MicroRNA Array Services Final Report

Project:	Prognostic impact of microRNAs in sarcomas
Customer:	Magnus Persson
Company/Institute:	University Hospital Northern Norway
Date (dd-Mmm-yyyy):	28-Feb-2011

Table of contents

Additional files provided:

- *Project_Summary_Report.xls;*
  - Expression_matrix: contains normalized Hy3™/Hy5™ ratios (log2 transformed) from all hybridizations.
  - Slide X: contains all data for each slide including normalized data, log ratios and SD-values of the 4 replicate microRNA measures per array.

- *RNA_QC.pdf* – Output file from Bioanalyzer 2100 for sample RNA Quality control.
- *Raw data folder* – containing raw data txt-files, GAL file, barcode list and legends for raw data files
- *Graphics folder* – presentation of data 'before' and 'after' normalization in MA plots for each slide

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Data quality assessment	**MTE**
Final report	**MTE**
Review	**SJE**

Summary

Dear Magnus Persson,

We have now finalized the analysis of the samples you submitted. After having passed sample quality control on the Bioanalyser2100 and RNA measurement on the Nanodrop instrument, the samples were labeled using the miRCURY LNA™ microRNA Hi-Power Labeling Kit, Hy3™/Hy5™ and hybridized on the miRCURY LNA™ microRNA Array (6th gen - hsa, mmu & rno). The samples were hybridized on a hybridization station following the scheme you outlined in the sample submission.
Analysis of the scanned slides showed that the labeling was successful as all capture probes for the control spike-in oligo nucleotides produced signals in the expected range. The quantified signals (background corrected) were normalized using the global Lowess (LOcally WEighted Scatterplot Smoothing) regression algorithm, which we have found produces the best within-slide normalization to minimize the intensity-dependent differences between the dyes. The positive effect of this normalization is illustrated in the MA-plots for each slide. An example is found in section 3 in this document (all MA plots are found in the folder named 'Graphics').

Samples # 6 and 7 were rerun due to poor performance of the first run. The re-runs show somewhat lower signal for sample 6, whereas sample 7 performs as the remaining samples.

In conclusion, the microRNA expression profiling has successfully been completed on your samples. The microRNA profiling identified a subset of the total number of microRNAs analyzed by the miRCURY LNA™ microRNA Array that are differentially expressed in the different samples.

Exiqon's miRCURY LNA™ product line offers many tools for validating potentially regulated microRNAs by qPCR, *in situ* hybridization, Northern blot or microRNA inhibition. Exiqon Services offers microRNA qPCR services where we can assist you in validating your microRNA array data. For more information please follow the link: www.exiqon.com/mirna-products.

If you have questions to this report, please do not hesitate to contact us at DxServices@exiqon.com.

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Work flow

RNA QC
Prior to initiating the analysis your samples have been subject to RNA quality control in order to assess the integrity of the RNA, its content of small RNA and its concentration.

Labeling and Hybridization
Our the miRCURY™ LNA Array Hi-Power labeling kit allows highly efficient and uniform labeling of microRNAs with minimal sequence bias. Using Tecan hybridization stations the hybridization and washing steps are fully automated for excellent reproducibility.

Data collection and analysis
Arrays are scanned with highly sensitive equipment in ozone free environment to reduce day-to-day variation to a minimum. Image analysis is then performed to quantify the signals on the array.

