

miRNA

Технические характеристики

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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’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

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.

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.



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

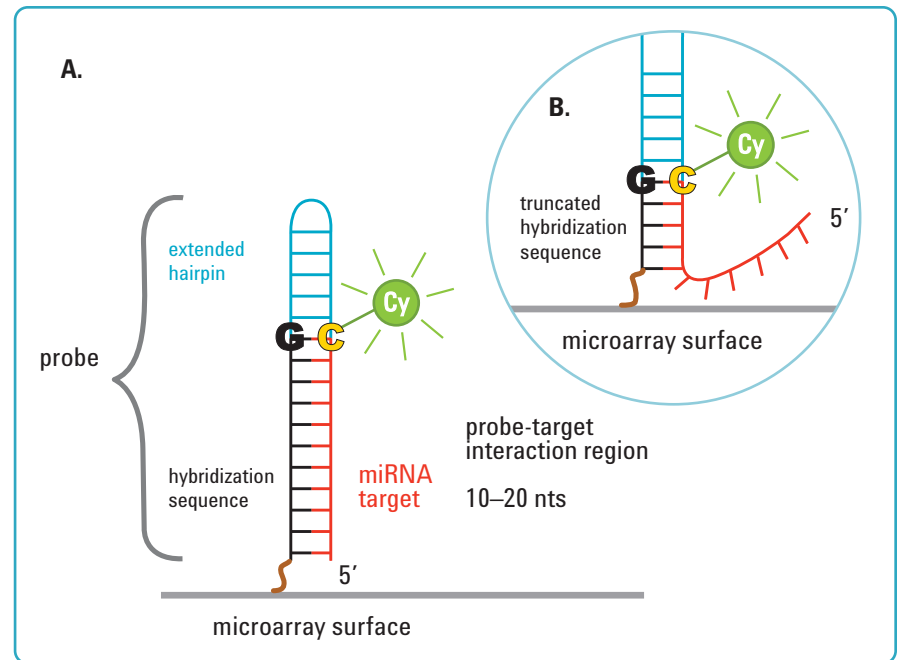


Figure 1. Components of the Agilent miRNA microarray probe design. An unmodified microarray probe (black) is a synthesized sequence that hybridizes to the target miRNA (red). Probes are anchored to the glass slide surface by a stilt (brown). **A.** Inclusion of a G residue (black) to the 5' end of the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

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 accommodate 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 a 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

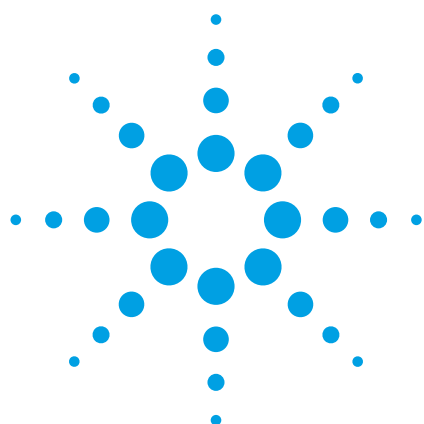
Specifications

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

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.





