Supplementary Material and Methods.

Setting up microRNA profiling.

For microRNA profiling we used the Exiqon (Vedbaek, Denmark) capture probe set. A combination of normal and Locked Nucleic Acid (LNA) oligonucleotides have been used for the construction of this capture probe set. LNA, a bicyclic DNA/RNA analogue in which the ribose ring is 'locked' by a methylene bridge connecting the 2' oxygen atom with the 4' carbon atom, displays high affinity duplex formation (ΔT_m of +2 – 8 ºC per base) and improved base pairing specificity obeying the Watson and Crick base-pairing rules. LNA-RNA hybrids are extremely stable, whereas a single mismatch significantly destabilizes the duplex. These properties make LNA modified oligonucleotides very suitable to be used for the detection of miRNAs. Discriminative properties are difficult to reach, because miRNAs are only ~22 nucleotides with a variable G/C content and a high degree of similarity in some miRNA families (e.g. let-7 family). Therefore, we used LNA oligonucleotide capture probes in which all capture probes are T_m (72ºC) normalized. Spotting and hybridization conditions were extensively tested on various types of slides, hybridization conditions, RNA labeling techniques etc. using a selected custom-made set of NH_2-C6 modified LNA capture probes with or without a hexaethylene glycol linker and including perfect match and single mismatch oligonucleotides (data not shown).

The LNA capture probe set was spotted in duplicate on Schott Nexterion Hi-Sense E glass slides in Nexterion Spot buffer using a Virtek Chipwriter Pro in a class 10000 cleanroom. Before hybridization the miRNA arrays were blocked according to manufacturer's (Schott Nexterion) protocols. Total RNA was isolated with Trizol (Invitrogen). Total RNA was labeled only with Cy3 containing dyes using the ULS aRNA labeling kit (Kreatech) according to the manufacturer's protocol in a total volume of 10 μl. Then 100 μl of salt-based hybridization buffer (Ocimum Biosolutions) was added and the sample was incubated for 5 minutes

at 95°C, spinned for 2 minutes at maximal speed and injected in the Hybridization station.

Hybridization was done in a Tecan HS4800 Pro Hybridization station according to the following protocol:

1) Washing of slides with a buffer that contains 0.05% SDS and 2X SSC (final concentrations) at 60°C for 30 s.

2) Sample injection (105 μl) at 60°C.

3) Hybridization at 60°C for 16 hours using agitation (cycles 1.1 minute of agitation followed by 3.5 minutes without agitation).

4) Washing of slides with a buffer that contains 0.05% SDS and 2X SSC (final concentrations) in which slides were 1 minute washed followed by 1 minute soaking (repeated once) at 60°C.

5) Washing of slides with 1X SSC (final concentration) in which slides were 1 minute washed followed by 1 minute soaking (repeated once) at 23°C.

6) Washing of slides with 0.2X SSC (final concentration) in which slides were 1 minute washed followed by 1 minute soaking (repeated once) at 23°C.

7) Washing of slides with 0.2X SSC (final concentration) for 30 s at 23°C.

8) Slide drying using pressurized N2 for 5 minutes at 23°C.

Hybridized slides were scanned in a Tecan LS Reloaded scanner. Data was extracted using Imagene 6.0 standard edition software. After background subtraction, raw data was normalized using a Quantile normalization using a script in R (available upon request). Normalized data was used for statistical analysis. During data extraction in Imagene, for each spot the quality was determined. Only correct spots were used for statistical analysis. Subsequent analysis was performed with TM4 microarray software suite (Saeed et al., 2003).

After setting the specific conditions as described above, the specificity of these arrays was extensively tested. MiRNAs that were reported to be tissue-specific were monitored in a subset of mouse tissues (liver, lung and brain). Indeed

reported specificity was confirmed in our arrays (data not shown). In order to determine discriminative power, the capture probe set contains various capture probes that contain 1 or 2 mismatches to their cognate miRNA. Using described hybridization and washing conditions there is almost no cross-hybridization as observed with these capture probes (data not shown). We also performed various experiments in which a total RNA sample was split in two, labeled independently and hybridized on different arrays (from different batches) on different days. This demonstrated that the arrays could be reproduced very well as seen in a XY-scatterplot (y=0.99x) when using raw data (data not shown). Finally, we verified array data using quantitative PCR (Applied Biosystems). Using these data we set cut-off values for arrays at >1.5 or <-1.5 fold change (data not shown).