miRCURY LNA™ microRNA Arrays

Sensitive and specific microRNA microarrays – truly global microRNA expression profiling. LNA™-enhanced and $T_m$-optimized capture probes give uniform detection of all microRNAs. Exiqon offers a streamlined workflow from RNA labeling to data analysis.

At a glance
- Truly global microRNA profiling – ~3100 capture probes cover all human, mouse and rat microRNAs in miRBase V18.0
- $T_m$-optimized for robust detection of all microRNAs, regardless of GC-content
- Validated LNA™-enhanced capture probes for increased sensitivity and specificity
- Excellent sensitivity - microRNA profiling starting from 30ng total RNA
- Efficient discrimination of closely related microRNA family members
- Leading-edge data analysis software customized to Exiqon arrays available

Product coverage
- miRCURY LNA™ microRNA Array, 7th gen - hsa, mmu & rno
  The 7th generation of our array contains about 3100 capture probes, covering all human, mouse and rat microRNAs annotated in miRBase 18.0, as well as all viral microRNAs related to these species. In addition, this array contains capture probes for miRPlus™ human microRNAs. These are proprietary microRNAs not found in miRBase.

Advantages of LNA™ capture probes
As a unique feature of Exiqon’s microRNA array, all capture probes are LNA™-enhanced. LNA™ probes have two important advantages over traditional DNA probes (Figures 1 & 2):

1. **High affinity** - The addition of LNA™ to the capture probes results in high melting temperatures $T_m$ of the probe-target duplex, thus increasing the specificity and sensitivity of the array.
2. **Uniform affinity** - Unlike DNA capture probes, $T_m$-normalized LNA™ probes bind to their target sequences with equal affinity regardless of the GC-content of the microRNA. This can be achieved by varying the positions and amount of LNA™ in each probe.

As a consequence, all probes will perform optimally under the same high-stringency hybridization conditions.

Hi-Power labeling offers close to double signal intensity
Exiqon’s miRCURY LNA™ Hi-Power microRNA Labeling Kit offers almost double signal-to-noise ratios compared to our standard labeling kit. This means that microRNAs that were previously just below the level of detection, can now be readily detected. More microRNAs can be detected from the same amount of input RNA. Furthermore, the same number of microRNAs can be detected with half the amount of RNA when using the Hi-Power kit (Figure 3).

Figure 1. LNA™-enhanced capture probes ensure robust detection of all microRNAs. With DNA-based capture probes, half of microRNAs were either undetected or poorly detected. Signal strength (log2 signal/100amol target) from 660 synthetic microRNAs hybridized to Exiqon’s microarray and Supplier A’s DNA-based array are compared.

Figure 2. LNA™ capture probes have high uniform $T_m$. LNA™ probes have substantially higher $T_m$ than DNA probes. In addition, they can be $T_m$-normalized which means that all capture probes perform well under the same high stringency conditions.
Unmatched sensitivity
In combination with the miRCURY LNA™ microRNA Hi-Power Labeling Kit, Exiqon’s array has unmatched sensitivity (Figure 4). More than half of the LNA™ capture probes on the array have a detection limit of ≤0.5 amol.

Exiqon’s microRNA arrays can produce reliable results from as little as 30ng of total RNA (Figure 5). Because of the high specificity of the platform, the sample size can be scaled up without compromising the quality of the data. This is especially important when studying microRNAs expressed at low levels.

High specificity with single nucleotide discrimination
miRCURY LNA™ microRNA Arrays are highly specific for their microRNA targets. The combination of Tm-normalized LNA™ capture probes and hybridization conditions optimized for high stringency binding, dramatically increases the specificity of the capture probes. As a result, Exiqon arrays provide superior discrimination between closely related microRNA family members (Table 1).

Broad dynamic range
miRCURY LNA™ microRNA Arrays offer superior dynamic range over more than 5 orders of magnitude, ensuring that microRNAs with high and low expression levels will be detected well within the linear detection range.

Table 1. Superior discrimination between microRNA family members.
There is very little cross-hybridization between let-7 family members. The experiments were performed with synthetic let-7 spike-in microRNA (300 amol) in a background of tRNA.

<table>
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<th>Let-7a</th>
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Figure 3. Detect the same number of microRNAs using half the RNA input. Signal intensities were compared between the Hi-Power and Power labeling kits. The experiments were conducted on Exiqon microRNA Arrays.

Figure 4. The most sensitive array available. Due to optimally designed Tm normalized capture probes and extremely efficient labeling, the Exiqon array detects a significantly higher percentage of microRNAs than competitor arrays.