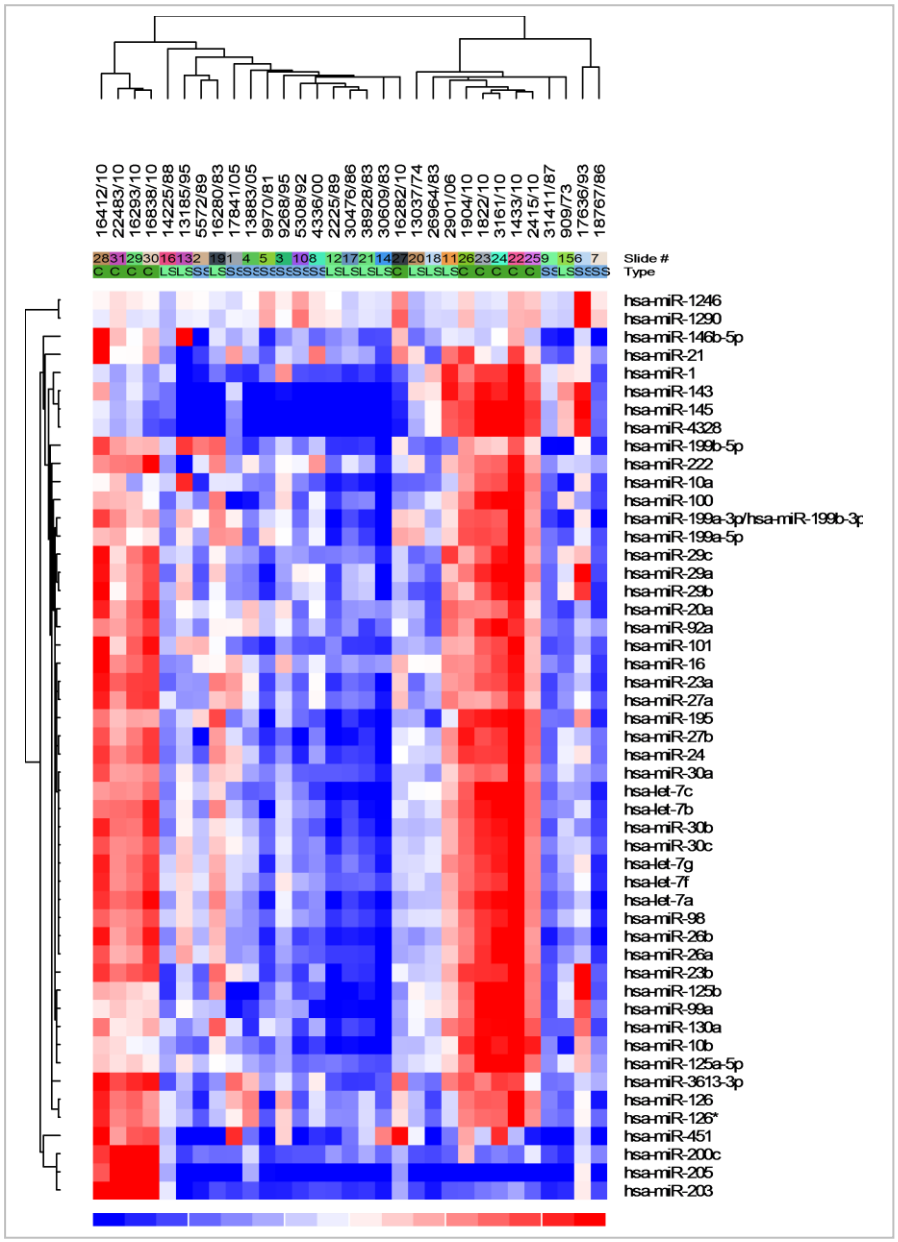
Technical quality assessment
Before proceeding with data normalization a technical quality assessment is performed based on results from spike in controls, flagging of spots, background intensity levels and signal intensity distribution.

Data collection and analysis
The data obtained is normalized using methods applicable to the performed experiment. Depending on the experimental setup and the size of the project the relevant data analysis will be applied.

Results

Heat map and unsupervised hierarchical clustering

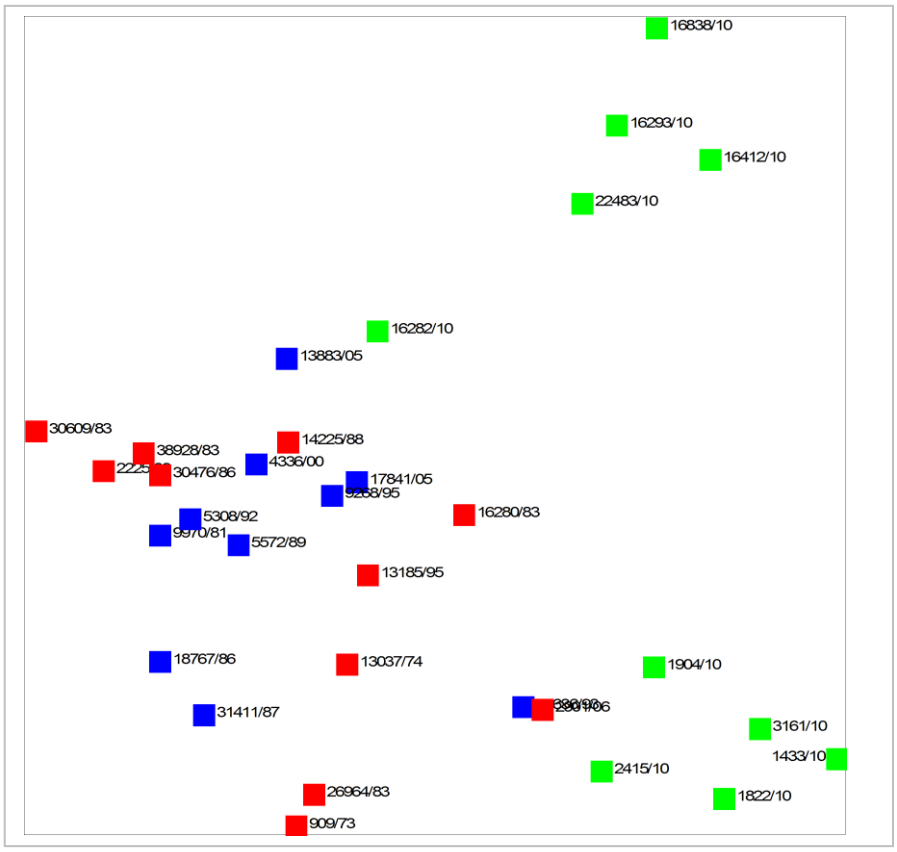
The heat map diagram shows the result of the two-way hierarchical clustering of microRNAs and samples. Each row represents one microRNA and each column represents one sample. The microRNA clustering tree is shown on the left. The color scale shown at the bottom illustrates the relative expression level of a microRNA across all samples: red color represents an expression level above mean, blue color represents expression lower than the mean.