Agilent Bioreagent Components for Processing Microarrays

Gene Expression and miRNA Reagents

Part Number	Description
5185-6000	Total RNA Isolation Mini Kit (50) Contains reagents, mini-pretreatment columns, mini-isolation columns, and final nuclease-free collection tubes for 50 total RNA isolations from mammalian cells (5×10^5 – 5×10^6), tissues (2.5–30 mg), yeast (10^7) and bacteria (10^8).
5188-2780	Plant RNA Isolation Mini Kit (50) Contains reagents, mini-pretreatment columns, mini-isolation columns, and final nuclease-free collection tubes for 50 total RNA isolations from a wide range of species and tissue types. ¹
5190-2305	Low Input Quick Amp Labeling Kit, one-color Contains Cyanine 3-CTP and enzyme kit to amplify and label RNA for oligo microarrays. Uses 25–200 ng total RNA input. Kit is sufficient for 24 reactions. Low Input Quick Amp Labeling Kit with Cyanine 5-CTP is also available (Part Number 5190-2307).
5190-2306	Low Input Quick Amp Labeling Kit, two-color Contains Cyanine 3-CTP, Cyanine 5-CTP, and enzyme kit to amplify and label RNA for oligo microarrays. Uses 25–200 ng total RNA input. Kit is sufficient for 48 reactions, 24 with each dye.
5190-2308	Low Input Quick Amp Labeling Kit, no dye Contains enzyme kit to amplify and label RNA for oligo microarrays. Uses 25–200 ng total RNA input. Kit is sufficient for 24 reactions. This product does not contain Cyanine CTP.
5190-0442	Quick Amp Labeling Kit, one-color Contains Cyanine 3-CTP and enzyme kit to amplify and label RNA for oligo microarrays. Uses 200 ng–1 µg total RNA input. Kit is sufficient for 20 reactions. Low Input Quick Amp Labeling Kit with Cyanine 5-CTP is also available (Part Number 5190-2307).
5190-0444	Quick Amp Labeling Kit, two-color Contains Cyanine 3-CTP, Cyanine 5-CTP, and enzyme kit to amplify and label RNA for oligo microarrays. Uses 50 ng–2 µg total RNA input. Kit is sufficient for 20 reactions, 10 with each dye.
5190-0447	Quick Amp Labeling Kit, no dye Contains enzyme kit to amplify and label RNA for oligo microarrays. Uses 25–200 ng total RNA input. Kit is sufficient for 20 reactions. This product does not contain Cyanine CTP.
5188-5282	One-Color RNA Spike-in Kit Contains dilution buffer and a pre-mixed cocktail in a predetermined ratio-generating concentration to serve as a positive microarray control. Kit sufficient for at least 200 microarray hybridizations.
5188-5279	Two-Color RNA Spike-in Kit Contains dilution buffer and two pre-mixed cocktails in predetermined ratio-generating concentrations that serve as positive microarray controls. Kit sufficient for at least 200 microarray hybridizations.
5188-5242	Gene Expression Hybridization Kit Contains 2X Hi-RPM Hybridization Buffer, 25X Fragmentation Buffer, and 10X Gene Expression Blocking Agent for at least 10 slides.
5190-0404	Hi-RPM Gene Expression Hybridization Kit, Large Volume Contains 2X Hi-RPM Gene Expression Hybridization Buffer, 25X Fragmentation Buffer, and 10X Gene Expression Blocking Agent for at least 100 slides. Components may be purchased separately.



Gene Expression and miRNA Reagents (continued)

Part Number	Description
5188-5325	Gene Expression Wash Buffer 1 Contains wash buffer 1 packaged in 4L container. Sufficient for 80 slides.
5188-5326	Gene Expression Wash Buffer 2 Contains wash buffer 2 packaged in 4L container. Sufficient for 80 slides.
5188-5327	Gene Expression Wash Buffer Kit Contains two 5188-5325 (Gene Expression Wash Buffer 1, 4L each), one 5188-5326 (Gene Expression Wash Buffer 2, 4L) and Triton X-102 detergent additive. Kit contains sufficient wash buffer for up to 80 slides. Wash buffers may be purchased separately.
5185-5979	Stabilization and Drying Solution, 500 mL Contains reagent that prevents ozone-induced degradation of cyanine dyes during processing and scanning of Agilent oligo-based microarrays. Sufficient stabilization and drying solution for up to 50 gene expression slides. Customer must purchase the following to use this product with oligo aCGH arrays: Acetonitrile (Sigma-Aldrich 271004-1L).
5190-0456	miRNA Complete Labeling and Hybridization Solution Contains a novel Cyanine 3-nucleotide along with all necessary array labeling and hybridization reagents. Contains sufficient reagents to label and hybridize 24 microarrays. Kit contents include T4 RNA Ligase, Calf Intestinal Phosphatase, Cyanine 3-pCp, Hi-RPM Hybridization Buffer, DMSO, and associated buffers.
750700	Universal miRNA Reference Kit High-quality human total RNA for miRNA and gene expression profiling on microarray and QPCR platforms. Comprised of 9 different tissues and cell lines for broad gene and miRNA coverage on human microarrays. Contains: 1 tube x 10 µg, in storage buffer, 10uL.
740000	Universal Human Reference RNA High-quality total RNA for human, mouse and rat microarray gene-expression profiling. Comprised of 10 cell lines for human, 11 for mouse, and 14 for rat giving broad gene coverage. Not for use with miRNA experiments. Contains 2 tubes x 200 µg each, RNase-free water, 1.5 mL.
5190-1934	microRNA Spike-In Kit Includes 2 vials of spike in controls (one labeling spike-in vial and one hybridization spike-in vial) and 2 vials of dilution buffer. Kit is sufficient for 384 reactions. Kit is to be used with miRNA Complete Labeling and Hyb Kit (part Number 5190-0456).

