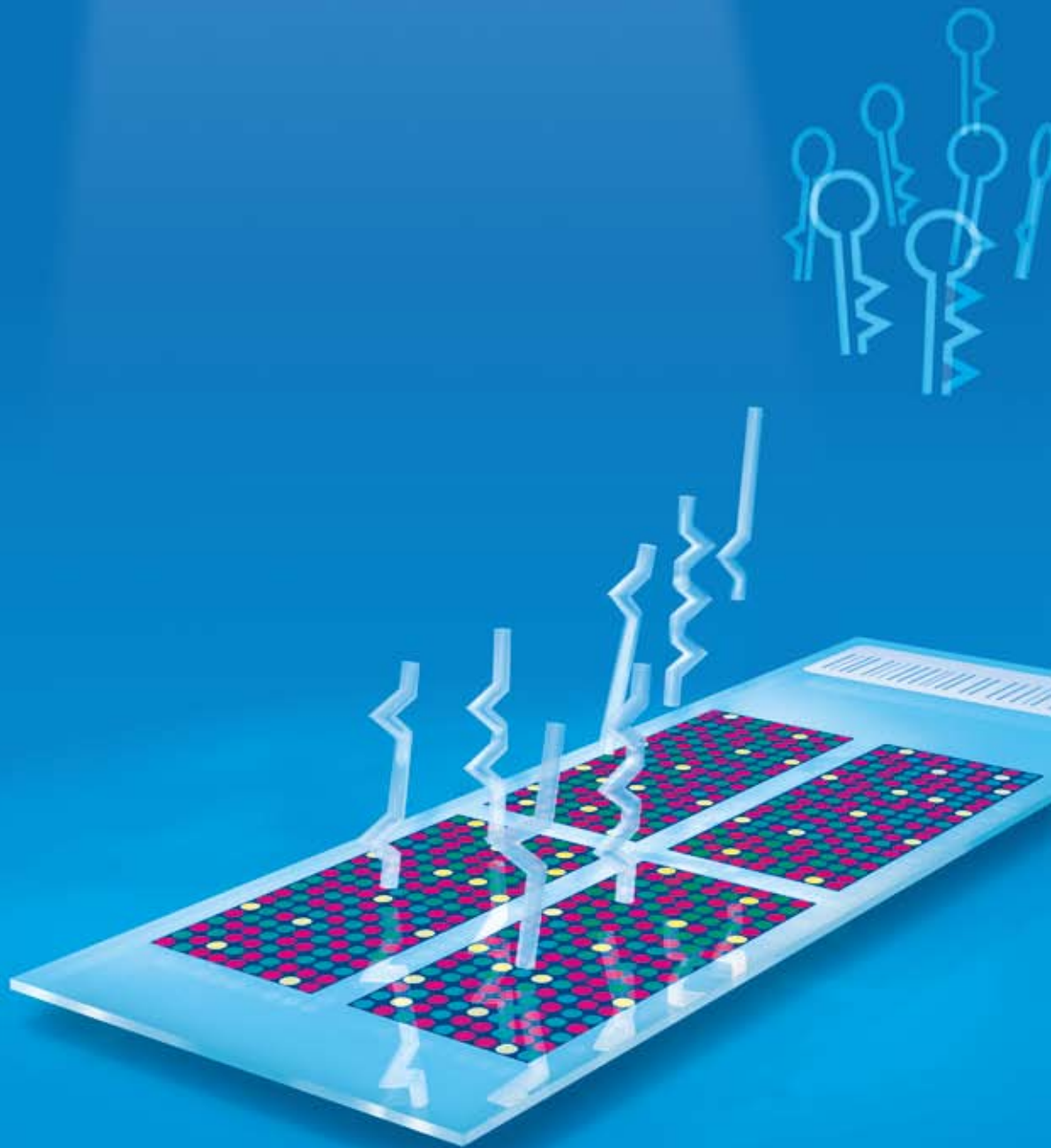




miRXplore™ Microarray

Products and services for
microRNA expression profiling



Miltenyi Biotec: ten-year microarray experience

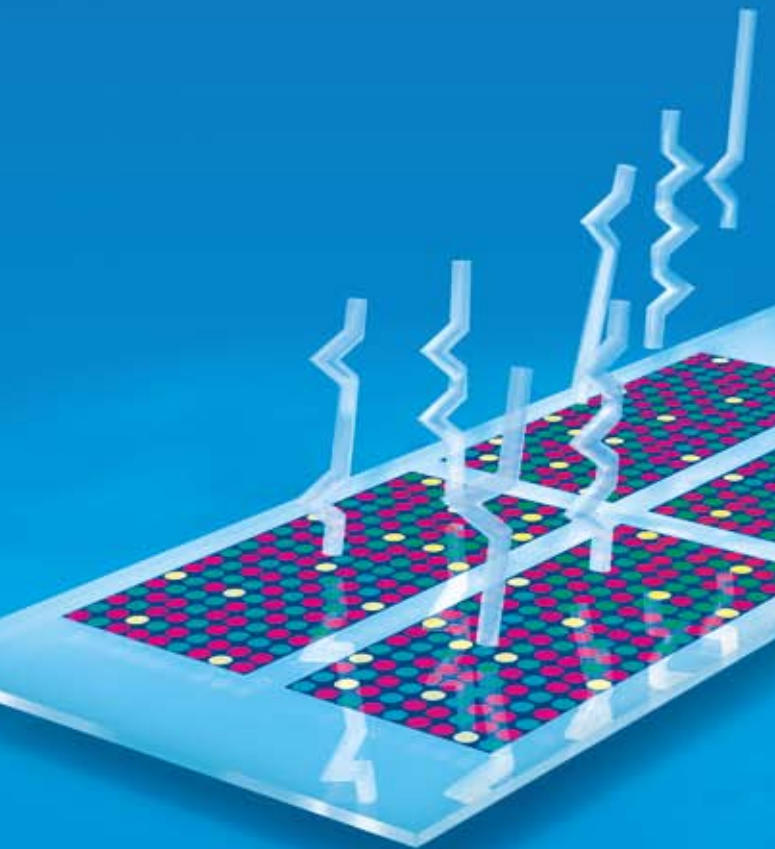
Since 1989, scientists of Miltenyi Biotec established MACS® Technology as the gold standard in cell separation and started developing microarrays ten years ago.

Multiple species—one slide

Premium content

Sample-independent normalization

Kits and comprehensive services



State-of-the-art microRNA expression profiling

miRNA analysis of multiple species on one slide

The microRNA sequences of human, mouse, rat as well as viral sequences are combined on the miRXplore™ Microarray, that was designed and validated in close cooperation with leading miRNA scientists at the Rockefeller University.¹

Premium content

Using the miRXplore Microarray, researchers are able to discriminate even between closely related miRNA sequences. The up-to-date miRNA content as published in the latest miRBase database release² is spotted on the slide, and miRNA signals can be detected down to 0.5 amol.

Sample-independent normalization

Synthetic spike-in control oligonucleotides are used for sample-independent normalization and experimental monitoring. Each microarray contains extensive positive, negative, and calibration controls.

Kits and comprehensive services

The miRXplore Microarray Kits include all necessary hybridization and wash buffers as well as controls. Also, researchers can profit from the cost-effective, reliable, and fast miRNA expression profiling services.

- The miRXplore Microarray Service directly compares both samples and controls.
- The miRXplore Universal Reference Service uses a synthetic miRNA pool to link different experiments.

¹ Landgraf, P. *et al.*, Tuschl, T. (2007) Cell 129: 1401–1414.

² <http://microrna.sanger.ac.uk>. Please inquire for updates, or visit www.mirxplore.com.

Services using miRXplore™ Microarrays

- miRXplore Microarray Service
- Service using the Universal Reference

Cost-effective, reliable, and fast

The trusted and competitive miRNA profiling services are offered by the scientists of Miltenyi Biotec's Genomics Services Department. Customers receive results within an average of two to three weeks.