Heat Map and Unsupervised Hierarchical Clustering. The clustering is performed on all samples, and on the top 50 microRNAs with highest standard deviation

PCA plot

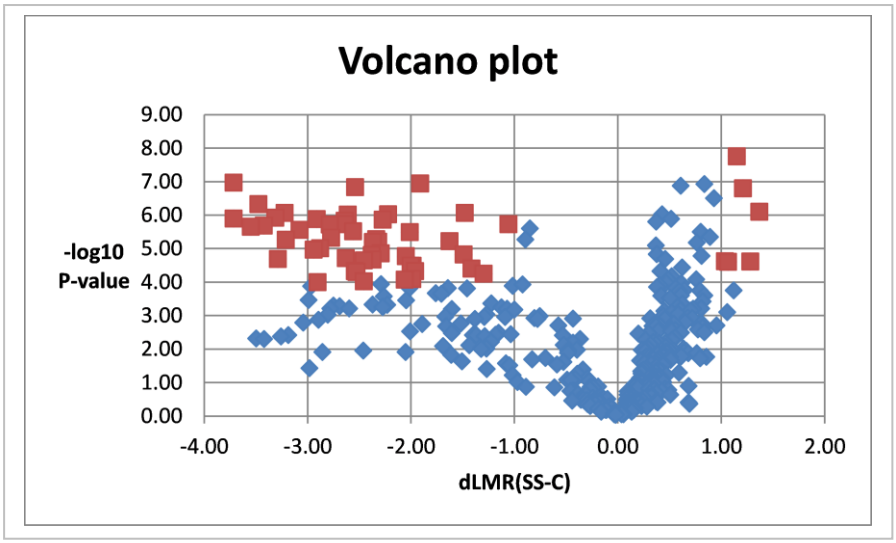
Principal Component Analysis (PCA) is a method used to reduce the dimension of large data sets and thereby a useful way to explore the naturally arising sample classes based on the expression profile. By including the top 50 microRNA that have the largest variation across all samples, an overview of how the samples cluster based on this variance is obtained. If the biological differences between the samples are pronounced, this will be a primary component of the variation. This leads to separation of samples in different regions of a PCA plot corresponding to their biology. If other factors, e.g., RNA quality, inflict more variation on the samples, the samples will not cluster according to the biology.



PCA plot. The principal component analysis is performed on all samples, and on the top 50 miRNAs with highest standard deviation. The normalized log ratio values have been used for the analysis.

Differentially expressed microRNAs – short survival versus control

A two-tailed t-test assuming unequal variance between the two groups short survival and controls, identified a number of microRNAs that have a low p-value and a deltaLogmedianRatio change of more than +/- 1.0 (= fold change +/- 2).
To quickly allow identification of significantly differentially expressed microRNA, the expression data have been plotted in a Volcano plot (see below). A volcano-plot shows fold-change against significance on x- and y-axis respectively and thus combines both the magnitude of change and significance to allow for quick identification of microRNAs that have both high differential expression (towards the right and left of the diagram) and with high significance (towards the upper part of the diagram).



Red squares indicate microRNAs that have p-values below the Bonferroni correction cutoff, and abs (dLMR) > 1.0.
Blue squares represent all microRNAs identified in all samples.

