microRNA Microarray Service FAQs

What is microRNA?

MicroRNAs (miRNAs) are a new class of small non-coding RNAs that are recently found to be negative regulators of gene expression in eukaryotic organisms. Newly synthesized primary microRNA transcripts (pri-miRNAs) are processed by the RNase III-like enzyme, Dicer, to generate ~70 to 100 nucleotide (nt) hairpin precursors (Pre-miRNAs). Pre-miRNAs which are further processed by another RNase III-like enzyme, yield mature microRNAs, averaging 21 to 23 nt in length. MicroRNAs are incorporated into the RNA interference (RNAi) effector complex, RISC, and target specific messenger RNAs for translational repression or mRNA cleavage. MicroRNAs show distinct expression patterns in different organisms, cell development stages, and disease models. Therefore, microRNAs play an important role in regulating gene expression [1-3].

References:

1. Bartel, D. P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281–-297.

2. Ambros, V. (2004) The functions of animal microRNAs. Nature 431, 350–-355.

3. He, L. & Hannon, G. J. (2004) MicroRNAs: small RNAs with a big role in gene regulation. Nature Rev. Genet. 5, 522–-531.

Microarray Design

What is the array platform used by LC Sciences?

These are not spotted arrays! Our microRNA microarray synthesis is based on a proprietary µParaflo® microfluidic technology developed by our partner, Atactic Technologies. This flexible technology enables fast, on chip synthesis of microarrays when ordered. (vs. an off-the-shelf spotted array) Please see our µParaflo® technology bulletin for further information.

What is available on the array?

LC Sciences offers the most comprehensive line of standard microRNA detection arrays – all species for which sequence data exist in the Sanger miRBase sequence database. Although the sequence database is being continually updated as new sequences are experimentally verified, the contents of our standard arrays are updated in synchronization with the Sanger miRBase. This synchronization is made possible by our flexible µParaFlo® microfluidic chip technology.

We also offer a NO-COST option to add up to 100 probes (of 25 nt or shorter) of your own design on each array. This option gives you the opportunity for discovering new small RNAs, adding internal controls, verifying hypothesis, or achieving any other enhancement that you would like to have.

We also offer totally custom arrays at a very reasonable cost. This service gives you total freedom for the choice of probes up to 3,918 sequences. For example, you can screen for new microRNAs

by adding predicted mature microRNA sequences or performing sequence walks along certain sequences sections. You can combine microRNA sequences of different species to identify cross-species conservations, add controls for the detection of customer-added spiking RNA sequences, or add probes for the detection of siRNAs and/or other small non-coding RNAs.

Please talk to our customer support about your requirements.

What is the Sanger miRBase?

miRBase is a sequence database that has been established by the Sanger Institute. Each entry in the microRNA Registry represents a predicted hairpin portion of a microRNA transcript (termed mir in the database), with information on the location and sequence of the mature microRNA sequence (termed miR).

The database was established with two broad aims:

- to provide microRNA gene hunters with unique names for novel microRNA genes prior to publication of results
- to provide a searchable database of published microRNAs

When was the latest update of array sequences?

The content of our arrays was updated to Sanger Version 9.2 in May, 2007.

How many redundancies are there of each sequence on the microarrays?

We include a minimum of four redundancies of each microRNA probe on the single species arrays. For multi species arrays, the number of redundancies is reduced due to the increased number of probe sequences. Please contact us before ordering multi species arrays.

What is the makeup of the detection probes on the array? Do they vary in length?

Each of our detection probes contains a coding segment and a spacer. The coding segment is a nucleotide sequence involving proprietary chemical modification for enhancing the detection of target transcripts. The spacer is a non-nucleotide molecule that extends the detection probe away from substrate and therefore further enhances the binding between the probe and the target. The length of the detection probes varies according to different targets.

Are the T_ms of the probes balanced? How?

Yes, the T_ms of our detection probes are balanced. By varying the number of chemically modified nucleotides in each probe, we can adjust the T_m of that probe. Our array detection signals are more uniform due to balanced T_m.

