microRNA Microarray Service

µParaflo®
Biochip Technology
High Performance Microfluidic
Custom Microarray Platform

COST EFFECTIVE 1-STOP SOLUTION
A comprehensive miRNA expression profiling service to save you time, labor and material costs. Includes data analysis and high level technical support.

100% OF V20 miRBASE PROBE CONTENT
The only profiling service offering the latest probe content and microarrays available for every organism in the miRBase sequence database.

OPTIMIZED RNA HYBRIDIZATION PROBES
Probes designed and experimentally tested to ensure uniform probe hybridization to their miRNA targets with enhanced specificity and sensitivity.

COMPLETE CONTENT FLEXIBILITY
On chip synthesis means microarrays are made to order. Validate predictions, add custom probes, or create totally custom tiling arrays for discovery of novel small RNAs.

HIGHER QUALITY DATA
A superior platform produces very-low-noise signals and reveals the true biological variations of your samples. Numerous customer publications to date.

www.lcsciences.com 1-888-528-8818
Detection of microRNAs using a microarray offers the opportunity to examine all known microRNA transcripts in a single experiment. A successful microRNA microarray detection system includes a comprehensive proven chip, small RNA preparation, labeling, and detection, and must meet several challenges that are unique to microRNAs. The service package provided by LC Sciences was developed to specifically address these challenges.

Our genome-wide microRNA expression profiling and detection “Total RNA to Data” comprehensive service includes:

**Sample QC**
Integrity of the received total RNA sample is determined via a thorough analysis process. Samples undergo QC analysis immediately after receipt as well as at each step through the process. Samples that do not pass QC are flagged and notification is sent with a recommendation not to proceed with the microarray assays.

**Sample Preparation**
Each total RNA sample is enriched for microRNAs. The enriched sample is then labeled with fluorescent dyes for one color or two color detection.

**microRNA Detection**
Microarray assay is performed on a µParaFlo® microfluidics chip. Each chip has passed rigorous quality analysis. Hybridization experiments are vigorously monitored to achieve high quality and stringency.

**Microarray Scan and Data Extraction**
Microarray images are carefully scanned for a balanced view. Numerical intensities are extracted for control, background, and microRNA probes.

**Data Analysis**
Full data analysis is included so that you can immediately use the information derived from the experiment. Background subtraction and normalization are performed. For a dual sample assay, a p-value calculation is performed and a list of differentially expressed transcripts is produced based on a predetermined p-value threshold. The results are organized and a summary is emailed. The complete data set is saved to a CD, which is shipped via overnight carrier.

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**Specifications**

<table>
<thead>
<tr>
<th>Comprehensive Service Available</th>
<th>✓</th>
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</thead>
<tbody>
<tr>
<td><strong>Microarray Platform</strong></td>
<td>µParaFlo® Microfluidic Biochip Technology</td>
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<tr>
<td>Dual Color Capable</td>
<td>✓</td>
</tr>
<tr>
<td>Sample Requirement</td>
<td>1-3 µg Total RNA*</td>
</tr>
<tr>
<td>Probe Content (miRBase Version)</td>
<td>20</td>
</tr>
<tr>
<td>Species Covered</td>
<td>All species for which data exists (in miRBase)</td>
</tr>
<tr>
<td>Probes Tₘ Equalized</td>
<td>✓</td>
</tr>
<tr>
<td>Add Custom Sequences</td>
<td>✓</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>10 attomoles</td>
</tr>
<tr>
<td>Dynamic Range</td>
<td>&gt;3.5 logs</td>
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<tr>
<td>Full Data Analysis Included</td>
<td>✓</td>
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<tr>
<td>Data Delivery Time</td>
<td>2 Weeks</td>
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*Total RNA must be extracted using a protocol that does not remove the low molecular weight RNA fractions.*
Reliability through Design and Control

Microfluidic Array Platform
These are not spotted arrays! A proprietary µParaflo® microfluidic biochip is used. The microfluidic technology produces a uniform distribution of the sample solutions on the array and enhances binding reactions and stringency wash processes. The microarray chip consists of thousands of three-dimensional chambers and is a closed system. Dye oxidation and deterioration are not an issue!

In situ Synthesis
In situ oligonucleotide synthesis using PGA (photogenerated acid) coupled with conventional DMT chemistry means high probe quality, tight process control, and complete content flexibility. Our advanced manufacturing process ensures highly uniform spots and high reproducibility across lots of arrays and yet permits total customization of contents on each individual array. In comparison, spotted microarrays tend to suffer from poor spot uniformity and large spot to spot and array to array variations, which lead to large data deviations. The spotting process requires significant up-front investment for oligo libraries and spotting equipment and permits no flexibility for content update or customization.

