cDNA for analysis by RainDrop Digital PCR System using the duplex assay.

**Figure 1**  
**Patient #1: Normal**



**Patient #2: Affected**



The top panel of Figure 1 shows very low number of positive droplets of the two targets in the normal sample. The bottom panel shows significantly higher counts and good spectral separation for the targets in the affected sample. One can clearly see that this duplex assay for miRNA targets #1 and #2 differentiates the 'normal' from the 'affected' patient. This provides initial validation for these miRNA molecules as disease-associated biomarkers, and shows how dPCR can potentially be used to assess patient health.

## Conclusion

MicroRNAs show tissue-specific character and remarkable stability in serum and other bodily fluids, creating promise as novel biomarkers across a variety of diseases and clinical applications. The ease of assay development, superior sensitivity and multiplexing capabilities of the RainDrop Digital PCR System will enable exciting research in this rapidly evolving field.

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