Does the presence of modified nucleotides negatively affect the specificity of binding to the probes?

No, we have done extensive studies and verified that these chemical modifications enhance weak signals without sacrificing binding specificity. We use specificity control probes on every chip and we show a perfect match to mismatch ratio of more than 10 for a spike-in control RNA

sequence. Customers can easily examine the binding specificity of their array results by looking at control signals in the data they receive from us.

Do I need to perform replicate arrays for more confidence in my results?

For single species arrays, we synthesize all sequences in a minimum of four replicates on the chip and our intra chip variability is very low. Further, because our chips are based on the µParaflo® technology, the spot uniformity is excellent both within the chip and from chip to chip. The decision of how many replicate chips to run is up to you. Generally, customers will run a single chip and make an assessment to determine if experimental design is OK or needs to be altered before multiple chips are run to validate results. If you think there might be variability in your sample, you may want to run multiple (3) arrays.

What quality control is used for manufacture of the arrays?

Probes on our arrays are synthesized using chemical reagents of the highest quality. Every array produced has to pass a rigorous QC process which involves the hybridization with two testing DNA oligos labeled with Cy3 and Cy5, respectively. There are 16 sets of control probes on each array for the production QC. Our QC criteria include a across-array uniformity at a spot-to-spot CV less than 15%, a minimum hybridization intensity at a predetermined testing oligo concentration, and a match to (single-base substitution) mismatch ratio of larger than 10.

What experimental controls are on the array?

We spike a 20 mer control RNA into each sample followed by labeling and hybridization. The control RNA has been computationally and experimentally verified not to cross-hybridize with the probes of any known microRNA transcript. On each array there are 16 sets of control probes spatially distributed across the array. Each set contains a perfect match and a single-base-substitution mismatch for the control RNA. Chip and assay qualities, such as uniformity and specificity, can be assessed by examining the signal intensities of these control probes. Typically, the CV of the spot-to-spot intensities of the perfect match probes is less than 15% and the intensity ratio of perfect match to mismatch probes is larger than 10.

Custom Sequences

Can I add my own sequences to the array? How many? What is the cost?

Yes, you can add custom sequences to any of the standard microRNA arrays. Assuming that your longest custom sequence is less than 25 nt, there is **no charge** for adding up to **100 additional sequences** to the array.

What is the cost of longer oligos?

If any of your sequences are longer than 25 nt (it doesn't matter how many), there will be $5 charge for each additional nucleotide over 25 (count from the longest oligo).

What if I want to add more than 100 sequences?

There are 3,748 open features available for synthesis of customer sequences. Subtract the total number of standard sequences from this number to determine the number of custom sequences

available. This number will vary slightly as each species array contains a different number of microRNA probes. Please talk to our customer support about your requirements.

How much does it cost to add a large number of custom sequences?

If more than 100 custom sequences are needed, we treat the array as if it is a custom array. See our custom array pricing page for details.

How many redundancies of each custom sequence do you recommend?

We recommend triplets although this will reduce the total number of sequences you can add to the array.

Sample Preparation

How much sample do I need to send to LC Sciences?

For each assay, we ask that you try to send in at least 5 µg of total RNA. Please transfer your sample to a 1.5ml microcentrifuge tube for shipment (0.5 ml or smaller PCR tubes can crack when frozen).

We prefer that you send total RNA but if you have already done the enrichment for microRNA, we ask that you try to send 500 ng of microRNA.

If you have difficulty in obtaining the above quantities, please call and talk to our customer support. We can use an optional amplification procedure and achieve results starting from as little as 100 ng total RNA, provided the RNA is very high quality.

How do I prepare my sample?

It is very important to use a total RNA preparation procedure that does not remove the low molecular weight RNA fractions.

