# In-Depth Data Analysis Report

## miRNA Microarray Service

Experiment:
Date: 2014-01-28
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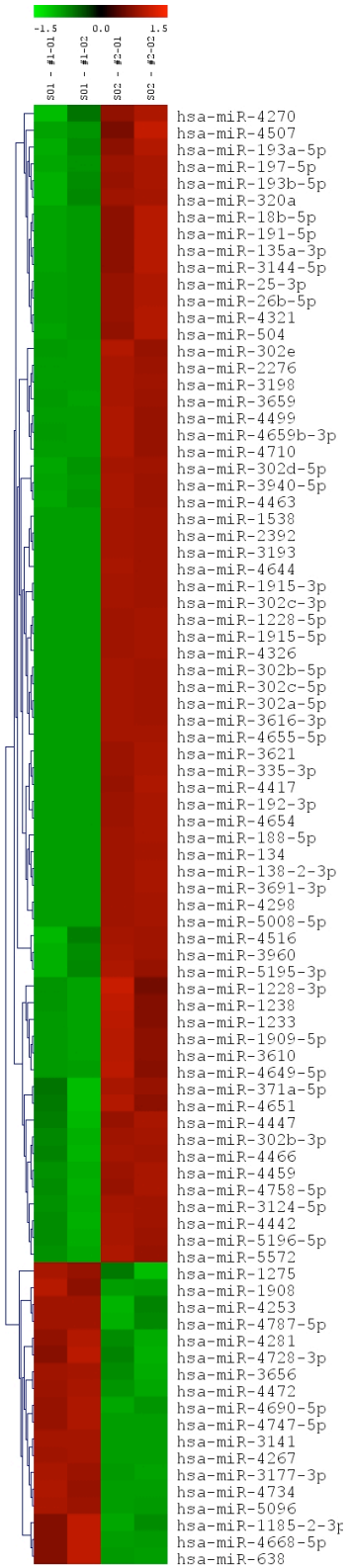
# I. Statistic Test and Clustering Analysis

1. T-test

Test No. T-test-01
Test Name: #2 vs #1

| Group No. | Sample Name | Group Name | Pair | Comment |
|---|---|---|---|---|
| 1 | #1 | #1 | | |
| 2 | #2 | #2 | | |

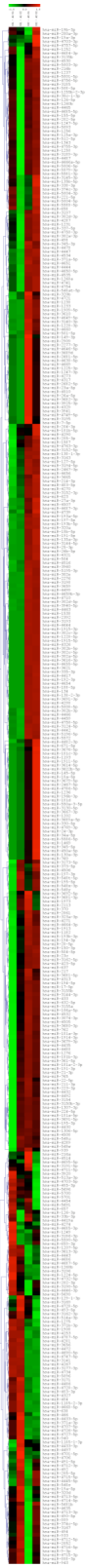

-1.5
0.0
1.5
SD1 - T0-1#
SD1 - 2C#
SD2 - T0-2#
SD2 - 2C#
hsa-miR-4270
hsa-miR-4507
hsa-miR-193a-5p
hsa-miR-197-5p
hsa-miR-193b-5p
hsa-miR-320a
hsa-miR-18b-5p
hsa-miR-191-5p
hsa-miR-135a-3p
hsa-miR-3144-5p
hsa-miR-25-3p
hsa-miR-26b-5p
hsa-miR-4321
hsa-miR-504
hsa-miR-302e
hsa-miR-2276
hsa-miR-3198
hsa-miR-3659
hsa-miR-4499
hsa-miR-4659b-3p
hsa-miR-4710
hsa-miR-302d-5p
hsa-miR-3940-5p
hsa-miR-4463
hsa-miR-1538
hsa-miR-2392
hsa-miR-3193
hsa-miR-4644
hsa-miR-1915-3p
hsa-miR-302c-3p
hsa-miR-1228-5p
hsa-miR-1915-5p
hsa-miR-4326
hsa-miR-302b-5p
hsa-miR-302c-5p
hsa-miR-302a-5p
hsa-miR-3616-3p
hsa-miR-4655-5p
hsa-miR-3621
hsa-miR-335-3p
hsa-miR-4417
hsa-miR-192-3p
hsa-miR-4654
hsa-miR-188-5p
hsa-miR-134
hsa-miR-138-2-3p
hsa-miR-3691-3p
hsa-miR-4298
hsa-miR-5008-5p
hsa-miR-4516
hsa-miR-3960
hsa-miR-5195-3p
hsa-miR-1228-3p
hsa-miR-1238
hsa-miR-1233
hsa-miR-1909-5p
hsa-miR-3610
hsa-miR-4649-5p
hsa-miR-371a-5p
hsa-miR-4651
hsa-miR-4447
hsa-miR-302b-3p
hsa-miR-4466
hsa-miR-4459
hsa-miR-4758-5p
hsa-miR-3124-5p
hsa-miR-4442
hsa-miR-5196-5p
hsa-miR-5572
hsa-miR-1275
hsa-miR-1908
hsa-miR-4253
hsa-miR-4787-5p
hsa-miR-4281
hsa-miR-4728-3p
hsa-miR-3656
hsa-miR-4472
hsa-miR-4690-5p
hsa-miR-4747-5p
hsa-miR-3141
hsa-miR-4267
hsa-miR-3177-3p
hsa-miR-4734
hsa-miR-5096
hsa-miR-1185-2-3p
hsa-miR-4668-5p
hsa-miR-638