Top genes table – short survival versus control

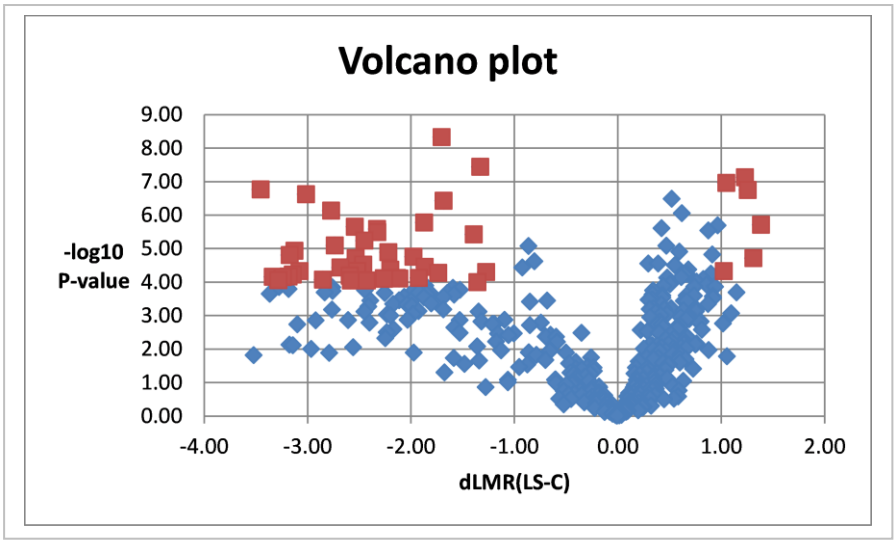
The table below lists the identified top genes.

		Average LMR		SS-C	
Annotation	p-value	SS	C	dLMR	SD dLMR
hsa-let-7b	4.75E-07	-1.438	2.037	-3.475	1.439
hsa-miR-185*	1.79E-08	0.232	-0.919	1.151	0.361
hsa-miR-191	1.37E-06	-0.696	1.578	-2.274	1.017
hsa-miR-24	8.62E-07	-1.238	1.982	-3.221	1.371
hsa-miR-26b	1.28E-06	-1.859	1.855	-3.714	1.555
hsa-miR-27b	1.07E-07	-1.801	1.914	-3.715	1.366
hsa-miR-30b	1.2E-06	-1.316	1.996	-3.312	1.362
hsa-miR-342-3p	1.48E-07	-0.880	1.659	-2.539	0.963
hsa-miR-3607-3p	9.71E-07	-0.879	1.732	-2.611	0.955
hsa-miR-3607-5p	9.6E-07	-0.650	1.572	-2.222	0.864
hsa-miR-3653	1.15E-07	-0.301	1.610	-1.911	0.714
hsa-miR-3660	1.58E-07	-0.067	-1.278	1.211	0.456
hsa-miR-552	8.01E-07	0.124	-1.246	1.369	0.584
hsa-miR-98	1.34E-06	-1.177	1.733	-2.910	1.260
hsa-miRPlus-A1015	8.68E-07	-0.269	1.211	-1.480	0.633

A dLMR below zero indicate expression levels lower in short survival than in control, and levels above zero indicate expression levels higher in short survival than control.

Differentially expressed microRNAs – long survival versus control

A two-tailed t-test assuming unequal variance between the two groups long survival and controls, identified a number of microRNAs that have a low p-value and a deltaLogmedianRatio change of more than +/- 1.0 (= fold change +/- 2).
To quickly allow identification of significantly differentially expressed microRNA, the expression data have been plotted in a Volcano plot (see below). A volcano-plot shows fold-change against significance on x- and y-axis respectively and thus combines both the magnitude of change and significance to allow for quick identification of microRNAs that have both high differential expression (towards the right and left of the diagram) and with high significance (towards the upper part of the diagram).



Red squares indicate microRNAs that have p-values below the Bonferroni correction cutoff, and abs (dLMR) > 1.0.
Blue squares represent all microRNAs identified in all samples.

