

Agilent Human, Mouse, Rat, and Custom miRNA Microarrays

Technical Overview

Regular miRBase content updates allow for flexibility in catalog and custom array designs

Low sample input preserves precious RNA

Highly sensitive platform detects broadest range of miRNAs, spanning over 5 orders of magnitude

Optimized probe design yields highest sensitivity

Agilent's miRNA microarray platform of human, mouse, rat, and custom arrays enables high-throughput miRNA profiling. Combining a unique miRNA direct labeling method with our innovative probe design and established high-performance SurePrint inkjet synthesis technology, our miRNA microarrays provide broad insight into gene expression regulation. Our human, mouse, and rat arrays are based on the latest miRBase public database release, which continues to grow as new miRNAs are discovered. Custom array designs are also available, offering researchers the broadest insight into the intricate networks that impact gene expression.

Direct and Sensitive miRNA Profiling

MicroRNAs (miRNAs) are a prevalent class of small single-stranded non-coding RNAs (19–30 nucleotides long). Precursor miRNAs have been found in nearly all species, including animal, plant, and viral, and as many as 60% of mammalian genes may be regulated by miRNAs. Playing a widespread role in post-transcriptional gene silencing, miRNAs have emerged as crucial regulators of gene expression, development, proliferation and differentiation, and apoptosis. miRNA expression signatures have become invaluable in cancer research, as many miRNAs have been discovered to play a role in both oncogenesis and tumor suppression.

Agilent's miRNA platform provides researchers a complete profiling solution based on an optimized probe design method and labeling protocols. Offered in both 8x15K and 8x60K formats, our arrays enable highly sensitive and specific miRNA expression analysis.



Agilent Technologies

Innovative Labeling and Probe Design

Agilent's microarray-based, high-throughput miRNA expression profiling system delivers 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 proprietary SurePrint inkjet technology, which synthesizes 40–60-mer oligonucleotide probes directly on the array. This results in exceptionally high-purity, high-fidelity probes.

The small size of miRNAs 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.

Because it uses a high-yield labeling method and does not require size fractionation or amplification steps that may introduce bias, the Agilent miRNA platform requires only small input amounts of total RNA—in the 100-ng range.

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.

Several key probe design features are illustrated in Figure 1A. Our labeling protocol adds a C residue to the 3' end of the miRNA. The inclusion of G residue at the 5' end increases the stability of binding to a labeled target miRNA. Empirical probe selection studies have shown that the incorporation of a 5' end hairpin provides valuable discrimination for increasing target size specificity because 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.

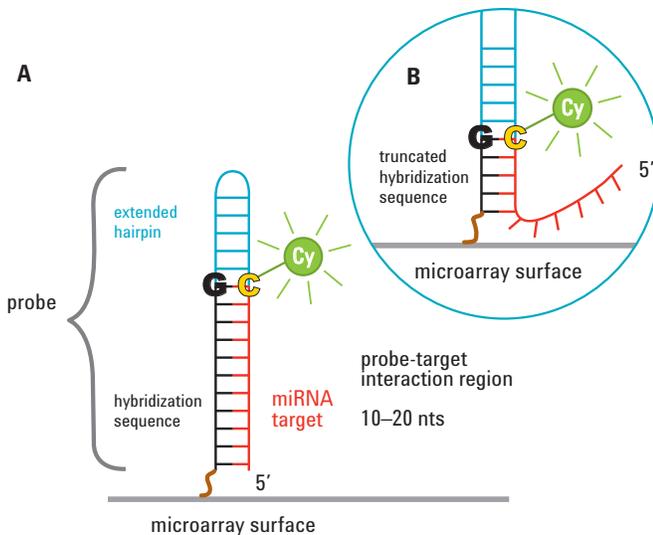


Figure 1. Components of the Agilent miRNA microarray probe design. An unmodified microarray probe (black) is a synthesized sequence that hybridizes to the target miRNA (red). Probes are anchored to the glass slide surface by a stilt (brown). A. Inclusion of a G residue (black) to the 5' end of the hybridization 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.

“Identification of tumor-suppressor miRNAs (TS-miRNAs) has been attracting major interest in the field of cancer research because of the crucial roles of miRNAs in carcinogenesis. We carried out functional genetic screens by using a lentivirus miRNA expression library and Agilent custom-made array, and successfully identified miR-22 that acts as a tumor-suppressor gene in human colon cancer. The Agilent microarray system is a powerful tool for rapid identification of TS-miRNAs, and it gives high-quality comprehensive genomic information with an advantage compared with other experimental methods, including deep sequencing.”

