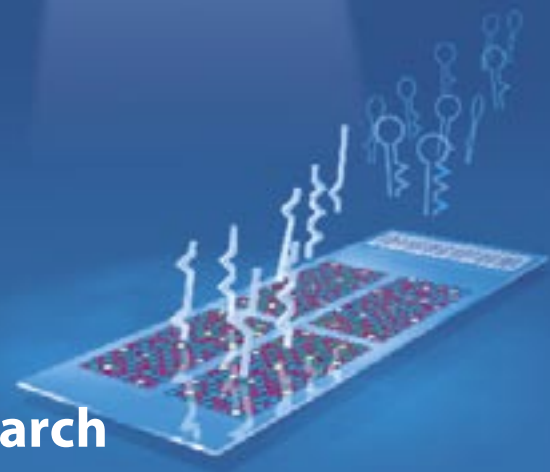




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# Universal reference for miRNA research

## Universal reference for microRNA research

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MicroRNAs (miRNAs) are presumed to be key regulators of gene expression. They are derived from endogenous transcripts that contain complementary or near-complementary 20–50 base pair-inverted repeats. Subsequent to gene transcription, the small, non-coding miRNAs serve as guides in translational repression or mRNA cleavage processes.<sup>1,2</sup>

For the global analysis of miRNA expression, microarray technology is the method of choice. To achieve reliable miRNA microarray results, certain characteristics have to be considered, i.e. the short length and differing melting temperature of the miRNAs. We have developed a universal reference miRNA pool in cooperation with leading miRNA experts<sup>3</sup>. Here we report that the miRNA pool is well-suited to be used as a general reference, hybridization control, and quality control for microarray hybridization.

### A synthetic universal miRNA reference pool

The newly designed miRNA reference pool comprises more than 950 single-stranded synthetic RNA oligonucleotides in equimolar concentrations matching mature microRNAs annotated in the miRBase 9.2 sequence database<sup>4</sup>. Therefore, it matches closely the recently developed miRXplore™ Microarray that contains quadruplicate spots of all human, mouse, rat, and viral miRNA sequences published in the latest miRBase version<sup>4</sup> (see also www.mirxplore.com).

### Applications of the universal reference

The miRXplore Universal Reference can be used as a hybridization control and as a quality control of miRNA microarrays. Furthermore, it can be applied as positive control for real-time PCR experiments. For absolute quantification of miRNAs, serial dilutions of the miRXplore Universal Reference can be used to generate standard curves for the miRNAs of interest.

### The reference strategy for two-color hybridizations

In microarray experiments, the direct comparison of absolute signal intensities of different microarrays can be

critical due to different hybridization efficiencies.

Generally, to avoid this obstacle, two-color microarray hybridizations are the method of choice.

In two-color microarray hybridizations, the sample, e.g. labeled with Cy5, and the control, e.g. labeled with Cy3, are hybridized on one microarray. As the labeled molecules compete for the same probes on the microarray, the hybridization efficiency stays the same, and allows a comparison of sample versus control. Basically, it is the ratio of the signal intensities of the two dyes that represents the ratio between sample and control. The principle of a microarray reference is based on the hybridization of each of several samples and controls versus the reference. Thereby, the reference can be used to compensate differences of the hybridization efficiency for each microarray.

### Saving sample material

The two-color microarray experiments achieve a high accuracy. Still, if all samples of a sample collection have to be compared with each other, a high number of hybridizations is necessary because for each pair of samples at least one microarray experiment has to be performed. With the miRXplore Universal Reference the comparison of many samples can now be performed without the need for pairwise hybridizations.

Each sample—or control—is hybridized together with the universal reference on an individual microarray. Then, signal ratios are calculated from the ratio of each sample(s) versus universal reference over the ratio of control versus universal reference, resulting in a so-called re-ratio, reflecting indirectly the ratio of the sample versus control (fig. 1). Thereby, the results of multiple microarray experiments can be arithmetically linked without the need for pairwise hybridizations. For larger experiment series, the miRXplore Universal Reference facilitates the comparison of multiple samples and helps to save sample material.

$$\text{Re-ratio} = \frac{\frac{\text{Sample}}{\text{UR}}}{\frac{\text{Control}}{\text{UR}}} = \frac{\text{Sample}}{\text{Control}}$$

Figure 1: Principle of the so-called re-ratio.

