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Benchmarking the FirePlex® miRNA Assay

A comparison with other platforms

Abstract

The FirePlex® microRNA (miRNA) Assay enables high-throughput, multiplexed analysis of miRNAs directly from complex biofluids to provide robust data for miRNA biomarker discovery and verification. Here, we demonstrate strong cross-platform concordance of the FirePlex miRNA Assay with other profiling technologies while showing excellent data reproducibility even with diminishing RNA input.

Background

Molecular biomarkers are commonly used as indicators of disease or measures of response to therapeutic intervention[1,2]. Historically, individual biomolecules were used to assess disease state. More recently, it has been shown that signatures of multiple biomarkers (obtained by multiplex biomarker profiling) can better account for patient heterogeneity, providing a more comprehensive indication of patient health[2].

Due to their presence in circulation and tissue-specific expression patterns, miRNAs have emerged as ideal biomarkers. Their stability in biofluids such as plasma, serum, urine, and cerebrospinal fluid (CSF) makes them well-suited for liquid biopsies. Further, miRNA expression has been found to be differentially regulated in response to circadian rhythm, inflammation, acute and chronic tissue injury, and in cancer[3], thus making miRNAs potential candidates for assessing patient health across a variety of disease states. While miRNAs show promise of clinical utility, reliably profiling these molecules poses several challenges.

Existing technologies for miRNA biomarker development require large sample input, laborious sample processing, and lack of multiplexing ability. FirePlex technology is designed specifically to circumvent these barriers. The assay enables multiplexed miRNA analysis directly from biofluids with minimal sample preparation and excellent sensitivity, providing an unprecedented combination of performance, throughput, and ease of use.

The FirePlex platform offers the following:

- Ability to detect miRNAs directly from biofluids including plasma, serum, exosomes, saliva and urine, as well as directly from FFPEs and cell suspensions
- A three-dimensional hydrogel particle substrate providing ideal hybridization thermodynamics and large nucleic acid binding capacity
- Flexibility of panel design, allowing detection of 5–400 miRNAs from a single sample
- Readout on standard flow cytometers, removing the need to purchase new equipment
- Streamlined analysis with a full-service software suite.

High-throughput analysis of multiple miRNAs over hundreds of samples

Most technologies for miRNA detection are designed for either an in-depth analysis of many targets across a few samples (eg sequencing or microarrays) or a shallow analysis of a few targets over many samples (eg qRT-PCR, Figure 1). However, because the number of samples required to make statistically-significant observations in validation studies increases dramatically with biomarker number[4], appropriately designed studies for multiplexed biomarker signatures require profiling across hundreds or even thousands of samples. The FirePlex technology provides a combination of high-throughput and multiplexing to address validation needs while requiring less input than other technologies (Figure 1).

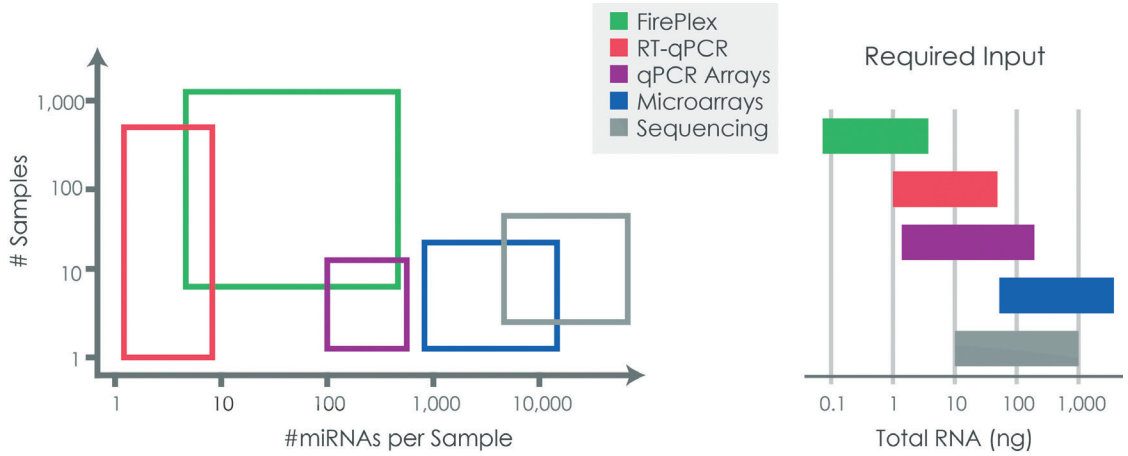


Figure 1. Position of FirePlex particle technology in the miRNA profiling landscape. (Left) Capability of different miRNA profiling technologies for profiling multiple miRNAs across multiple samples, (right) required input RNA amounts suggested for major platforms.