Probe Design
Each of our detection probes contains a coding segment and a long spacer. The coding segment is a nucleotide sequence involving proprietary chemical modification for enhancing the sensitivity and specificity for the detection of target transcripts. The spacer is a non-nucleotide molecule that extends the detection probe away from the substrate and therefore reduces surface effects and further enhances the binding between the probe and the target. Probe repeats are used on each array to allow statistical analysis of the data. A layout and an image of a mouse miRNA array are shown on the left below.

Optimized RNA Hybridization Probes
The Tms of our detection probes are balanced by incorporation of modified nucleotides with increased binding affinities. These are not standard modified nucleotides that often have an undesirable "stickiness" characteristic. We have improved detectability and specificity in our arrays compared to those made from regular DNA probes. By varying the number of modified nucleotides in each probe, we can adjust the Tm of that probe.

Quality Control
Multiple QC steps are implemented at various stages of array manufacturing and assay processes. Before being released for customer sample assays, each array must pass a stringent QC test involving hybridization with a group of control oligos. Based on the reading from 16 sets of control probes spatially distributed across the array, signal intensities, spot uniformity, cross-array spot-to-spot uniformity, and perfect-match vs. mismatch specificities are thoroughly evaluated. For the QC of the entire assay process, a fixed amount of several 20-mer RNA oligos is spiked into each customer sample as external controls. Multiple sets of control probes are designed to detect the spiked-in controls.
100% Coverage of the latest miRBase Version

Comprehensive - Any Species in miRBase

- The most comprehensive line of standard microRNA detection microarrays.
- Based on the experimentally verified microRNAs that are listed in the miRBase sequence database.
  (http://www.mirbase.org/)
- Standard arrays available for all individual species listed in the database (a partial list is shown to the left) as well as groups of related species (i.e. mammals, plants, viruses).

Current - 100% Coverage of the Latest miRBase Version

The field of microRNA is rapidly evolving and the sequence database is being updated frequently with newly discovered sequences. Many scientists predict that thousands more microRNAs have yet to be discovered. To provide the most updated contents for our customers, we use a highly flexible microfluidic chip manufacturing technology. The fast and flexible µParaflo® technology enables us to synthesize microarrays when ordered. Made to order microarrays mean yours will always contain the latest microRNA sequence information from the miRBase sequence database.

miRBase

The miRBase sequence database is a comprehensive database of miRNA sequence data, annotation, and predicted gene targets and is the primary public repository for these data. Updates to the database are released approximately every 4-6 months.
**Customizable Probe Content**

**Novel Small RNA Discovery**

Non-coding (ncRNAs) are a class of RNAs that do not encode proteins but instead possess regulatory function at the level of RNA in the cell. Custom microarrays have been shown to be an effective experimental approach to discovery of novel ncRNAs as well as a method to validate candidate ncRNAs.

LC Sciences provides a small non-coding RNA discovery service using innovative µParaflo® biochip technology and proprietary probe design, which enable highly sensitive and specific direct detection of small RNAs in your sample.

**Comprehensive Service**

From array design to sample preparation to data analysis, enabling the most efficient novel discovery of small regulatory/functional RNA embedded within ncRNAs or genomic RNAs.

**Completely Custom Probe Content**

Allows you to design an array cover your specific area of interest in the genome of your species of choice. These are not spotted arrays. Utilizing our flexible microfluidic on-chip synthesis technology, we will custom synthesize your microarray to order when you order it.

**High Performance Microarray Platform**

µParaflo® microfluidic chip technology is sensitive enough to detect low abundance sequences and specific enough to differentiate single base mismatch from perfect match sequences.

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**Potential microRNA or other small regulatory RNA genes**

- Confirm existence and generate expression data for putative novel microRNAs uncovered by high-throughput sequencing.
- Discover novel microRNAs by adding predicted mature microRNA sequences or performing tiling along a certain section of genome.
- Add controls of your choice for the detection of customer-added spiking RNA sequences and use as customer-selected internal controls.
- Add probes for the detection of siRNAs and/or other small non-coding RNAs.

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Results in 2 Weeks

Assay Results

We can generally have data back to the customer about 2 weeks from the date we receive their total RNA sample. Expedited service is available.

Full data analysis is included with our array service so that the customer can immediately use the information derived from the experiment without any further analysis.