2100 Bioanalyzer RNA and DNA Kits

Part Number	Description
5067-1511	Agilent RNA 6000 Nano Kit For the analysis and quantitation of total and mRNA samples of 25 to 500 ng/µL in concentration. The kit includes microfluidic chips, reagents and consumables sufficient for 300 samples.
5067-1513	Agilent RNA 6000 Pico Kit For the analysis of low concentrated RNA samples down to 50 pg/µL of total RNA or 250 pg/µL of mRNA. The kit includes microfluidic chips, reagents and consumables sufficient for 275 samples.
5067-1548	Agilent Small RNA Kit For the analysis and quantitation of small RNA samples of 6 and 150 nt in size and 50 to 2000 pg/µL in concentration. The kit includes microfluidic chips, reagents and consumables sufficient for 275 samples.
5067-1504	Agilent DNA 1000 Kit For the sizing and quantitation of dsDNA fragments from 25 to 1000bp. The kit includes microfluidics chips, reagents, and consumables sufficient for 300 samples.
5067-1506	Agilent DNA 7500 Kit For the sizing and quantitation of dsDNA fragments from 100 to 7500bp. The kit includes microfluidics chips, reagents, and consumables sufficient for 300 samples.
5067-1508	Agilent DNA 12000 Kit For the sizing and quantitation of dsDNA fragments from 100 to 12000bp. The kit includes microfluidics chips, reagents, and consumables sufficient for 300 samples.
5067-4626	Agilent High Sensitivity DNA Kit For the sizing and quantitation of dsDNA fragments from 50 to 7000bp. The kit includes microfluidics chips, reagents, and consumables sufficient for 110 samples.