Long-time expertise

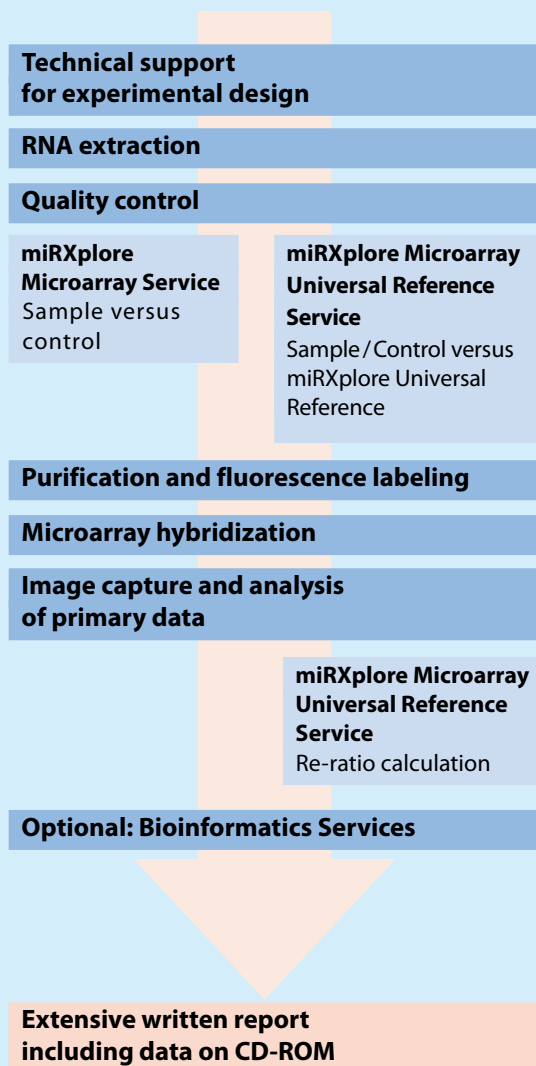
Benefit from long-time expertise for:

- fast processing of samples
- reliable profiling from 400 ng of total RNA*
- extensive quality controlling

Service with the Universal Reference

Complementary to the miRXplore Microarray Service, the service using the Universal Reference features the processing of samples versus the Universal Reference, a synthetic miRNA pool. Thus, an indirect comparison between samples is possible by calculation of re-ratios.

Send samples—receive results!



Kits with miRXplore™ Microarrays

Multiple species coverage

Sequences of diverse species are combined on this unique microarray: human, mouse, rat and viral miRNAs allow cross-species miRNA expression profiling.

Controls on the miRXplore Microarray

Validate your results with extensive controls:

- Negative mismatch controls 13
- Positive controls 36
- Hybridization controls 5
- Calibration controls 18

More than 2000 human, mouse, rat, and viral microRNA sequences

Take advantage of the comprehensive sequence coverage.²

	Human	Mouse	Rat	Viral
No. of miRNA sequences	872	634	427	130

Number of miRNA sequences according to latest miRBase version 12.0. Note that sequences are constantly updated, please inquire or check www.mirxplore.com.

* The recommended amount is 2–5 µg of total RNA.

microRNA research

Subsequent to gene transcription, small, non-coding microRNAs (miRNAs) serve as guides in translational repression or mRNA cleavage processes. miRNAs play key roles in cellular differentiation and proliferation pathways as well as apoptosis.^{3,4}

The miRXplore Microarray products and services are ideally suited to support microRNA research.¹

miRXplore™ Microarray — premium features

The high-quality miRXplore™ Microarray Kits are designed to fast-track microRNA research.

One microarray for multiple species

The miRXplore Microarray comprises currently known mature human, mouse, rat, and viral microRNA sequences.

Expert coverage of sequences

The sequences on the miRXplore Microarray fully match the latest miRBase sequence database release.² The quadruplicate spotting ensures a robust data analysis.

Guaranteed 100% spot content

An integrated camera system in the spotting device detects and qualifies each single spot of the miRXplore Microarray.

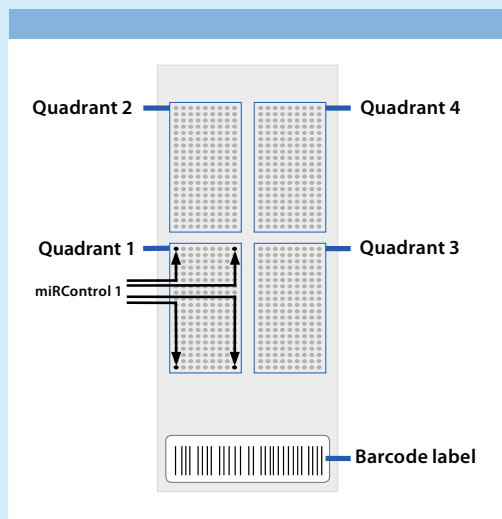


Figure 1: Position of control RNA miRControl 1 on miRXplore Microarray. The oligonucleotide probes on the miRXplore Microarrays detect the respective mature microRNAs. All sequences on the miRXplore Microarrays are spotted in quadruplicates.