Data Report

For each array, the customer receives the original and processed images, an array layout file, a raw intensity data file in Excel and a fully analyzed data file in Excel. Additionally, for each batch of samples, the customer receives a Data Summary containing a catalog of data files, images of representative regions of corresponding arrays, and descriptions of specific features of the arrays. The Data Summary is emailed to the customer, and all files are stored on a CD and delivered by express mail. (See http://www.lcsciences.com/applications/transcriptomics/mirna-profiling/mirna/mirna-example-data/ for a full set of sample data.)

Along with the processed data, we provide the customer with a list of up and down regulated transcripts that are called based on a statistical analysis. The result of the data analysis helps our customers to save significant down-stream cost by quickly zooming in on relevant target microRNA transcripts for further studies.

Normalization

Data normalization, using a cyclic LOWESS (Locally-weighted Regression) method is used to remove system related variations, such as sample amount variations, dye labeling bias, and signal gain differences between scanners, so that biological relevant variations can be faithfully revealed.

P-value

The data analysis also includes the statistical calculation of the p-values. Based on a predetermined p-value threshold, a list of differentially expressed transcripts is produced.

Sensitivity and Detectivity

Low System Noise

Spot density is accurately controlled during production and has been optimized for maximum signal with minimal background noise. The low system noise means reliable calls for the expression differentials.

Detection Range and Limit

Our microRNA detection dynamic range is no less than 3.5 logs and the lower detection limit is less than 10 attomoles.

Specificity

Very high detection specificity is ensured on every assay performed using our µParaFlo® microfluidic chip technology.

On each chip we have multiple perfect match and mismatch QC (quality control) probes detecting spiked-in (20 mer) RNA controls which are added into every sample and co-labeled and co-hybridized with the sample. Even with a single-base mismatch (1MM) in a detection probe most signal drops by more than 30 fold and a perfect match (PM) to 1MM ratio of more than 100 fold is achieved.
We can provide you with both microarray and qRT-PCR profiling services for the most complete picture of microRNA expression in your samples. While microarrays provide efficient genome-wide screening of miRNA expression, qRT-PCR is the gold standard for validation of microarray expression data and provides quantitative analysis of microRNA expression in real time which can help you to zero in on the miRs of interest in your samples revealed by microarrays. In addition, the specificity of real time PCR expression data can provide further understanding of the true biological significance of these miRs.

**Comprehensive Service**

Our service is comprehensive and includes small RNA enrichment from your total RNA sample, TaqMan® microRNA assay (in triplicate), and fully analyzed quantitative data.

**TaqMan® Reliability**

TaqMan® MicroRNA Assays quantitate microRNAs with the specificity and sensitivity of TaqMan® assay chemistry. The assays target only mature microRNAs, not their precursors, ensuring biologically relevant results. Every TaqMan® MicroRNA Assay has been functionally validated under laboratory conditions to ensure accurate results.

**Results**

- For individual samples, we can generate standard curve(s) and provide absolute quantitation of expression levels.
- For multiple sample experiments (e.g., treated and untreated) we can perform statistical analysis to provide relative expression levels.
- In all cases both raw and fully analyzed data will be provided to you.

**QPCR Validation**

We performed a "color reversal" experiment using mouse brain and mouse thymus RNA samples purchased from Ambion. The results are compared with QPCR data for the same two RNA samples, also purchased from Ambion, published by Applied Biosystems (ABI)*. The comparison data includes all 12 microRNA transcripts published by ABI without any selective picking of the transcripts. As shown in the bar graphs below, the relative intensities of different microRNA transcripts of our array data are in excellent agreement with ABI’s QPCR results.

* Data is obtained from [http://www.appliedbiosystems.com](http://www.appliedbiosystems.com)

![Graph showing QPCR validation results](image-url)
The Proof is in the Publications

Our customers, using the company’s microarray service for analyzing microRNA expression profiles and for discovery of novel small RNAs, have been publishing routinely since we became the first company to offer this service back in 2005. Numbering over 300 now, these studies by leading researchers in the field contribute to a fast growing body of knowledge defining this recently discovered class of regulatory RNAs.

Recent Customer Publications


LC Sciences is a biotechnology company providing products and services to the genomics and proteomics markets for nucleic acid/protein analysis, biomarker-discovery, novel drug screening, and development of diagnostics and biosensing devices. Our innovative products and comprehensive services are based on a unique, proprietary technology platform that, for the first time, integrates multiple bioanalyses on a single platform.