Top genes table – long survival versus control

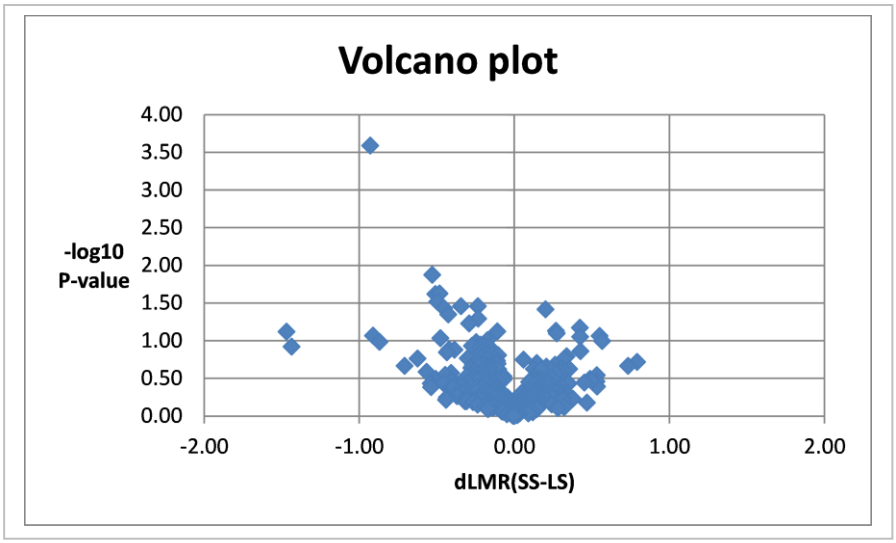
The table below lists the identified top genes.

		Average LMR		LS-C	
Annotation	p-value	LS	C	LS-C	LS-C
hsa-miR-126	2.42E-07	-1.141	1.873	-3.014	1.161
hsa-miR-126*	7.46E-07	-1.023	1.748	-2.770	1.065
hsa-miR-16	2.25E-06	-0.723	1.819	-2.541	1.235
hsa-miR-185*	1.8E-07	0.340	-0.919	1.259	0.462
hsa-miR-2113	1.1E-07	0.056	-0.995	1.051	0.414
hsa-miR-342-3p	3.22E-06	-0.665	1.659	-2.324	1.158
hsa-miR-3613-3p	1.72E-07	-1.444	2.009	-3.453	1.395
hsa-miR-365	2.63E-06	-0.528	1.798	-2.327	1.132
hsa-miR-3651	4.74E-09	-0.692	1.012	-1.704	0.549
hsa-miR-3653	1.67E-06	-0.263	1.610	-1.873	0.883
hsa-miR-3654	3.62E-08	-0.297	1.033	-1.330	0.480
hsa-miR-3660	7.47E-08	-0.047	-1.278	1.231	0.470
hsa-miR-4317	3.78E-06	-0.328	1.063	-1.391	0.692
hsa-miR-552	1.96E-06	0.141	-1.246	1.386	0.668
hsa-miRPlus-A1015	3.72E-07	-0.472	1.211	-1.683	0.721

A dLMR below zero indicate expression levels lower in long survival than in control, and levels above zero indicate expression levels higher in long survival than control.

Differentially expressed microRNAs – short survival versus long survival

A two-tailed t-test assuming unequal variance between the two groups short survival and long survival, identified a number of microRNAs that have a low p-value and a deltaLogmedianRatio change of more than +/- 0.5 (= fold change +/- 1.4).
To quickly allow identification of significantly differentially expressed microRNA, the expression data have been plotted in a Volcano plot (see below). A volcano-plot shows fold-change against significance on x- and y-axis respectively and thus combines both the magnitude of change and significance to allow for quick identification of microRNAs that have both high differential expression (towards the right and left of the diagram) and with high significance (towards the upper part of the diagram).



Blue squares represent all microRNAs identified in all samples. None of the microRNAs pass the cutoff for differential expression (p-value < Bonferroni cutoff, and +/- dLMR> 0.5)

Re-run samples 6 and 7

Due to poor performance of the initial runs for sample 6 and 7, these were re-run. The re-runs showed perfect performance of the included spike-ins. The signal intensity of sample #6 is somewhat reduced for Hy3 (see MA plot), but this does not appear to affect the results obtained for this sample. The re-run of sample #7 passes all quality controls.

Sample # 16 passes all quality controls concerning the spike-ins. The signal intensities obtained from the sample in the Hy3 channel for this sample is reduced – like for sample #6. This results in deviating MA plots and could result in skewed data upon normalization

Array quality control and assessment

Array slide quality control using spike-ins (labeling controls)

Spike-in controls were added in various concentrations in both Hy3 and Hy5 labeling reactions giving the opportunity to evaluate the labeling reaction, hybridization and the performance of the array experiment in general. The high correlations shown in the tables below for both the Hy3 and Hy5 channels indicate that both labeling and hybridization were successful. Spike-ins were added in concentrations covering a full signal range (extended_spike_control_1 to 52). Each of the 52 spike-in control has 4 replicates of capture probes on the array. We have evaluated 10 out of the 52 in the tables below.