p-value < 0.10

| | | | Group 1 | Group 2 | |
|---|---|---|---|---|---|
| | | | #1 | #2 | |
| Rpter Index | Reporter Name | p-value | Mean | Mean | Log2 (G2/G1) |
| 331 | hsa-miR-302c-5p | 1.28E-03 | 0 | 52,804 | 22.65 |
| 327 | hsa-miR-302a-5p | 1.38E-03 | 0 | 26,579 | 21.85 |
| 326 | hsa-miR-302a-3p | 1.52E-03 | 0 | 66,718 | 22.92 |
| 330 | hsa-miR-302c-3p | 3.51E-03 | 0 | 15,973 | 20.71 |
| 333 | hsa-miR-302d-5p | 2.23E-02 | 3 | 22,124 | 12.87 |
| 329 | hsa-miR-302b-5p | 3.09E-02 | 1 | 940 | 3.75 |
| 332 | hsa-miR-302d-3p | 4.86E-02 | 0 | 9,579 | 18.53 |
| 328 | hsa-miR-302b-3p | 4.88E-02 | 6 | 1,963 | 2.23 |
| 381 | hsa-miR-367-3p | 8.78E-02 | 4 | 6 | 0.52 |
| Following transcripts are statistically significant but have low signals (signal < 500) | | | | | |
| 1657 | hsa-miR-4655-5p | 7.47E-05 | 0 | 126 | 15.60 |
| 948 | hsa-miR-1228-5p | 8.61E-04 | 0 | 147 | 15.78 |
| 1087 | hsa-miR-1915-5p | 1.25E-03 | 0 | 51 | 14.55 |
| 1268 | hsa-miR-3616-3p | 1.48E-03 | 0 | 340 | 16.77 |
| 1474 | hsa-miR-4326 | 2.00E-03 | 0 | 35 | 14.11 |
| 1086 | hsa-miR-1915-3p | 2.76E-03 | 0 | 188 | 16.07 |
| 1446 | hsa-miR-4298 | 4.77E-03 | 0 | 62 | 14.78 |
| 1919 | hsa-miR-5008-5p | 5.42E-03 | 0 | 88 | 15.18 |
| 1110 | hsa-miR-2392 | 6.06E-03 | 0 | 159 | 15.88 |
| 1237 | hsa-miR-3193 | 6.56E-03 | 0 | 62 | 14.79 |
| 1609 | hsa-miR-1538 | 7.09E-03 | 0 | 56 | 14.66 |
| 167 | hsa-miR-134 | 8.27E-03 | 0 | 154 | 15.84 |
| 177 | hsa-miR-138-2-3p | 9.03E-03 | 0 | 102 | 15.36 |
| 1639 | hsa-miR-4644 | 9.44E-03 | 0 | 299 | 16.62 |
| 1340 | hsa-miR-3691-3p | 9.62E-03 | 0 | 46 | 14.44 |
| 239 | hsa-miR-188-5p | 1.18E-02 | 0 | 99 | 15.33 |
| 1243 | hsa-miR-3198 | 1.35E-02 | 0 | 105 | 15.40 |
| 245 | hsa-miR-192-3p | 1.51E-02 | 0 | 36 | 14.14 |
| 1103 | hsa-miR-2276 | 1.69E-02 | 0 | 161 | 15.90 |
| 1655 | hsa-miR-4654 | 1.76E-02 | 0 | 69 | 14.90 |
| 374 | hsa-miR-3621 | 2.00E-02 | 0 | 110 | 15.46 |
| 1211 | hsa-miR-3177-3p | 2.06E-02 | 402 | 76 | -2.40 |
| 357 | hsa-miR-335-3p | 2.18E-02 | 0 | 32 | 14.02 |
| 1479 | hsa-miR-4417 | 2.25E-02 | 0 | 94 | 15.28 |
| 1573 | hsa-miR-4499 | 2.26E-02 | 0 | 33 | 14.04 |
| 1661 | hsa-miR-4659b-3p | 2.43E-02 | 0 | 33 | 14.06 |
| 63 | hsa-miR-25-3p | 2.91E-02 | 0 | 44 | 14.39 |
| 69 | hsa-miR-26b-5p | 2.94E-02 | 0 | 25 | 13.75 |
| 334 | hsa-miR-302e | 3.34E-02 | 0 | 40 | 14.29 |
| 1469 | hsa-miR-4321 | 3.47E-02 | 0 | 28 | 13.90 |
| 1530 | hsa-miR-4459 | 3.59E-02 | 66 | 135 | 1.04 |
| 520 | hsa-miR-504 | 4.00E-02 | 0 | 32 | 14.05 |
| 1511 | hsa-miR-4442 | 4.52E-02 | 32 | 65 | 1.02 |
| 244 | hsa-miR-191-5p | 4.63E-02 | 0 | 38 | 14.27 |