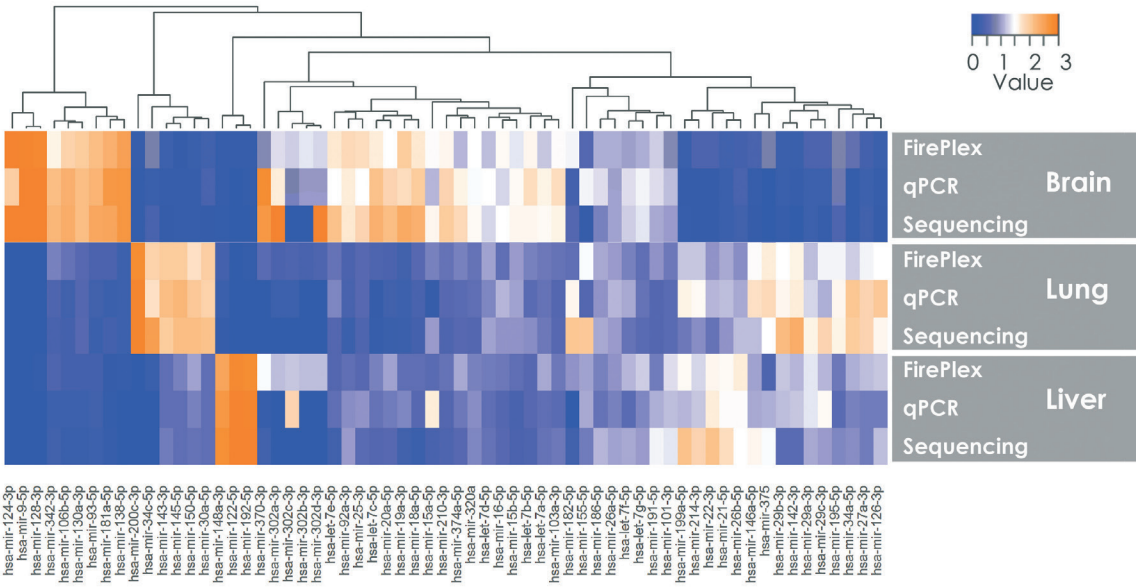
Strong cross-platform concordance

miRNA profiling platforms exhibit platform-specific effects with respect to sensitivity, specificity and sequence-dependent detection efficiency[5]. While direct comparison of platforms across miRNAs in a single sample rarely provides strongly-correlating data, the ability of a platform to capture differential expression of miRNAs across samples is of utmost importance in biomarker development, and can serve as a more reliable means of cross-platform comparison.

To this end, we compared cross-sample expression analysis obtained using the FirePlex miRNA Assay with those obtained using sequencing (Illumina) and qPCR (TaqMan Low Density Array (TLDA), Figure 2). We profiled total RNA isolated from three diverse human tissues known to vary appreciably in miRNA expression (RNA from human brain, lung and liver tissue, Cell Applications, Inc.). Input amounts for the FirePlex miRNA Assay, qPCR and sequencing assays were 5 ng, 60 ng and 1 µg, respectively.

For comparison of miRNA expression, qPCR signals were first transformed to linear space and signals for each platform were geometric-mean normalized across targets, then for each target across the three samples. This approach is used to account for differences in RNA input amounts and overall analytical signal magnitude. Using these normalized data, cross-platform concordance was measured by Pearson correlation (Figure 2, bottom). Overall, platforms showed good concordance yielding correlations above 88% in all cases, with the FirePlex miRNA Assay giving correlations consistently above 90%.

These data demonstrate that for differential expression analysis, the FirePlex miRNA Assay compares very well with both sequencing and qPCR-based approaches. Similar results were obtained with microarray-based approaches (data not shown).



