MicroRNA Expression Profiling Version 2 Panels

A highly multiplexed assay deployed on flexible multi-sample readout formats create human- and mouse-specific panels containing the most current content for profiling miRNA expression.

Highlights of The MicroRNA Expression Profiling Panels

- **High Sensitivity:** Detect low-abundance miRNA and small differences between samples
- **Low Sample Input:** 100–200 ng total RNA from cultured cells, fresh frozen, or FFPE tissue
- **Robust Assay:** Validated with qPCR, sequencing, and reproducibility experiments
- **High Specificity:** Two-step discrimination based on hybridization followed by enzymatic primer extension

Introduction

Gene expression regulation by miRNA is an essential mechanism contributing to normal cell physiology. Not surprisingly, there have been many descriptions of miRNA dysregulation implicated in the etiology and progression of diseases, such as cancer1,2, heart disease3, and Parkinson disease4. Thus, there is a strong need for a tool that facilitates widespread analysis of the expression levels of the rapidly growing list of miRNAs that have been identified so far in mice and humans.

The Illumina MicroRNA Expression Profiling Assay is an efficient and cost effective system for high-throughput multiplexed miRNA expression profiling. The assay is an adaptation of the proven DASL® (cDNA-mediated Annealing, Selection, Extension, and Ligation) Assay. To accurately quantify levels of a diverse population of miRNAs, the unique methodology and chemistry of the DASL Assay are incorporated with a simple sample preparation for collecting total RNA from cultured cells or tissue. Illumina has developed content panels for expression profiling in human and mouse composed of miRNA described in the Sanger Institute’s miRBase database5 plus additional novel content derived using Illumina sequencing technology. The human v2 miRNA panel contains 1,146 assays, for detecting more than 97% of the miRNAs described in the miRBase database. The mouse v2 miRNA panel contains 656 assays to cover more than 96% of miRNA described in miRBase. The unique assay design and flexible platform multiplex capacity allow for product updates to continue incorporating future discovered miRNA.

Simple Workflow

The MicroRNA Expression Profiling Assay requires an input of only 100–200 ng of isolated total RNA. The input RNA is polyadenylated and converted to cDNA by standard methods. A single miRNA-specific oligo (MSO) is used to assay each miRNA on the panel. All MSOs are hybridized to the sample in parallel, and a solid-phase primer extension step further increases specificity and reduces noise (Figure 2). Universal amplification of extended products only after this point avoids PCR-induced detection bias. The universal PCR step creates fluorescently labeled products identifiable by their unique MSO sequence. These labeled assay products correspond to, and are in relative abundance to, specific original miRNA in the sample. The address sequence from each MSO is used to hybridize specific miRNA products to specific locations on the BeadArrayTM substrate for readout.

Researchers have two BeadArray substrates to choose from, depending on the number of samples being run. A single Universal Array Matrix permits processing 96 independent samples simultaneously and a Universal BeadChip processes 12 samples at a time (Figure 1). Both substrates provide equivalent assay results (Figure 3). For either substrate, the Illumina iScanTM or BeadArray Reader measures the signal intensity at each address location, which corresponds to the quantity of the respective miRNA in the original sample.

The probe-target recognition and chemistry of the MicroRNA Expression Profiling Assay are fully compatible with formalin-fixed paraffin-
miRNA profiling assays yield nearly identical results whether they are read on the 96-sample Universal Array Matrix or the 12-sample Universal BeadChip.

High Performance

This assay takes advantage of enzymatic primer extension in addition to hybridization to achieve high mismatch discrimination. The BeadArray platform has the highest built-in feature redundancy of any currently available array, which further increases reproducibility and improves the signal-to-noise ratio.

To ensure data derived from these products are of the highest quality, miRNA-specific query oligos were subjected to rigorous probe screening. First, probes were selected for maximum predicted success in the assay. Then, to maximize assay specificity, candidate probes were examined collectively to minimize sequence similarity between probes, particularly at their 3' ends. Additionally, both human and mouse MicroRNA Expression Profiling Panels have undergone rigorous functional testing.

Illumina scientists empirically validated the high accuracy of miRNA quantitation. Results from MicroRNA Expression Profiling Panels were compared with those from qPCR assays (Figure 4). Expression differences for 12 miRNAs were measured by each method in four independent samples of both human and mouse cell lines. The results show high concordance between the two methods ($r^2 > 0.9$), indicating that this highly multiplexed assay yields quality data that are comparable with other lower throughput methods of detection. Using Illumina sequencing technology to conduct digital miRNA expression profiling provides further comprehensive cross-platform confirmation of the miRNA profiling assay. These experiments showed highly concordant results between the two completely separate assay methodologies using human cell lines or mouse tissue (Figure 5).

To confirm that the MicroRNA Expression Profiling Assay provides reproducible data from many different types of sample sources, several repeat experiments were conducted (Figure 6). Regardless of whether samples were prepared from RNA extracted from cell lines, fresh frozen tissues, or FFPE tissues, they all show high reproducibility ($r^2 > 0.99$). Consistent results are also supported by quality controlled reagent sets.

Since miRNA represent a relatively small fraction of total cellular RNA, specificity and sensitivity of detection are crucial factors to the success of miRNA profiling assays. This MicroRNA Expression Profiling assay is highly sensitive, and exhibits a fold-difference detection limit of 1.2 to 1.3-fold. The system dynamic range is approximately 4 logs.
**Full Software support**

MicroRNA Expression Profiling is fully supported by the BeadStudio software package. Image data scanned by the iScan or BeadArray Reader are analyzed using BeadStudio. There, a set of analysis tools allow standard evaluation of results, such as measurement of expression levels of specific miRNA or determination of differential miRNA expression between two experimental samples. Additionally, BeadStudio supports correlation analysis between mRNA and miRNA expression levels to integrate both types of experiments.

**Summary**

The MicroRNA Expression Profiling Panels are a highly sensitive solution for accurate measurement of miRNA. This assay offers quantitation accuracy comparable to qPCR with a high-throughput, highly multiplexed platform for broad miRNA profiling in humans or mice.

With this addition to the already strong portfolio of Illumina RNA Analysis products, researchers are now able to look at miRNA and mRNA easily in the same study. This product uses the same scanner for detection, and uses a highly analogous assay to existing Illumina Gene Expression Profiling products. This continuity enables researchers to easily expand their scope of RNA expression profiling.

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**Figure 5: High Concordance With Digital MIRNA Expression Profiling**

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<th>A: HUMAN HEK293 VS. PC3</th>
<th>B: MOUSE LIVER VS. BRAIN</th>
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All miRNAs on the profiling panel were measured with Illumina sequencing technology from several human cell lines and mouse tissues. In these examples, differential expression was compared between the two assays for HEK293 and PC3 cells (A) and for mouse liver and brain samples (B). Highly correlated results confirm that the miRNA profiling assay provides specific and quantitative measurements of miRNA abundance.

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**Figure 6: High Reproducibility**

Scatter plots of technical replicates exhibit high reproducibility using Illumina MicroRNA Expression Profiling Panels with different sample sources. High reproducibility is measured \( r^2 > 0.95 \) for all cases. Importantly, common fixation techniques do not prevent high assay performance.
References


Ordering Information

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