Correlation between Spike-in signals (raw signals) on different slides

Slide #

Hy3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	-																										
2	1.00	-																									
3	1.00	1.00	-																								
4	1.00	1.00	1.00	-																							
5	0.99	0.99	0.99	1.00	-																						
6	0.98	0.98	0.98	0.98	0.99	-																					
7	0.99	0.99	0.99	0.99	0.99	0.99	-																				
8	1.00	1.00	1.00	1.00	1.00	0.98	0.99	-																			
9	1.00	1.00	1.00	1.00	1.00	0.98	0.99	1.00	-																		
10	0.99	0.99	0.99	0.99	0.98	0.96	0.98	0.98	0.99	-																	
11	1.00	1.00	1.00	1.00	0.99	0.98	0.99	1.00	1.00	1.00	-																
12	0.99	1.00	1.00	1.00	0.99	0.97	0.98	0.99	1.00	1.00	1.00	-															
13	0.99	1.00	1.00	1.00	1.00	0.97	0.98	1.00	1.00	0.99	1.00	1.00	-														
14	0.99	1.00	1.00	1.00	0.99	0.97	0.98	1.00	1.00	0.99	1.00	1.00	1.00	-													
15	1.00	1.00	0.99	1.00	1.00	0.98	0.98	1.00	1.00	0.98	0.99	0.99	1.00	1.00	-												
16	0.99	1.00	1.00	0.99	0.99	0.97	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	-											
17	1.00	1.00	1.00	1.00	0.99	0.97	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	-										
18	0.99	1.00	1.00	1.00	0.99	0.97	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	-									
19	0.99	1.00	1.00	0.99	0.98	0.96	0.98	0.99	1.00	1.00	1.00	1.00	0.99	0.99	0.98	1.00	1.00	1.00	-								
20	1.00	1.00	1.00	1.00	0.99	0.97	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	-							
21	0.99	0.99	0.99	0.99	0.98	0.96	0.98	0.99	0.99	1.00	1.00	1.00	0.99	1.00	0.98	1.00	1.00	1.00	1.00	1.00	-						
22	1.00	1.00	1.00	1.00	0.99	0.97	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	-					
23	1.00	1.00	1.00	1.00	1.00	0.98	0.99	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0.99	1.00	-				
24	0.99	1.00	1.00	1.00	0.99	0.97	0.98	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	-			
25	1.00	1.00	1.00	1.00	0.99	0.98	0.99	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-		
26	1.00	1.00	1.00	1.00	0.99	0.97	0.98	0.99	1.00	0.99	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	
27	1.00	1.00	1.00	1.00	1.00	0.98	0.99	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0.99	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	-
28	0.98	0.98	0.99	0.98	0.96	0.94	0.97	0.97	0.98	1.00	0.99	0.99	0.98	0.98	0.97	0.99	0.99	0.99	1.00	0.99	1.00	0.99	0.98	0.98	0.99	0.99	0.98
29	0.99	0.99	1.00	0.99	0.98	0.96	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	0.99
30	1.00	1.00	1.00	1.00	0.99	0.97	0.98	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00
31	0.99	1.00	1.00	1.00	0.99	0.96	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99