Extensive control system

Total RNA isolation and microRNA labeling reactions can be monitored by using provided spike-in controls.

Hybridization monitoring is enabled with several positive and negative controls on the slide.

Sample-independent normalization is facilitated using the calibration oligos.

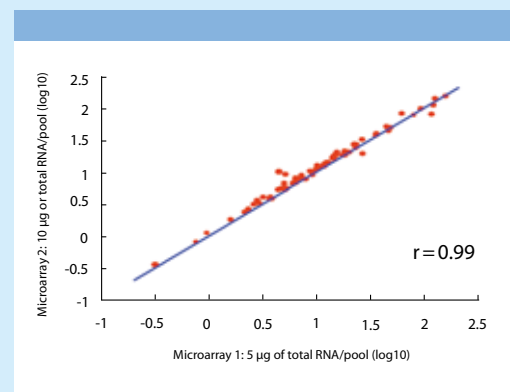
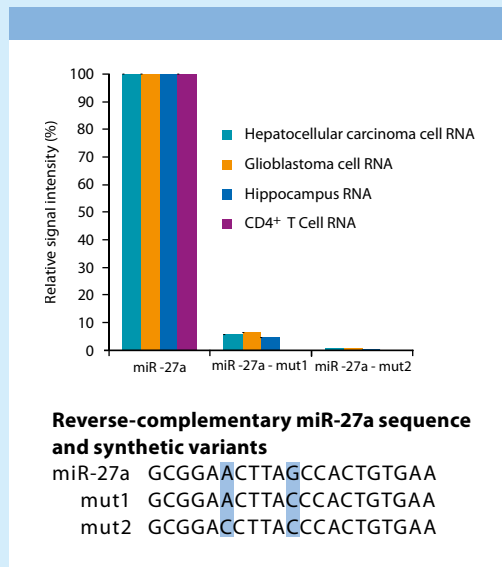


Figure 2: Highly reproducible results. Total RNA was isolated from human CD4⁺ T cells, and aliquots of 5 µg and 10 µg were labeled with Cy5 fluorescent dye. The miRXplore Universal Reference, a synthetic miRNA pool, was labeled with Cy3 fluorescent dye. Labeled Universal Reference was hybridized together with either the 5 µg or the 10 µg labeled RNA sample to miRXplore Microarray 1 and 2, respectively. The scatter plot shows the log ratios of signal intensities of the 5 µg-sample/pool (microarray 1) versus the 10 µg-sample/pool (microarray 2). The high Pearson correlation coefficient ($r = 0.99$) demonstrates near-identical signals on both microarrays.

miRXplore™ Microarray — designed for high specificity

Discrimination of closely related microRNAs

The miRXplore™ Microarray enables a highly specific distinction of miRNA sequence variants differing by even only one nucleotide. This was proven by microarray experiments with mismatched sequences of miR-27a: When compared to perfect-match oligonucleotides, cross hybridization signals of the single- and double-mismatch sequences were below 10% and 2%, respectively (fig. 3).



Reverse-complementary miR-27a sequence and synthetic variants

miR-27a GCGGA~~A~~CTTAGCCACTGTGAA
mut1 GCGGA~~A~~CTTACCCACTGTGAA
mut2 GCGGAC~~CT~~TACCCACTGTGAA

Figure 3: Probe-target specificity of miRXplore Microarray. 5 µg of human total RNA from hepatocellular carcinoma cells, glioblastoma cells, hippocampus cells, and CD4⁺ T cells were individually fluorescently labeled and hybridized to four miRXplore Microarrays. The figure illustrates the relative signal intensities of the single-mismatch (mut 1) and double-mismatch (mut 2) probes, with the perfect-match signal intensities set to 100%.

