GENOMICS INFORMATICS PROTEOMICS METABOLOMICS

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G A A T C T G A T C C T T G A A C T A C C T T C C A A G G T G

The Agilent Human miRNA Microarray

Pioneer the Role of miRNAs in Your Genomics Story

Agilent has developed a new microarray-based application for studying microRNAs (miRNAs) that combines a unique miRNA direct labeling method with our innovative probe design and established high-performance SurePrint inkjet synthesis technology. miRNA is the latest addition to our integrated and comprehensive repertoire of proven genomics tools. The creation of complete miRNA expression profiles using robust and highly sensitive microarrays allows you to be the first to gain broad insight into human miRNA expression and regulation. This new capability is a unique opportunity to develop a confident and clear picture of the intricate expression networks and systems that impact your genomics research.

"Agilent's miRNA expression profiling platform includes a straightforward and easy sample preparation procedure combined with their well-established inkjet-printed arrays. An advantage of their technology compared to others is that a very low amount of starting total RNA sample is required, thus enabling clinical sample profiling."

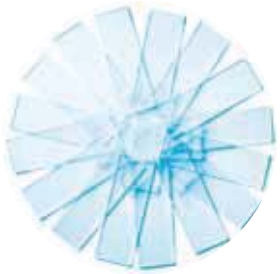
—**Dr. Zora Modrusan**
Scientist, Head of Microarray Laboratory
Molecular Biology
Genentech

Implications for Cancer Research

MicroRNAs (miRNAs) are a prevalent class of small single-stranded non-coding RNAs (19-30 nts long). They serve widespread functions as regulatory molecules in post-transcriptional gene silencing and have recently emerged as crucial regulators of gene expression, development, proliferation and differentiation, and apoptosis.

Since the discovery of miRNAs in 1993, the number of miRNAs in the Sanger miRBASE database has rapidly increased. ~4400 precursor miRNAs (based on miRBASE) have been found to date in virtually all species—animals, plants, and viruses. More than 470 human miRNAs have been publicly identified, and as many as one-third of all human genes may be miRNA-regulated. This diverse yet fundamentally conserved group of small RNAs may rival classical transcription factors in their role and involvement in modulating the complex regulatory circuitry found in cells.

Much recent human cancer research has been intensely focused on studying and understanding miRNA expression. Gene expression pattern changes resulting from altered and/or aberrant miRNA expression fingerprints may be a key determinant of their ultimate function—oncogene or tumor suppressor. Clearly, miRNA expression signatures are invaluable and hold great promise in human disease characterization, potentially as prognostic indicators for chemotherapy, diagnostic markers for tumor classification, and biomarkers.



Explore in detail further research highlights from the publication **"Direct and Sensitive miRNA Profiling From Low Input Total RNA"** (Wang *et al*) from RNA (2007) 13(1):151-59. This scientific publication can be found at: www.opengenomics.com/miRNA