	FirePlex	qPCR	Sequencing
FirePlex	100%		
qPCR	90%	100%	
Sequencing	92%	88%	100%

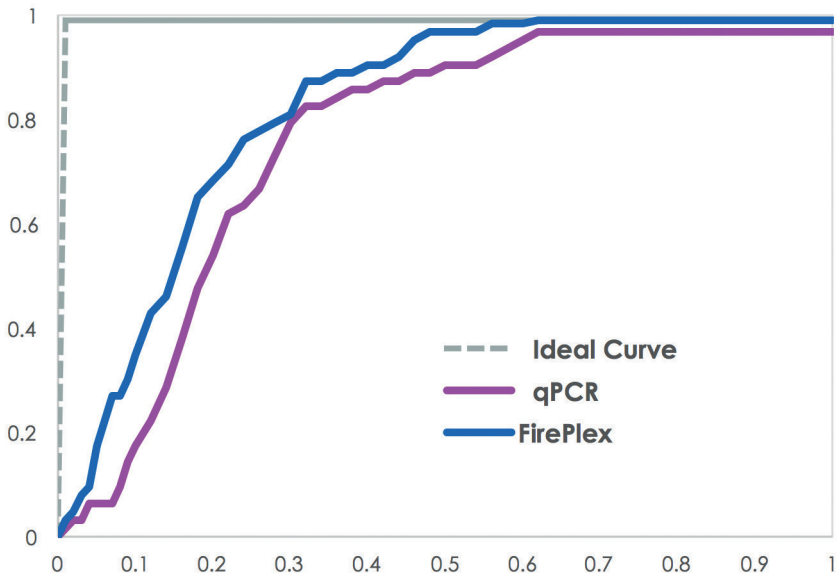
Figure 2. Cross-platform comparison of miRNA profiling assays.
(Top) Heat map of normalized expressions across the FirePlex miRNA Assay, TaqMan TLDA and Illumina assays. (Bottom) Pearson correlations between normalized platform expression profiles, averaged across three sample types. The FirePlex miRNA Assay was performed in-house, while sequencing was performed by LCSciences and qPCR analysis was performed by the Shannon McCormack Molecular Diagnostics Laboratory at Dana Farber Cancer Institute.

Assay reproducibility and sensitivity

To assess assay reproducibility, we performed replicate analysis on single samples and quantified the number of targets measured within a given fold change[5]. For this study, 5 ng each of total RNA from brain, lung and liver was input for duplicate analysis with the FirePlex miRNA Assay and qPCR (TLDA). Data were analyzed for expression differences across replicates: the fraction of miRNA targets showing smaller deviation than a given fold-difference were plotted against that fold-difference (Figure 3, top). Platforms with very good reproducibility yield a cumulative target fraction that quickly approaches a value of 1 (ideal curve shown).

Both platforms show good reproducibility in this study. While 90% of the targets assayed show less than 0.5-fold difference across duplicates for qPCR, the same number show less than 0.4-fold for the FirePlex miRNA Assay.

The reproducibility of a platform across input amounts demonstrates assay robustness and indicates dynamic range. Using a 1:1:1 mixture of brain, lung and liver total RNA, we assessed intra-platform reproducibility across a broad range of input amounts. In all cases, we observed Pearson correlations >94% for all input amounts (Figure 3, bottom). This experiment also demonstrates the sensitivity of the FirePlex miRNA Assay. While most microarray platforms use micrograms of RNA, and most qPCR platforms require nanogram inputs, we show the robust detect of miRNAs with as little as 39 picograms.



	2500ng	625ng	156ng	39ng
2500ng	100%			
625ng	100%	100%		
156ng	98%	99%	100%	
39ng	96%	97%	95%	100%

Figure 3. Reproducibility of the FirePlex multiplex assay.
(Top) Replicate fold-change analysis for duplicate measurements of brain, lung, liver total RNA mix. (Bottom) Pearson correlation values for the FirePlex miRNA Assay across total RNA input amounts.

Conclusion

The experimental results provided demonstrate that the FirePlex miRNA Assay produces data that strongly correlate with common miRNA profiling platforms, with equal or improved reproducibility in comparison to qPCR. Given the sensitivity, throughput, and robustness of the assay, along with the ability to profile directly from crude biofluids, the FirePlex miRNA Assay is an ideal platform that can address all stages of miRNA biomarker development.

References

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Notes

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