CGH, ChIP-on-Chip, and DNA Methylation Reagents

Part Number	Description
5190-0449	Genomic DNA Enzymatic Labeling Kit Contains Cyanine 3- and Cyanine 5-dUTP and reagents to label genomic DNA for Oligo aCGH microarrays. Kit is sufficient for 50 reactions (or 100 reactions for the 8-pack). Customer must purchase the following to purify the labeled DNA: Amicon Ultra-0.5, Ultracel-30 Membrane, 30 kDa filter units (Millipore part numbers UFC503008, UFC503024, UFC503096 or UFC5030BK) or AutoScreen-96A well plates (GE Healthcare part number 25-9005-98).
5190-0450	Genomic DNA High-Throughput ULS Labeling Kit Genomic DNA labeling kit validated and optimized for DNA isolated from FFPE, cells, blood, and tissue. Contains sufficient two-color labeling reaction reagents for 16 — 1-pack, 24 — 2-pack, 48 — 4-pack, and 96 — 8-pack microarrays. Customers should purchase either the Genomic DNA Purification Module (5190-0418) or the Genomic DNA 96-well Purification Module (5190-0451) to purify the labeled genomic DNA.
5190-0419	Genomic DNA ULS Labeling Kit Genomic DNA labeling kit validated and optimized for DNA isolated from FFPE, cells, blood, and tissue. Contains sufficient two-color labeling reaction reagents for 5 microarray slides of the formats 1-pack, 2-pack, 4-pack or 8-pack. Contains sufficient purification columns for processing 5 — 1-pack microarrays. Additional columns and tubes should be ordered separately when using multi-pack microarrays (5190-0418).
5190-0451	Genomic DNA 96-well Purification Module Used for ULS-labeled genomic DNA purification. Contents include 1 96-well purification plate, 1 wash plate, and 1 collection plate.
5190-0418	Genomic DNA Purification Module Used for ULS-labeled genomic DNA purification. Contents include 10 purification columns and 10 collection tubes.
5188-5220	Oligo aCGH/ChIP-on-Chip Hybridization Kit (25) Contains 2X Hi-RPM Hybridization Solution and 10X Blocking Agent for 25 slides. Customer must purchase the following to prepare hybridization solution: Human Cot-1 DNA (Invitrogen part numbers 15279-011 or 15279-101 and Kreatech part number EA-020) or Mouse Cot-1 DNA (Invitrogen part number 18440-016) or Rat Hybloc (Applied Genetics part number RHB). DNase/RNase-Free Distilled Water (Invitrogen part number 10977-015).
5188-5380	Oligo aCGH/ChIP-on-Chip Hybridization Kit, Large Volume Contains 2X Hi-RPM Hybridization Solution and 10X CGH Blocking Agent for 100 slides. Customer must purchase the following to prepare hybridization solution: Human Cot-1 DNA (Invitrogen part numbers 15279-011 or 15279-101 and Kreatech part number EA-020) or Mouse Cot-1 DNA (Invitrogen part number 18440-016) or Rat Hybloc (Applied Genetics part number RHB). DNase/RNase-Free Distilled Water (Invitrogen part number 10977-015).
5188-5221	Oligo aCGH/ChIP-on-Chip Wash Buffer 1, 4L Contains wash buffer (Buffer 1) packaged in 4L container. Sufficient for 20 slides.
5188-5222	Oligo aCGH/ChIP-on-Chip Wash Buffer 2, 4L Contains wash buffer (Buffer 2) packaged in 4L container. Sufficient for 40 slides.
5188-5226	Oligo aCGH Wash Buffer Kit This kit contains Oligo aCGH Wash Buffer 1 (4L, part number 5188-5221) and Oligo Wash Buffer 2 (4L, part number 5188-5222). This kit is available in two configurations: Option 1 for manual processing contains 2 containers of Wash Buffer 1 and 1 container of Wash Buffer 2. The kit is sufficient for processing up to 40 oligo aCGH slides. Option 2 for Tecan processing contains 1 container of Wash Buffer 1 and 2 containers of Wash Buffer 2. The kit is sufficient for processing up to 120 oligo aCGH slides.
5190-0402	Oligo aCGH Prehybridization Buffer, 4L For use in Tecan processing protocols only. Sufficient prehybridization buffer for approximately 120 slides when processed on the Tecan HS 400 Pro or HS 4800 Pro with compatible hybridization chambers.
5190-0401	Wash Buffer Additive For use in Tecan processing protocols only. Solution is used to prepare Conditioning Solution and Wash Buffer 2 for processing oligo aCGH arrays on the Tecan HS 400 Pro or HS 4800 Pro with compatible hybridization chambers. Sufficient for approximately 120 slides.
5185-5979	Stabilization and Drying Solution, 500 mL Contains reagent that prevents ozone-induced degradation of cyanine dyes during processing and scanning of Agilent oligo-based microarrays. Sufficient reagent for up to 25 oligo aCGH slides. Customer must purchase the following to use this product with oligo aCGH arrays: Acetonitrile (Sigma-Aldrich 271004-1L).

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