Slide #

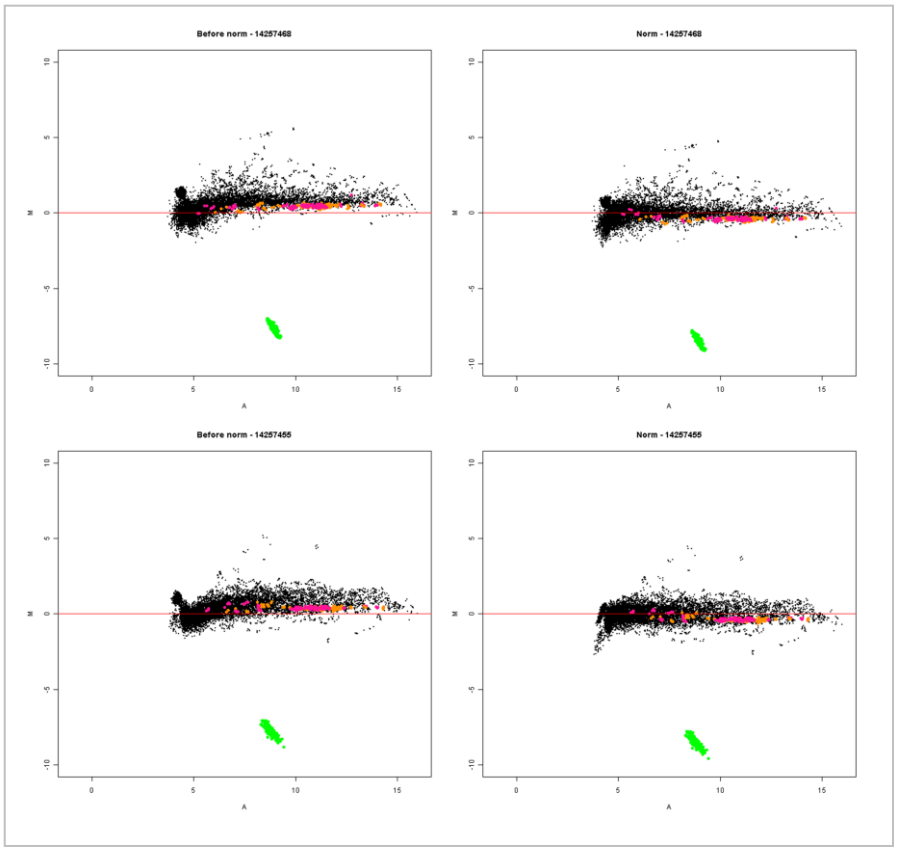
Hy5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	-																										
2	1.00	-																									
3	1.00	1.00	-																								
4	1.00	1.00	1.00	-																							
5	0.99	1.00	1.00	1.00	-																						
6	1.00	1.00	1.00	1.00	1.00	-																					
7	0.99	1.00	1.00	1.00	0.99	1.00	-																				
8	1.00	1.00	1.00	1.00	1.00	1.00	0.99	-																			
9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-																		
10	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	-																	
11	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	-																
12	0.99	1.00	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00	1.00	-															
13	0.99	0.99	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.99	0.99	0.99	-														
14	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	0.99	1.00	-													
15	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	-												
16	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-											
17	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	-										
18	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	-									
19	0.99	0.99	0.99	0.99	0.98	0.99	1.00	0.99	0.99	1.00	1.00	1.00	0.98	0.99	0.99	0.99	1.00	1.00	-								
20	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	-							
21	0.99	0.99	0.99	0.99	0.98	0.99	1.00	0.98	0.99	0.99	0.99	1.00	0.98	0.98	0.99	0.99	0.99	0.99	1.00	0.99	-						
22	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00	1.00	1.00	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-					
23	0.99	0.99	1.00	0.99	1.00	0.99	0.99	1.00	0.99	0.99	0.99	0.98	1.00	1.00	1.00	1.00	0.99	0.99	0.98	0.99	0.97	0.99	-				
24	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0.99	1.00	1.00	-			
25	1.00	1.00	1.00	1.00	1.00	0.99	0.99	1.00	0.99	0.99	0.99	1.00	1.00	1.00	1.00	1.00	0.99	0.99	0.98	0.99	0.98	0.99	1.00	1.00	-		
26	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0.99	1.00	0.99	1.00	1.00	-	
27	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	-
28	0.99	0.99	0.99	0.99	0.98	0.99	1.00	0.99	0.99	1.00	1.00	1.00	0.98	0.99	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.98	0.99	0.98	0.99	0.99
29	0.99	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0.99	1.00	0.99	1.00	1.00
30	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0.99	1.00	0.99	1.00	0.99	1.00	1.00
31	0.99	0.99	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	0.99	1.00	0.99	1.00	0.99	0.99	0.99	1.00	0.99	1.00	1.00

The tables show the correlation of all spike-ins between different slides. The correlation coefficients are calculated between e.g. slide 1 and each of the other slides in the study. The calculation is carried out for both Hy3 and Hy5 channels.

Effect of normalization

Data normalization is performed using Lowess (Locally Weighted Scatterplot Smoothing) normalization.
Lowess uses a locally established regression to smooth the M/A (log ratio/log mean-intensity) scatterplot toward a linear distribution. The Lowess algorithm works under the assumption that the majority of the signals between samples do not differ and it enforces equal overall means on all signal intensities. Therefore, Lowess allows the correction of systematic deviations in the MA plot giving an intensity-dependent adjustment of MA-data to a straight line. We have found this normalization to produce the best within-slide normalization and thereby minimize the intensity-dependent differences between the dyes.
The positive effect of this normalization is illustrated in the MA-plots for each with a MA plot before and after normalization. After normalization the spots appear symmetrically scattered around the horizontal line M=0. The difference between the two channels (M) is now independent of the average intensity level of the two channels (A). The MA plots are placed in the folder named 'Graphics'.

MA plots



The normalization is performed to minimize differences between the colors in an intensity-dependent manner. The shown plots are M versus A plots ("MA plot"); M=log2ratio(Hy5/Hy3) and A=log2(Hy5*Hy3)/2 (combined signal from both channels). The green spots are Hy3 controls spotted directly on the array surface the orange/pink spots are the 52 different spike-in controls.

