MicroRNA profile using miRCURY™ LNA Array

The miRCURY™ LNA Array allows comprehensive profiling of all known microRNAs (miRNAs) in 54 organisms registered and annotated in miRBase. Here we show that data originated from the miRCURY LNA Array platform are in line with published in situ hybridization and sequencing data, confirming the high specificity of the miRCURY LNA microRNA Array platform.

miRNAs are short noncoding RNA molecules that affect stability and/or translation of mRNAs. miRNAs have been shown to be involved in the regulation of many biological processes, including development, differentiation and apoptosis, and are implicated in the pathogenesis of several human diseases including cancer.

The miRCURY LNA Array allows comprehensive profiling of all known miRNAs in 54 organisms registered and annotated in miRBase (The Wellcome Trust Sanger Institute). Additionally, the miRCURY LNA Arrays contain several miRPlus™ capture probes for miRNAs not yet annotated in the miRBase. Thus, the miRCURY LNA Arrays have a strong discovery potential and are very well suited for generation of miRNA molecular signatures.

Sensitive and specific miRNA profiling

Microarrays are one of the fastest and most comprehensive methods for determining the miRNA profile of a given sample.

The miRCURY LNA microRNA Arrays provide researchers with the ability to conduct genome-wide profiling of miRNAs in various samples including tissue, blood and formalin-fixed paraffin-embedded (FFPE) samples, and to identify miRNA signatures associated with development, differentiation and metabolism, providing valuable diagnostic and prognostic indicators of disease.

miRNA profiling differs from global mRNA expression profiling in several important aspects. First of all, it is extremely difficult to target RNA molecules as short as miRNAs (16–29 nucleotides). Standard DNA probes are not always able to discriminate sequences with single-nucleotide differences. This can result in many nonspecific signals. Another difference between global mRNA expression profiling and miRNA profiling is the number of expressed targets at a given time point in a given tissue (call rate). mRNA gene expression arrays contain thousands of different capture probes, and in most cases the call rate is higher than 50%. In contrast, the call rate for miRNA samples is usually low because only 20–35% are expressed per cell type.

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Figure 1 | Excellent correlation of 91% between 30 ng and 1,000 ng total RNA samples. Log₂ ratios of tumor versus normal adjacent tissue (T/N) of an esophagus cancer using 1,000 ng total RNA material are plotted against log₂ ratios in identical experiments using 30, 100 and 300 ng total RNA. The correlations to the miRNA profile are indicated.

The miRCURY LNA microRNA Arrays are optimal for detection of miRNAs with superior sensitivity and specificity. The LNA capture probes are designed with optimal content and positioning of LNA to obtain efficient target recognition and have a normalized melting temperature ($T_m$), ensuring that all miRNA targets hybridize to the array with equal affinity under high-stringency hybridization conditions. Without prior knowledge of the miRNA content in the sample, it is recommended to use around 250 ng of total RNA to facilitate the most robust expression profiles. However, the high sensitivity of the miRCURY LNA microRNA Arrays allows generation of reliable miRNA expression profiles from only 30 ng of total RNA (Fig. 1).

The specificity of the miRCURY LNA microRNA Array platform has previously been illustrated by an miRNA profile of breast cancer tissue (Litman, T. et al. MicroRNA profiling of breast cancer tissue using an LNA-based microarray. Nat. Methods 4, 2007). The analysis revealed many differentially expressed miRNAs, including those...
reported earlier to be associated with cancer, such as several members of the let-7 family and miR-21 (ref. 5). Some of these miRNAs may represent new molecular biomarkers with diagnostic and prognostic promise for individuals with cancer (Fig. 2). Additionally, a randomly selected subset of the thousands of samples that have been analyzed at Exiqon’s miRNA profiling service department shows an average call rate of 29% (data from 187 human samples from a variety of different tissues; Fig. 3). This is in line with published in situ hybridization and sequencing data, and confirms the high specificity of the miRCURY LNA microRNA Array platform14.

The list of publications using the miRCURY LNA microRNA Arrays is continuously growing. A few are mentioned below. An miRNA profile on Dicer knockdown in human endothelial cells using the miRCURY LNA microRNA Arrays revealed 25 highly expressed miRNAs in human endothelial cells6. Using miRNA mimicry it was shown that miR-222/221 regulate endothelial nitric oxide synthase protein levels after Dicer silencing. Collectively, these results indicate that maintenance and regulation of endogenous miRNA levels via Dicer-mediated processing is critical for endothelial cell gene expression and function in vitro.

The miRCURY LNA microRNA Array was used to show that exosomes (vesicles of endocytic origin released by many cells) do contain miRNAs and that exosomes contain both miRNA and miRNA that can be delivered to another cell and can be functional in this new location7.

A global miRNA profiling study showed that Drosha overexpression in cervical squamous cell carcinomas seems to be of functional importance8. Unsupervised principal component analysis of a mixed panel of cervical squamous cell carcinoma cell lines and clinical specimens showed clear separation according to Drosha overexpression. The miRNAs most significantly associated with Drosha overexpression are implicated in carcinogenesis in other tissues, suggesting that they regulate fundamental processes in neoplastic progression.

These data, as well as those from many other publications that used the miRCURY LNA microRNA Arrays, confirm that these arrays yield sensitive, specific and reliable data.

An updated list of published papers using the miRCURY LNA Arrays can be found at http://www.exiqon.com/array.

Conclusions

The miRCURY LNA Arrays have permitted many studies of miRNAs by providing researchers with highly sensitive, specific and potent product. Several peer-reviewed publications demonstrate the effectiveness and validity of the miRCURY LNA Array and show how the product is allowing miRNA science to advance.

Additional information about the miRCURY LNA microRNA product portfolio from Exiqon is available at http://www.exiqon.com.


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