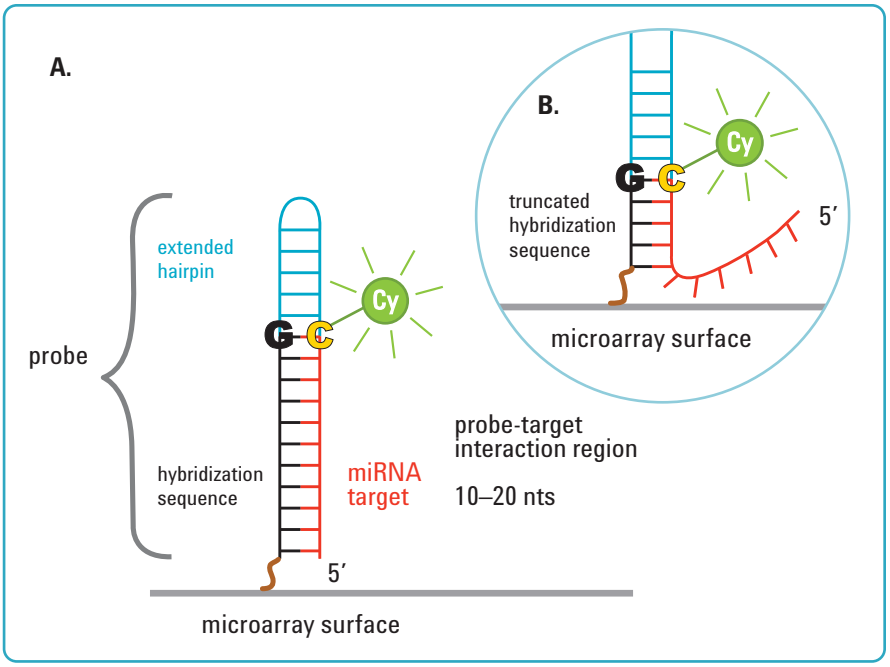


Figure 1. Components of the Agilent miRNA microarray probe design. An unmodified microarray probe (black) is a synthesized sequence that hybridizes to the target miRNA (red). Probes are anchored to the glass slide surface by a stilt (brown). **A.** Inclusion of a G residue (black) to the 5' end of the hybridization sequence complements the 3' end C residue (yellow) introduced in labeling. This additional G-C pair in the probe-target interaction region stabilizes targeted miRNAs relative to homologous RNAs. Additionally, all probes contain a 5' hairpin (blue), abutting the probe-target region, to increase target and size miRNA specificity. **B.** Destabilization of probes that are too stable. For probes requiring it, reduction of probe-target base-pairing is achieved through sequential elimination of base pairing from the 5' end of the miRNA.

Innovative Labeling and Probe Design

Agilent's miRNA microarray is the only commercially available high-throughput system that delivers the optimal sensitivity and specificity for both sequence and size discrimination, even between closely-related mature miRNAs. This superior performance results from our unique probe design, highly efficient direct labeling method, and our proprietary SurePrint inkjet technology, which, unlike competitive platforms, synthesizes 40–60-mer oligonucleotide probes directly on the array, resulting in high-purity, high-fidelity probes.

The small size of miRNA represents a particularly unique challenge for hybridization-based detection methods, requiring a novel labeling and design strategy compared to those used with conventional genomic and mRNA targets. Agilent's innovative probe design and *in situ*-synthesized probes have minimal sequence bias and use unmodified DNA oligonucleotides.

Our miRNA platform requires small input amounts of total RNA—in the 100 nanogram range—because it uses a high-yield labeling method, and does not require size fractionation or amplification steps that may introduce undesired bias during miRNA profiling. The simple, straightforward experimental protocol allows sample dephosphorylation and direct-labeling to take place in the same tube. Unlike conventional polymerase-based methods, this end-labeling method is insensitive to nucleotide damage within the substrate RNA and is advantageous for working with preserved or chemically treated samples.

There are several key probe design features illustrated in **Figure 1A**. Our labeling protocol adds a C residue to the 3' end of miRNAs. The inclusion of G residue at the 5' end increases the stability of binding to labeled target miRNAs. Empirical probe selection studies have shown that the incorporation of a 5' end hairpin provides valuable discrimination for increasing target size specificity, as it destabilizes probe hybridization to larger, non-target RNAs.

To achieve highest sequence specificity, all probe-target interactions should ideally have the same stability under the assay conditions. In situations where the probe-target duplex is too stable (potentially resulting in non-specific interactions), the hybridization is optimized through reduction from the 5' end of the miRNA (**Figure 1B**). This design optimization improves the final specificity of the probes.