Conclusion and next step

The Principal Component Analysis showed that the control samples are very similar for the first component, whereas the second component (in the Y-axis plane) differs between two sets of control samples.When inspecting these data in detail, it is apparent that the microRNAs that differ within the controls can all be related to sampling. For example miR-451, and miR-203 are highly expressed in erythrocytes and endothelia respectively.
The short- and long survival samples are not separated on the PCA plot.
In conclusion the microRNA profiling showed that a high number of microRNAs are differentially expressed between control and short- or long survival. The expression differences between short- and long survival samples are limited, but a sub-classification of the samples may reveal pronounced differences.

The selection of the most appropriate microRNA candidates for validation and further study requires some careful consideration. We would like to help you understand the data presented in this report and how best to proceed with your subsequent experiments. If you would like to arrange a time to discuss the data with us in more detail, please do not hesitate to contact DXServices@exiqon.com and we will be happy to arrange a phone call with you.

Sample RNA quality control

Bioanalyzer

Sample RNA Quality Control is performed using Bioanalyser2100. The Bioanalyzer provides an electropherogram for each sample (see the RNA_QC.pdf – file) giving a good estimate on the RNA integrity. As well as the traditional measurement of the rRNA ratio (s28/s18), the Bioanalyzer provides a RIN value (RNA Integrity Number ranging from 0 to 10), which gives a reliable impression of the quality of the RNA[1]. We normally recommend RIN values higher than 7 for good array performance.

The microRNA fraction is expected to lie in the range 25 to 30 sec (see the RNA_QC.pdf – file). Degradation of total RNA is observed as increased number of peaks or a smear in the range between 25 and 50 sec.

Nanodrop

The Nanodrop instrument is used for accurate for measurement of concentrations (abs 260), protein contamination (ratio abs260/abs280) or contamination with organic compounds (ratio abs260/abs230). Compounds like phenol, Trizol and others aromatics may inhibit the labeling reaction giving inconclusive or negative results. For labeling and array hybridization we normally recommend ratios above 1.8 for good array performance when Trizol is used for purification.

[1] Schroeder A., et. al., BMC Mol Biol. 2006 Jan 31;7:3.

Frequently asked questions

Which controls were spiked-in?
The miRCURY™ array contain a number of capture probes for hybridization of a range of synthetic spike-in controls. The spike-in controls extended_spike_control_1 to 52 were spiked into the labeling reaction at various concentrations (some very low).

What is probe ID 13138 named Hy3?
These are control spots with dye printed directly on the arrays for orientation of the grid in analysis. They only give signal in the Hy3 channel and should be removed prior to normalization.

Where can I find the raw data from the scanning of my slides?
The raw data can be found in the txt-files for each slide, in the folder named Raw data files (*X_Exiqon_barcode.txt*).

What are probes named miRPlus?
The miRPlus sequences are in licensed human sequences not yet annotated in miRBase. The sequences will probably be submitted to miRBase soon.
We can provide the sequences when a Non-Disclosure Agreement (NDA) is signed by Exiqon and you. The NDA is valid for 1 year or until third party (e.g. miRBase) disclose the sequences. For requests please contact Exiqon Support, support@exiqon.com.

Material and methods

All experiments were conducted at Exiqon Services, Denmark. The quality of the total RNA was verified by an Agilent 2100 Bioanalyzer profile. 500 ng total RNA from sample and reference was labeled with Hy3™ and Hy5™ fluorescent label, respectively, using the miRCURY LNA™ microRNA Hi-Power Labeling Kit, Hy3™/Hy5™ (Exiqon, Denmark) following the procedure described by the manufacturer. The Hy3™-labeled samples and a Hy5™- labeled reference RNA sample were mixed pair-wise and hybridized to the miRCURY LNA™ microRNA Array (6th gen - hsa, mmu & rno /) (Exiqon, Denmark), which contains capture probes targeting all miRNAs for human, mouse or rat registered in the miRBASE 16.0. The hybridization was performed according to the miRCURY LNA™ microRNA Array instruction manual using a Tecan HS 4800™ hybridization station (Tecan, Austria). After hybridization the microarray slides were scanned and stored in an ozone free environment (ozone level below 2.0 ppb) in order to prevent potential bleaching of the fluorescent dyes. The miRCURY™ LNA array microarray slides were scanned using the Agilent G2565BA Microarray Scanner System (Agilent Technologies, Inc., USA) and the image analysis was carried out using the ImaGene 9.0 software (BioDiscovery, Inc., USA). The quantified signals were background corrected (Normexp with offset value 10, see Ritchie *et al.* 2007) and normalized using the global Lowess (LOcally WEighted Scatterplot Smoothing) regression algorithm.