— Dr. Naoto Tsuchiya
Laboratory Head, Division of Multistep Carcinogenesis
National Cancer Center Research Institute

Flexibility in a Dynamic miRNA Landscape

Our 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 miRNA sequences from the most current miRBase database. Agilent printing formats can accommodate significant increases in the number of sequences for comprehensive yet convenient coverage.

Customizable Arrays

Our custom offerings are extensive and enable the most flexibility for miRNA experimental analysis. Depending on your research needs, you can choose from a variety of catalog kits or design a custom array for any organism in miRBase using new sequences from the latest miRBase release. In addition to being able to design probe sets for any organism, Agilent can also design a microarray that contains multiple organisms on a single array, providing even higher throughput.

Quality Support

Agilent offers an optional miRNA Spike-In Kit (P/N 5190-1934) that provides positive controls for monitoring the labeling and hybridization efficiencies of the miRNA workflow. The efficiency of the reactions are evaluated and reported in the Feature Extraction QC Report. In addition, the Universal miRNA Reference Kit (P/N 750700) is an ideal reference control for miRNA microarray experiments. Used for optimizing and standardizing miRNA analysis, the Reference RNA sample contains an extensive range of miRNAs, including those for mRNA, large intergenic non-coding RNA, and piwi-interacting RNA.

Integrated Platform

Agilent's miRNA microarrays are an integral part of our microarray portfolio. From sample labeling and hybridization to data analysis and visualization, we offer a complete solution for performing microarray analysis. Using Agilent's leading GeneSpring GX software, which now enables multi-omic analysis, comparing miRNA profiling data to other transcriptomic data helps researchers see the whole story.

miRNA Microarray General Specifications

	8x15K Format	8x60K Format
Microarrays per slide	8	8
Slide format	1" x 3"	1" x 3"
Probe length	40–60-mer	40–60-mer
Feature size	65 µm	30 µm
Number of features per miRNA	16–20	40
Total features	~15,000	~60,000
Input amount	100 ng	100 ng
Starting sample input	Total RNA	Total RNA
Labeling type	Direct end labeling using Cyanine 3-pCp	Direct end labeling using Cyanine 3-pCp
Overall assay time	<2 days	<2 days
Storage condition for microarray	Room temperature (in the dark)	Room temperature (in the dark)
Storage condition for Cyanine 3 pCp	–20°C	–20°C

miRNA Ordering Information

Microarrays						
Product – Single Slides	Product #	Design ID	Format	Composition	Probes	
Human						
SurePrint G3 Human miRNA Microarray, Release 21.0	G4872A	070156	8 x 60K	miRBase 21.0	2,549 human	
SurePrint G3 Human miRNA Microarray, Release 19.0	G4872A	046064	8 x 60K	miRBase 19.0	2,006 human	
Mouse						
SurePrint G3 Mouse miRNA Microarray, Release 21.0	G4872A	070155	8 x 60K	miRBase 21.0	1,881 mouse	
SurePrint G3 Mouse miRNA Microarray, Release 19.0	G4872A	046065	8 x 60K	miRBase 19.0	1,247 mouse	
Rat						
SurePrint Rat miRNA Microarray, Release 21.0	G4471A	070154	8 x 15K	miRBase 21.0	758 rat	
SurePrint Rat miRNA Microarray, Release 19.0	G4471A	046066	8 x 15K	miRBase 19.0	719 rat	
Custom Microarrays						
<i>New!</i> Custom microarrays can be designed using the latest content from miRBase 21.0						
SurePrint G3 Custom miRNA Microarray, 8x60K	G4871A		8 x 60K	N/A	As many as the format permits	
Sureprint Custom miRNA Microarray, 8x15K	G4474A		8 x 15K	N/A	As many as the format permits	

Bioreagents and Instrumentation	
Product	P/N
Sample QC	
microRNA Spike-in Kit	5190-1934
Universal miRNA Reference Kit	750700
Sample preparation and isolation	
Absolutely RNA miRNA Purification Kit	400814
2100 Bioanalyzer System	Contact local account manager for details
RNA 6000 Nano Kit	5067-1511
Small RNA Kit	5067-1548
Sample labeling	
miRNA Complete Labeling and Hybridization Kit	5190-0456
Microarray processing	
Gene Expression Wash Buffer Kit	5188-5327
Hybridization Chamber Kit	G2534A
Hybridization Gasket Slide Kit	G2534-60014 (See web site for additional pack sizes)
Hybridization Oven	G2545A
Microarray scanning	
SureScan Microarray Scanner	Contact local account manager for details
Microarray data analysis	
GeneSpring GX Bioanalysis Software	Contact local account manager for details
Validation	
AriaMx QPCR System	Contact local account manager for details
Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix	See web site for details

Learn more:

www.agilent.com/genomics/miRNA21

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