Highly reproducible signal intensities Intra-microarray variance

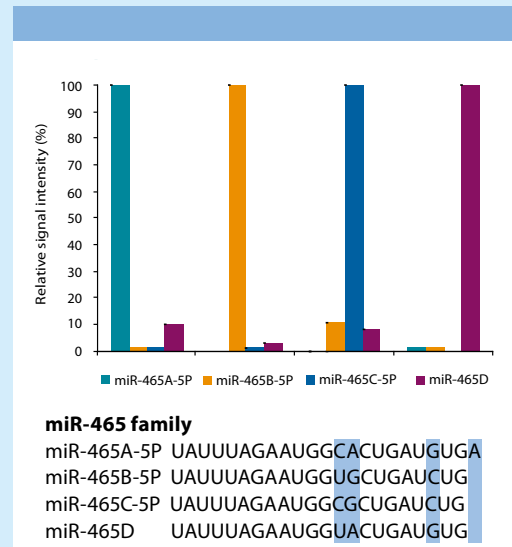
The mean coefficient of variation of the signals detected on replicate spots on an individual miRXplore Microarray is usually below 10%.

Inter-microarray variance

The correlation coefficient of repeated multiple-color hybridizations using several microarrays is on average 0.99.

Easy differentiation of microRNA family members

Closely related microRNAs such as the miR-465 family members show substantial sequence homologies. Using miRXplore Microarrays, the individual member of the miR-465 family can be easily distinguished (fig. 4).



miR-465 family

miR-465A-5P UAUUUAGAAUGGCACUGAUGUGA
miR-465B-5P UAUUUAGAAUGGUGUGAUGUG
miR-465C-5P UAUUUAGAAUGGCGCUGAUGUG
miR-465D UAUUUAGAAUGGUACUGAUGUG

Figure 4: Specific detection of miR-465 family members. Four synthetic microRNAs representing the miR-465 family, differing by one to four nucleotides, were labeled and hybridized individually on four miRXplore Microarrays (rows 1–4). The relative signals from each probe for the different miR-465 miRNAs are displayed with the corresponding perfect-match signal intensity set to 100%.

Accurate tissue-specific microRNA identification

A variety of microRNAs show tissue-dependent expression patterns, for example, miR-133a is only detected in heart tissue.⁵ In experiments with miRXplore Microarrays, the highly tissue-specific expression of miR-133a and other selectively expressed miRNA was confirmed (fig. 5).

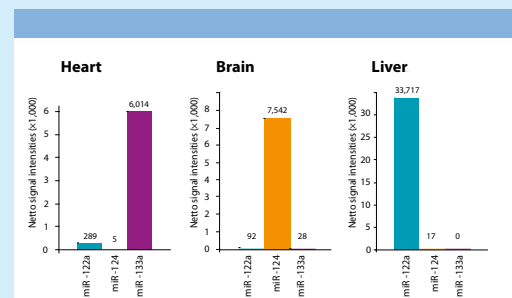


Figure 5: Tissue-specific signal detection using miRXplore Microarrays

Examples of specifically expressed miRNAs are miR-133a in heart, miR-124 in brain, and miR-122a in liver.⁵ From each tissue, 5 µg of total RNA was hybridized to individual miRXplore Microarrays.

³ Jovanovic, M. (2006) *Oncogene* 25: 6176–6187.

⁴ Wiemer, E.A. (2007) *Eur. J. Cancer* 43: 1529–1544.

⁵ Lagos-Quintana, M. *et al.* (2002) *Current Biol.*, 12: 735–739.

miRXplore™ Universal Reference

Skilled control tool for miRNA analysis

Complementing the miRXplore™ Microarray, the miRXplore Universal Reference is a unique synthetic microRNA pool that contains more than 950 miRNA sequences from human, mouse, rat, and virus to be used as a

- microarray reference
- hybridization control
- quality control for microarray hybridization
- quantification tool for real-time PCR¹
- positive control for microRNA cloning¹

Ready-to-use RNA oligonucleotide pool

The content of the Universal Reference matches the mature microRNAs annotated in miRBase 9.2 sequence database.² Delivered as a lyophilizate, the pool needs only to be reconstituted in the provided RNase-free water and is ready to use.

Re-ratio: strategy to save sample material

In two-color hybridizations, the sample material and the reference are individually labeled and hybridized to the same microarray. The control sample is processed likewise. Subsequently, re-ratios are calculated to compare sample versus control*. Thus, multiple samples can be compared without the need of pairwise hybridization, independent of sample size and any given time.

$\text{Re-ratio} = \frac{\frac{\text{Sample}}{\text{UR}}}{\frac{\text{Control}}{\text{UR}}} = \frac{\text{Sample}}{\text{Control}}$
<p>Principle of the so-called re-ratio using the miRXplore Universal Reference versus the experimental sample and control sample.</p>

* Please note further info on backpage, reference 7.