Make sure to use one of the commercially available RNA extraction kits that are specifically developed for micro RNA studies. Please refer to corresponding manufacturer's manuals. Use only one type of extraction kit for all the samples of your project. We recommend the use of miRNeasy Mini Kit from Qiagen. Please make sure to choose “miRNeasy” and NOT “RNeasy”.

Many laboratories have obtained excellent results from total RNA samples extracted using Trizol methods. However, skills, experiences and sometime sample types may become critical factors in obtaining consistently good sample qualities. According to our statistics, the method has an overall higher failure rate than column-based commercial methods, although the rate varies among different laboratories. If you must use a Trizol method, we recommend modifying precipitation step by doubling the usual isopropanol volume and leaving the RNA at -80°C for 10-20 minutes so as to ensure the precipitation of small nucleic acids. Some laboratories perform the precipitation step twice and/or perform a post-precipitation wash twice in order to clean up the sample. You will need to perform some tests in order to find a proper protocol for your sample.

There is no need to perform a small RNA enrichment step. We will perform small RNA enrichment in our own lab when we receive your sample. We can accept fractionated microRNA but in this case, certain controls can not be included in your experiment.

Please transfer your sample to a 1.5ml microcentrifuge tube for shipment (smaller tubes can crack when frozen). Be sure the tube labels match those listed on your sample submission form.

Yes, you can check the RNA quality with a Bioanalyzer, or a 1-1.5% agarose gel. High quality RNA will show a 28S rRNA band at 4.5kb that should be twice the intensity of the 18S rRNA band at 1.9kb. Excessive smearing indicates degraded RNA.

Additionally, you can check the UV spectrum of your sample and make sure that the 260 nm/230 nm intensity ratio is above 1.0 and that the 260 nm/280 nm ratio is above 1.8.

We have found that sample failure is often related to the method of sample preparation used. There are several different proven methods and commercial kits now available however, some researchers may be very comfortable with one procedure but not with another. If your sample fails quality control, we recommend not to try to re-extract the RNA using the same method, but instead try one of the other methods available.

If your RNA sample fails one of our quality control checks, we will not proceed with the array, but there will be a small processing charge to cover costs incurred to that point in the process.

Sample Submission

What is required for sample submission?

Please complete a sample submission form prior to sending your sample to us. Email the form to us at orders@lcsciences.com. A copy of the form should also be sent in the package along with your sample. (Note – Please place the form in a separate waterproof bag from your samples.)

We cannot begin work on your array without a **PO Number** or **Credit Card** Number.

How do I pack and ship my sample?

Please transfer your sample to a 1.5ml microcentrifuge tube for shipment (0.5 ml or smaller PCR tubes can crack when frozen).

If you want to seal the tubes or hold them in a rack, please don't use tape (it will crack when frozen), use parafilm.

Pack the sample with dry ice in a thermal insulated shipping box. Ship by overnight carrier for delivery the next day.

Note: Do not ship samples on Friday as they will sit over the week-end and deteriorate. Wait until the following Monday to ship the package for Tuesday delivery.

Where do I send my sample?

Mail your package to: Attn: microRNA Array Sample

LC Sciences

2575 West Bellfort Street Ste 270

Houston, TX 77054

Sample Labeling

2575 West Bellfort St.
Suite 270
Houston, TX 77054
USA

PHONE (888) 528-8818
FAX (713) 664-8181
E-MAIL info@lcsciences.com
WEB SITE http://www.lcsciences.com

Do you amplify the microRNA?

Currently we do not amplify the sample but instead we use a signal amplification strategy to detect small amounts of microRNA.

How is the sample labeled?

We use a proprietary labeling method which utilizes an affinity tag for signal amplification after miRNA hybridization to the chip. In the case of a dual-sample experiment, the two sets of RNA sequences are labeled with different affinity tags to allow simultaneous detection of both samples.

What does dual sample or dual color mean?