Precise miRNA Discrimination

Agilent miRNA probes can accurately discriminate between similar sizes and sequences, as demonstrated by studies with 19 synthetic human miRNAs with high sequence homology to other miRNAs. These show low cross-hybridization for miRNAs differing by > 1 nt. With the well-studied human *let-7* family of miRNAs, probe-target sequence cross-hybridizations > 5% were observed in less than 10% of 56 potential cross-hybridization events. miRNA families such as the hsa-miR-196 and hsa-miR-30 showed cross-hybridizations of < 1%.

Flexibility for the Evolving miRNA Landscape

Agilent's SurePrint technology, probe design methods, and printing formats are powerful components of the Agilent integrated platform that allow for regular and ongoing content updates to accommodate newly discovered sequences in the continuously evolving miRNA landscape. Our printing formats can accomodate significant increases in the number of sequences for comprehensive yet convenient coverage.

Integrated Platform

As the latest addition to our integrated and comprehensive portfolio of proven microarray-based genomics tools, miRNA profiling is synergistic with our gene expression and array-based CGH solutions. Agilent's core microarray technology for miRNA encompasses sample labeling and an integrated experimental workflow, as well as data analysis, visualization, and comparison across multiple applications. By enabling you to answer complex questions at the intersection of transcriptomics, genetics, and proteomics you get the whole story.

Key Features and Benefits

Significant advantages such as optimized probe design method and labeling protocols, as described in Wang et al., are the basis for Agilent's commercial miRNA profiling solution. Our microarray contains ~15,000 features printed in an 8-plex format (eight individual microarrays on a 1" x 3" glass slide). It contains probes and annotation information for all human miRNAs sourced from the Sanger miRBASE public database (Release 9.1, February 2007).

- **Low sample input** – 100 ng total RNA requirement enables analysis of limited samples (fine needle aspirates, blood, plasma, etc.)
- **High sensitivity and specificity** – confident detection of both low-abundance and highly homologous miRNAs
- **Broad linear dynamic range** – spans over four orders of magnitude and ensures thorough and comprehensive profiling of all miRNAs across their biologically occurring range of expression
- **Low detection limit** – detection of synthetic miRNAs at concentrations less than 0.1 amol

"Lung cancer is the leading cause of cancer-related deaths in Japan. We have shown for the first time that *let-7* expression is frequently reduced in lung cancers and that alterations in miRNA expression may have an prognostic impact on survival of surgically-treated lung cancer patients. Agilent gives us a comprehensive miRNA expression profile with excellent performance on sensitivity and accuracy. I expect that studies with the Agilent miRNA array may ultimately provide a foundation for a new paradigm of the involvement of miRNA in human oncogenesis."

—**Dr. Takashi Takahashi**
Professor of Oncology,
Molecular Carcinogenesis
Nagoya University

Specifications

Format	8 x 15K
Microarrays per slide	8 (8-plex)
Slides per kit	3
Slide format	1" x 3" (25mm x 75mm)
Average probe length	~ 40-60 nucleotides (depends on probe)
Replicate features per miRNA	20–40
Feature size	65 µm
Total features	~ 15,000
Sequence source	Sanger miRBASE (Release 9.1, February 2007)
Human miRNA Microarray Kit (3 slides)	G4470A
miRNA Labeling Reagent and Hybridization Kit	5190-0408
Hybridization Chamber	G2534A
Hybridization Gasket Slide	G2534-60014
Starting sample input for labeling	100 ng total RNA
Labeling type	Direct end labeling using Cyanine 3 pCp
Overall assay time	< 2 days
Storage condition for microarray	Room temperature (in the dark)
Storage condition for Cyanine 3 pCp	–20° C

Connect to the latest version of the Sanger miRBASE at:
http://microRNA.sanger.ac.uk

About Agilent Technologies

Agilent Technologies is a leading supplier of life science research systems that enable scientists to understand complex biological processes, determine disease mechanisms, and speed drug discovery. Engineered for sensitivity, reproducibility, and workflow productivity, Agilent's life science solutions include instrumentation, microfluidics, software, microarrays, consumables, and services for genomics, proteomics, and metabolomics applications.

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