Experimentally validated capture probes
Most capture probes of the miRCURY LNA™ microRNA Arrays have been experimentally validated using synthetic microRNAs. Probes not performing according to our strict quality standards are replaced and never make it onto the final product.

Spike-in miRNA Kit v2 for data quality improvement
The 7th gen microRNA array includes a kit with 52 synthetic spike-in microRNAs that can be detected on the array by specifically designed capture probes. When the spike-in microRNAs are added to the labeling reactions before array hybridization, the signals from the spike-in capture probes can be used as controls for the labeling reaction and hybridization, scanner settings, data normalization, array replicates and technical variability.

Figure 5. Reliable microRNA expression profiles with as little as 30 ng total RNA. Four different microarray experiments with varying amounts of input RNA from oesophagus cancer (T) and normal adjacent (N) tissue were compared. A very high correlation is obtained when plotting the results from the experiment using 1000ng input RNA against those using 300, 100 and 30 ng.

Log2 ratios Tumor vs. Normal (oesophagus) - correlation between various RNA input amounts

- correlation between various RNA input amounts

Log2 ratios Tumor vs. Normal (oesophagus) - correlation between various RNA input amounts
A robust system with high reproducibility

The miRCURY LNA™ microRNA Arrays feature very high reproducibility due to a stringent manufacturing process that ensures high quality uniform spots. This results in very low coefficient of variation (CV) values of the four replicate spots as well as excellent correlation between individual array slides. This makes the array ideal for single as well as dual color array experiments.

Validate your results with Exiqon’s qPCR system

Our qPCR system offers the best available combination of performance and ease-of-use on the microRNA qPCR market and is the ideal solution for validating your microarray results. The combination of a Universal RT reaction and LNA™-enhanced PCR primers results in unmatched sensitivity and specificity. Ready-to-use microRNA PCR panels enable fast and easy microRNA expression profiling (Figure 6). Identical positive controls on both platforms allows for robust cross-platform comparison of results.

Selected publications

Esquerra et al. PLoS ONE 2011
Huang et al. RNA Biol. 2011
Ralfkiaer et al. Blood 2011

For more publications and updated product information, go to www.exiqon.com/array

Did you know?

Exiqon can perform your microRNA profiling and data analysis for you. Find out more at www.exiqon.com/microRNA-array-profiling-services.

Figure 6. Excellent correlation between microarray and qPCR results.

The results you get with our array system can be validated using our qPCR system - Get results you can trust. The array data was normalized (quantile normalization) and microRNAs with log2 ratios > or < 0.5 were included in the study. The qPCR data was normalized to reference genes. Only microRNAs that were detected (Cp < 36 for all replicates) were included. A total of 26 microRNAs were included in the study.

Ordering information

<table>
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<tr>
<th>miRCURY LNA™ microRNA Array, 7th gen, hsa/mmu/rno</th>
<th>Product description</th>
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<td>3, 6 or 24 slides</td>
<td>Microarray slides, hyb &amp; wash buffer and spike-in miRNA</td>
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<td>Detergent solution</td>
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miRCURY LNA™ microRNA Array Analysis Software

At a glance
- Fast and accurate analysis of miRCURY LNA™ microRNA Array data
- ImaGene® 9 and Nexus Expression™ 3 in a package specifically adapted to Exiqon’s microarrays
- Easy workflow – no bioinformatics skills required
- Best-in-class grid placement, automatic analysis and quality check of all spots
- Quick and easy identification of differentially expressed microRNAs
- Direct links to microRNAs in miRBase and TargetScan

Leading-edge microarray analysis software
This comprehensive microarray data analysis package is ideal for use with the miRCURY LNA™ microRNA Arrays. It consists of the well-known ImaGene® 9 and Nexus Expression™ 3 from BioDiscovery, as well as settings files for rapid and easy analysis of Exiqon microarrays. Go from raw data to publication-ready results in an easy, step-by-step process without the need for advanced bioinformatics skills.

Overview of ImaGene® 9 and Nexus Expression™ 3

ImaGene® 9
ImaGene® is a powerful tool for microarray image analysis. Through an intuitive interface, it lets the user extract signal intensities from the scanned array and flag (poor) spots either automatically or manually (Figure 1). ImaGene® can also be used for easy visualization of microarray data in scatter or M-A plots (Figure 2).

Nexus Expression™ 3
Nexus Expression™ is a comprehensive but easy-to-use program for the analysis of microarray experiments. Using a simple workflow, raw data from ImaGene® is background-subtracted and normalized, after which the data can be visualized in heat maps and differentially expressed microRNAs identified (Figure 3).

Ordering information

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