By "dual sample" we mean that you can hybridize two samples at the same time to a single array chip. Each sample would be labeled with a different fluorescent dye (Cy3 or Cy5) so that when hybridized, array spots appear red or green or yellow in a ratio image. When a transcript is abundant in the Cy3 labeled sample, its corresponding spot (probe) would appear in green color. When a transcript is abundant in the Cy5 labeled sample, its corresponding spot would appear in red color. When a transcript has similar express levels in both Cy3 and Cy5 labeled samples, its corresponding spot would appear in yellow color. This is very useful whenever comparison of two samples is needed such as wildtype vs. mutant or samples treated in two different ways.

What is color reversal?

"Color reversal" involves two chips. On the first chip you would label your sample "A" with Cy5 and your sample "B" with Cy3, respectively. On the second chip you would reverse the color by labeling your sample "A" with Cy3 and your sample "B" with Cy5, respectively. By correlating the results from two chips you would be able to eliminate or cancel most of the labeling, handling, and system related biases and therefore narrow down your calls to true biological differences. The color reversal method significantly improves the reliability of your results and will save your time, effort, and money from looking at falsely called genes or transcripts in any following up studies. Therefore, we strongly recommend the use of this method for any significant experiments.

Results

How long will it take to get results?

We can generally have data back to you about **2-3 weeks** from the date we receive your total RNA sample.

What will my data look like?

For each array, you will receive the original and processed microarray scan images, an array layout file, a raw intensity data file in Excel, a fully processed data file in Excel, and a list of up and down regulated transcripts that are called based on a statistical analysis. (dual sample arrays only)

Additionally, for each batch of samples, you will receive 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 above files will be stored on a CD and will be delivered to you by express mail. Click on Example Data Files to download and view them.

Data Analysis

Does the comprehensive service include data analysis?

Yes, standard data analysis which consists of sample to sample comparison on the same array is included in the comprehensive service. In-depth analysis of samples across multiple arrays is not included.

What is involved in the data analysis and what type of analysis result will I receive?

We have in house software for routine array data processing that follows the common practices of DNA array data treatment.[4-7] The data are processed in a MS Excel spreadsheet using a program routine that performs raw signal background subtraction using a local regression method (Xiaochuan Zhou, unpublished results; note that the photolithographically fabricated arrays do not have peripheral areas for background values);

Data normalization, using a cyclic LOWESS (Locally-weighted Regression) method[8] 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. Detected signals l greater than background plus 3 times the standard deviation will be derived for each color channel; the mean and the co-variance (CV = stdev x100/replicate mean) of each probe having a detected signal will be calculated.

For two color experiments, the ratio (log transformed) of the two sets of detected signals, and p-values of the t-test, will be calculated. Differentially detected signals are generally accepted as true when the ratio of the p value is less than 0.01.

References:

4. Ball, C. A.; Sherlock, G.; Parkinson, H.; Rocca-Sera, P.; Brooksbank, C.; Causton, H. C.; Cavalieri, D.; Gaasterland, T.; Hingamp, P.; Holstege, F.; Ringwald, M.; Spellman, P.; Stoeckert, C. J., Jr.; Stewart, J. E.; Taylor, R.; Brazma, A.; Quackenbush, J. (2002) Standards for microarray data. Science 298, 539.

5. Quackenbush, J. (2001) Computational analysis of microarray data. Nature Rev. Genet. 2, 418-27.

6. Quackenbush, J. (2002) Microarray data normalization and transformation. Nat. Genet**.** 32 Suppl., 496-501.

7. Sturn, A.; Quackenbush, J.; Trajanoski, Z., (2002) Genesis: cluster analysis of microarray data. Bioinformatics 18, 207-8.

8. Bolstad, B. M.; Irizarry, R. A.; Astrand, M.; Speed, T. P. (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19, 185-93.

What is a p-value?

p-value is a statistic parameter that measures the similarity of Cy3 and Cy5 labeled transcripts. The smaller the p-value is, the less possible the Cy3 and Cy5 labeled transcripts are similar. If a spot has a p-value less than 0.01, the Cy3 and Cy5 labeled transcripts detected by this spot are considered to be deferentially expressed in the two corresponding samples.