| | | | | | |
|---|---|---|---|---|---|
| 35 | hsa-miR-16-5p | 4.65E-02 | 0 | 41 | 14.36 |
| 1826 | hsa-miR-4758-5p | 4.83E-02 | 7 | 74 | 3.45 |
| 169 | hsa-miR-135a-3p | 5.26E-02 | 0 | 23 | 13.68 |
| 1166 | hsa-miR-3144-5p | 5.31E-02 | 0 | 24 | 13.71 |
| 1077 | hsa-miR-1909-5p | 5.37E-02 | 0 | 35 | 14.19 |
| 108 | hsa-miR-92a-1-5p | 5.40E-02 | 0 | 21 | 13.60 |
| 1537 | hsa-miR-4466 | 5.70E-02 | 58 | 137 | 1.23 |
| 1647 | hsa-miR-4649-5p | 5.76E-02 | 187 | 385 | 1.04 |
| 1951 | hsa-miR-5195-3p | 5.81E-02 | 49 | 316 | 2.68 |
| 1401 | hsa-miR-4253 | 5.86E-02 | 296 | 136 | -1.12 |
| 248 | hsa-miR-193a-5p | 5.93E-02 | 7 | 62 | 3.21 |
| 952 | hsa-miR-1233 | 6.40E-02 | 0 | 34 | 14.16 |
| 1682 | hsa-miR-4668-5p | 7.08E-02 | 315 | 70 | -2.17 |
| 959 | hsa-miR-1238 | 7.34E-02 | 0 | 25 | 13.82 |
| 1518 | hsa-miR-4447 | 7.41E-02 | 13 | 89 | 2.76 |
| 1777 | hsa-miR-4728-3p | 7.96E-02 | 42 | 16 | -1.42 |
| 381 | hsa-miR-367-3p | 8.78E-02 | 4 | 6 | 0.52 |
| 388 | hsa-miR-371a-5p | 8.97E-02 | 23 | 88 | 1.92 |
| 1581 | hsa-miR-4507 | 9.15E-02 | 0 | 44 | 14.54 |
| 1418 | hsa-miR-4270 | 9.39E-02 | 29 | 113 | 1.95 |
| 1650 | hsa-miR-4651 | 9.46E-02 | 25 | 116 | 2.19 |
| 947 | hsa-miR-1228-3p | 9.72E-02 | 0 | 52 | 14.74 |

Clustering graph of all miRNAs with signal intensity > 32



# I. Data List

Chip01

| Data File | |
|---|---|
| Folder Name | Chip01_H20_131413CD |
| Data File | 01_H20_131413CD-140127-Wen-mir302-390_Data.xls |
| Layout File | MRA-1001B2_miRHuman_20.xls |
| Original Image File | 01_H20_131413CD-140127-Wen-mir302-390.tif |
| Processed cy3 File | 01_H20_131413CD-140127-Wen-mir302-390cy3.tif |
| Processed cy5 File | |
| **Assay Information** | |
| Assay Date | 01/27/2014 |
| Sample A | |
| Sample ID | mir-302 |
| Receiving Date | 01/16/2014 |
| Labeling Dye | cy3 |
| Sample B | |
| Sample ID | |
| Receiving Date | |
| Labeling Dye | |

## II. Chip Content

The content of each chip is listed in a Layout File of a corresponding folder. Multiple redundant regions are included. Each region further comprises a miRNA probe region, which detects miRNA transcripts listed in Sanger miRBase Release 20.0 (http://www.miRBase.org/).