## Results

In the second figure, different amounts of an identical sample were hybridized on two microarrays versus the miRxplore Reference. The high Pearson correlation coefficient of the ratios of both microarrays ( $r = 0.99$ ) demonstrates the robustness and general reproducibility of independent microarray hybridizations.

A comparison of two different samples hybridized versus each other and versus the universal reference is shown in figure 3. These results show the nearly identical results generated using the miRxplore Universal Reference compared to direct hybridizations and proves the validity of our reference strategy.

## Summary

The miRxplore Universal Reference allows the indirect comparison of many samples without the need of pairwise hybridizations. The presented results demonstrate the comparability of data generated by direct hybridizations and via the universal reference strategy. Thus, the miRxplore Universal Reference can be used as a reference, hybridization control, and as quality control for microarray hybridizations.

## Literature

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- <http://microrna.sanger.ac.uk>

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miRxplore Universal Reference 5	130-094-407
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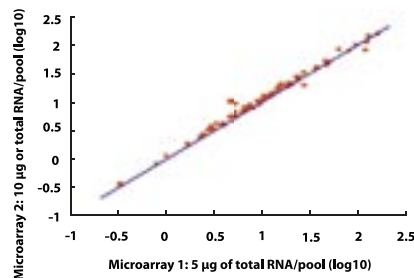


Figure 2: Total RNA was isolated from human CD4<sup>+</sup> T cells; aliquots of 5 µg and 10 µg were labeled with Cy5 fluorescent dye. The miRxplore Universal Reference, a synthetic miRNA pool, was labeled with Cy3 fluorescent dye. Labeled Universal Reference was hybridized together either with 5 µg or 10 µg labeled sample to miRxplore Microarray no. 1 and 2, respectively. The scatter plot shows log ratios of signal intensities of 10-µg/pool versus 5-µg/pool.

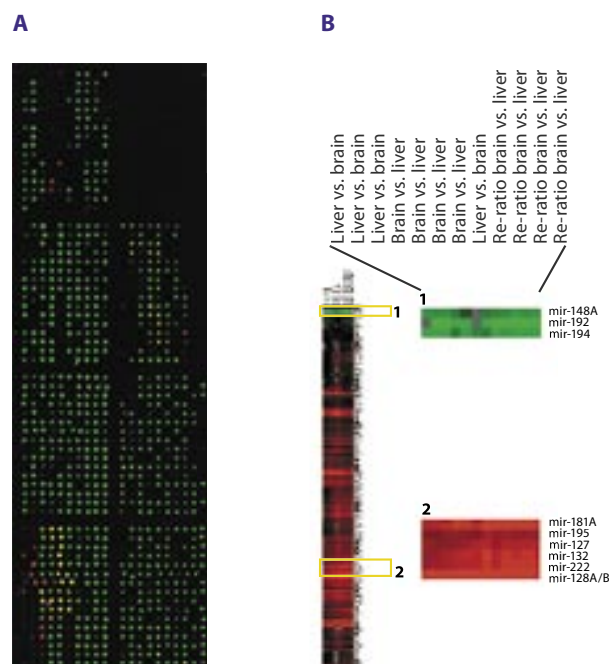


Figure 3: Each 5 µg of total RNA isolated from mouse brain and liver as well as miRxplore Universal Reference (5 fmol/miRNA) were fluorescently labeled with Cy5 or Cy3, respectively, and hybridized to miRxplore Microarrays.

**A** Cy5/Cy3 false-color image of miRxplore Microarray showing indirect tissue-versus-reference hybridization of brain vs. miRxplore Universal Reference.

**B** Cluster analysis of direct tissue-vs.-tissue signals and indirect tissue-vs.-reference signals. miRxplore Microarrays hybridized with total RNA derived of mouse liver vs. brain (lane 1–3, 8), and the dye-swap control brain vs. liver (4–7). Also shown are the re-ratios calculated from the primary ratios of liver vs. miRxplore Universal Reference and of brain RNA vs. miRxplore Universal Reference. To allow the direct comparison, reciprocal values of the ratios of dye-swap experiments have been used.