Is clustering analysis available for multiple chip orders?

Yes, for clustering analysis of multiple datasets, data adjustment includes data filtering, Log2 transformation, and gene centering and normalization. Data filtering will remove clustering values from the data set (detected signals or detected ratios that are below a threshold value). Data centering and normalization will transform Log2 values using the mean and the standard deviation for individual miRNA across all samples.

We will perform clustering with a hierarchical method using average linkage and Euclidean distance metric. The clustering data can be visualized using one of the several microarray programs, such as TIGR MeV (Multiple Experimental Viewer) (the Institute for Genomic Research).

Can I analyze my data myself?

Yes, the data package that we send to you contains sufficient raw data and array layout information for you to carry out you own data analysis.

Additional Information

Can I run the array myself?

Currently the array chips are not available for separate purchase without the service. Our technology utilizes a microfluidics chip that requires additional liquid handling equipment not available outside our lab. In the future we hope to be providing this equipment so that users may perform their own experiments.

We have a very well-trained and knowledgeable staff of scientists here at LC Sciences. If there is some specific experimental work you need done, we would be more than happy to put together custom service package designed specifically for your research needs.

Are there any publications?

Yes, they are listed on our publications page. (http://www.lcsciences.com/support/publications.html) There are currently several other groups working on manuscripts that will include data from LC Sciences arrays. We expect these to be published in the near future.

Can LC Sciences provide assistance with summarizing the microRNA microarray expression assay for my publications?

Sure, below is a suggestion:

Microarray assay was performed using a service provider (LC Sciences). The assay started from 2 to 5 µg total RNA sample, which was size fractionated using a YM-100 Microcon centrifugal filter (from Millipore) and the small RNAs (< 300 nt) isolated were 3'-extended with a poly(A) tail using poly(A) polymerase. An oligonucleotide tag was then ligated to the poly(A) tail for later

fluorescent dye staining; two different tags were used for the two RNA samples in dual-sample experiments. Hybridization was performed overnight on a μParaflo microfluidic chip using a micro-circulation pump (Atactic Technologies)[9]. On the microfluidic chip, each detection probe consisted of a chemically modified nucleotide coding segment complementary to target microRNA (from miRBase, http://microrna.sanger.ac.uk/sequences/) or other RNA (control or customer defined sequences) and a spacer segment of polyethylene glycol to extend the coding segment away from the substrate. The detection probes were made by *in situ* synthesis using PGR (photogenerated reagent) chemistry. The hybridization melting temperatures were balanced by chemical modifications of the detection probes. Hybridization used 100 μL 6xSSPE buffer (0.90 M NaCl, 60 mM Na_2HPO_4, 6 mM EDTA, pH 6.8) containing 25% formamide at 34 °C. After hybridization detection used fluorescence labeling using tag-specific Cy3 and Cy5 dyes. Hybridization images were collected using a laser scanner (GenePix 4000B, Molecular Device) and digitized using Array-Pro image analysis software (Media Cybernetics). Data were analyzed by first subtracting the background and then normalizing the signals using a LOWESS filter (Locally-weighted Regression)[10]. For two color experiments, the ratio of the two sets of detected signals (log2 transformed, balanced) and p-values of the t-test were calculated; differentially detected signals were those with less than 0.01 p-values.

9. (a) Gao, X., Gulari, E., and Zhou, X. (2004) In situ synthesis of oligonucleotide microarrays. Biopolymers 73, 579-596; (b) Zhu, Q., Hong, A., Sheng, N., Zhang, X., Jun, K.-Y., Srivannavit, O., Gulari, E., Gao, X., and Zhou, X. (2006) Microfluidic biochip for nucleic acid and protein analysis. in Methods Mol. Biol. Ed. Rampal, J. B. in press.

10. Bolstad, B. M., Irizarry, R. A., Astrandand, M., Speed, T. P. (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinfo. 19, 185-193.