Normalization procedures using miRXplore Universal Reference

When using a reference pool for microarray analysis, an adapted normalizing procedure has to be applied since the labeled reference will generate a plethora of signals whereas the labeled sample will result in only few signals.

The miRXplore Microarray Kits provide spike-in controls. Used also with the miRXplore Universal Reference, the signals of these control oligonucleotides are ideally suited for normalization¹.

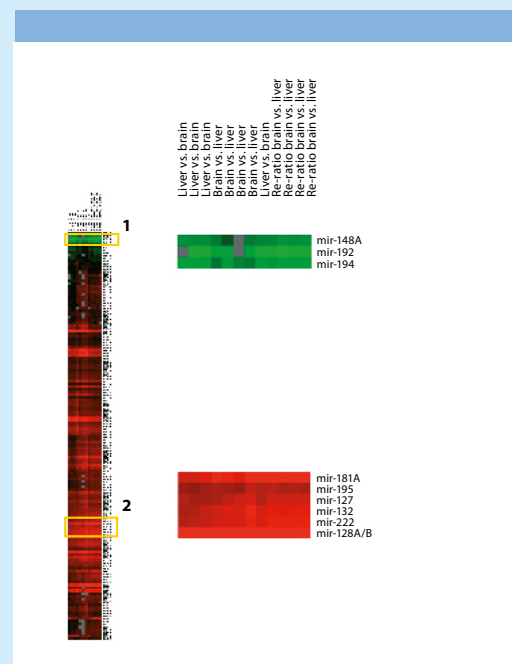


Figure 6: Cluster analysis of direct tissue-vs.-tissue signals and indirect tissue-vs.-reference signals. Each 5 µg of total RNA isolated from mouse brain and liver as well as miRXplore Universal Reference (5 fmol/miRNA) were fluorescently labeled with Cy5 or Cy3, respectively, and hybridized to miRXplore Microarrays: mouse liver total RNA vs. brain total RNA (lane 1–3, 8), and the dye-swap control brain total RNA vs. liver total RNA (4–7). Also shown are the results of the re-ratios calculated from the primary ratios of liver total RNA vs. miRXplore Reference and of brain total RNA vs. miRXplore Reference. To allow the direct comparison, reciprocal values of the ratios of dye-swap experiments have been used.

The ideal companion for miRXplore Microarrays: the a-Hyb™ Hybridization Station

The all-in-one instrument for automated slide processing

- Proprietary active circulation of sample solution and wash buffers
- Tight sandwich temperature control system
- Less user-variability

The easy way for standard slide processing

- miRXplore Microarrays
- Other microRNA microarrays
- PIQOR™ Microarrays
- Whole-genome microarrays
- array CGH microarrays
- Protein or tissue microarrays
- *In situ* hybridizations

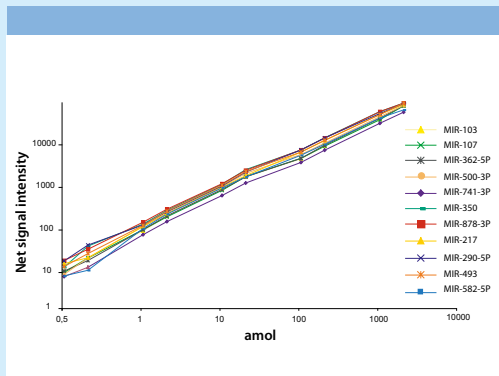


Figure 7: Detection down to 0.5 attomole using the a-Hyb Hybridization Station. Dilutions of labeled miRXplore Universal Reference were hybridized to individual miRXplore Microarrays. Calibration controls as well as microRNAs were detectable down to 0.5 attomole.



The a-Hyb™ Hybridization Station—ideally suited for automated processing of the miRXplore Microarrays.

Reliable performance is at your fingertips

Up to four slides and four independent hybridization conditions can be processed in parallel with the a-Hyb™ Station. Efficient mixing of solutions and reliable air bubble removal—due to proprietary active sample circulation—lead to greater reproducibility and sensitivity of the assays.

Data consistency is further enhanced by the unique tight temperature control of heating and cooling peltier elements embedding the hybridization chamber.

Simply touch the screen to use the intuitive control software.

Two-fold increased sensitivity

Using the a-Hyb Station, a two-fold increase in sensitivity was observed as compared to static hybridization using framed coverslips.

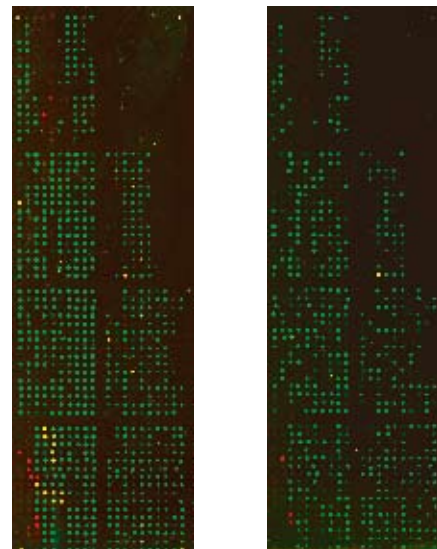


Figure 8: False-color overlay of miRXplore Microarrays hybridized with labeled miRXplore Universal Reference using the a-Hyb Station (left) and manually processed using framed cover slips and MACSmix™ Tube Rotator (right). Each scan shows one of four quadrants of the corresponding microarray.

miRXplore Microarray Kits and Services	Components	Order no.
miRXplore Microarray Kit, 4	Four miRXplore Microarrays; buffers for pre-hybridization, hybridization, and washing; PIQOR™ Navigator software, miRXplore Annotation file and configuration overview. Please note that reagents for labeling are not included.	130-093-254
miRXplore Microarray Kit, 8	Eight miRXplore Microarrays; further components see above.	130-093-272
miRXplore Universal Reference 5	Lyophilized pool of synthetic, unmodified HPLC-purified RNA oligonucleotides ⁶ . Sufficient for five microarray applications.	130-094-407
miRXplore Universal Reference 25	Lyophilized pool of synthetic, unmodified HPLC-purified miRNA oligonucleotides ⁶ . For 25 microarray applications.	130-093-521
miRXplore Hyb Frames	Five hybridization frames, wooden spatula, seal taps for manual slide hybridization.	130-094-454
miRXplore Microarray Kit, 4 plus miRXplore Universal Reference 5	Package contains one miRXplore Microarray Kit, 4 and one miRXplore Universal Reference 5	130-094-455
miRXplore Microarray Kit, 8 plus miRXplore Universal Reference 25	Package contains one miRXplore Microarray Kit, 8 and one miRXplore Universal Reference 25	130-094-456
miRXplore Microarray Service	Per sample: total RNA extraction, quality control (QC) of total RNA, labeling, QC of labeled RNA, hybridization to miRXplore Microarray, read out, and primary data analysis. A written report of all experimental steps and data analysis plus all relevant data files on CD-ROM are sent to the customer within an average of two to three weeks.	160-001-143
miRXplore Microarray Universal Reference Service (UR)	See miRXplore Microarray Service package. Here, the miRXplore Universal Reference, a synthetic miRNA pool, is used. Samples as well as controls are hybridized versus the miRXplore Universal Reference, on individual microarrays. Subsequently, re-ratios are calculated to compare samples versus controls.	160-001-161
miRXplore Additional Total RNA Extraction		160-001-162

Related products	Order no.
a-Hyb™ Hybridization Station for automated processing of standard glass slides	130-092-181
Topic-defined and custom PIQOR Microarray Kits and Services including SuperAmp™ Amplification ⁸	
Agilent Whole Genome Microarray Services for human, mouse, rat, and custom including SuperAmp Amplification ⁸	
Agilent CGH Microarray Service for human, mouse, and rat	

⁶ Each of the synthetic, unmodified HPLC-purified RNA oligonucleotides was controlled by mass spectroscopy and PAGE analysis. The reference was set up after thorough concentration measurements of each microRNA. The lyophilized pool has to be reconstituted in RNase-free water and is ready to use.

⁷ By calculating the ratio of signals from sample versus reference over the ratio of control versus reference, the resulting so-called re-ratio reflects indirectly the ratio of sample versus control. Thus, the amount of necessary sample material can be reduced if multiple samples need to be compared. For details on data analysis please contact TecSupport at macs@miltenyibiotec.de.

⁸ PIQOR Microarray Kits are not available in US and CAN.

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