Multiple control probes are included in each chip. The control probes are used for quality controls of chip production, sample labeling and assay conditions. Among the control probes, PUC2PM-20B and PUC2MM-20B are the perfect match and single-based match detection probes, respectively, of a 20-mer RNA positive control sequence that is spiked into the RNA samples before labeling. One may assess assay stringency from the intensity ratio of PUC2PM-20B and PUC2MM-20B, which is normally larger than 30.

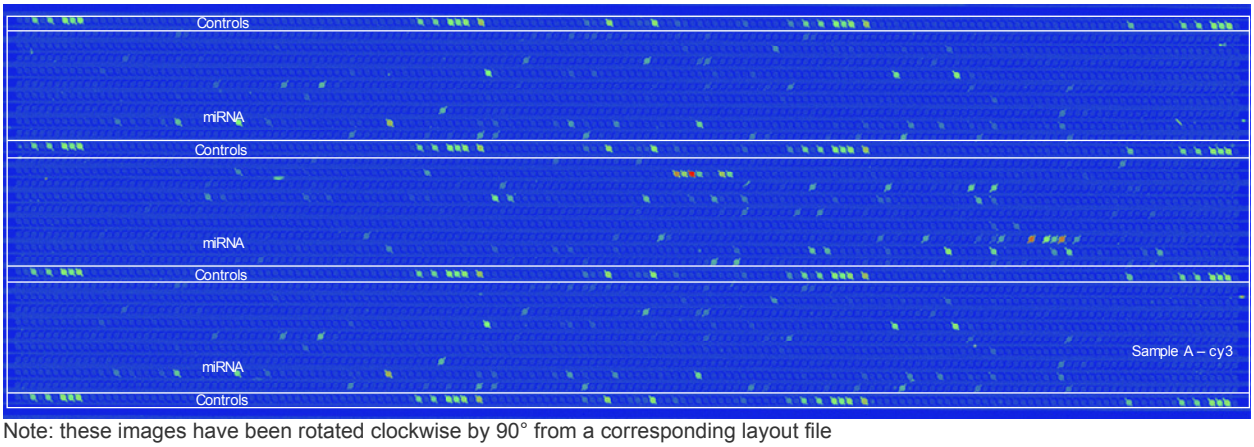
When the option for custom probes is selected, custom probes are also included.

## III. Summary of Results

Following are representative regions of chips images. From Cy3 images one may directly read miRNA profiles. The images are displayed in pseudo colors so as to expand visual dynamic range. In the images, as signal intensity increases from 1 to 65,535 the corresponding color changes from blue to green, to yellow, and to red.

Chip: 01_H20_131413CD

Images: Sample A: mir-302 - cy3




Note: these images have been rotated clockwise by 90° from a corresponding layout file

## IV. Data Analysis

We provide the result of a data analysis in data files (Chip#_Data.xls). There are five worksheets in each file as described in the following.

- Worksheet "File Info" – provides information on data files, samples, and data analysis parameters.
- Worksheet "Simple Differential" – lists all differentially expressed transcripts with p-value < 0.01. Mature miRNAs are sorted separately according to differential ratios. The ratio values are presented in $\log_2$ scale for quick and easy assessing differential direction as well as magnitude. A positive $\log_2$ value indicates an upper regulation and a negative $\log_2$ value indicates a down regulation. One can easily convert a $\log_2$ value into a arithmetic ratio on a calculator by typing in 2^(value). Detailed data processing statistics are listed in Worksheet "Differential Data".
- Worksheet "Summary" – lists average signal values all transcripts on the array. The signal mean values are derived by background subtraction. Signal p-values are provided to indicate how significantly the signal is differentiated from background. In general, a signal with p-value less then 0.01 is considered to be detectable. When a signal has a detection p-value larger than 0.01, its mean value is written in grey color. Differential log2 ratios and p-values are provided for those reporters (or probes) that are detectable in at least one of the Cy3 (or Af3) and Cy5 (or Af5) samples. In the differential calculation, the p-value is an indication on how significantly Cy3 (or Af3) and Cy5 (or Af5) signals are different from each other. In general, a p-value of less then 0.01 indicates a significant difference between Cy3 (or Af3) and Cy5 (or Af5) signals. When a reporter has a differential p-value larger than 0.01, its log2 ratio is written in grey color.
- Worksheet "Raw Data" – lists raw data extracted from image files with corresponding probe, sequence, and location information.
- Worksheet "Processed Data" – lists processed data, including background-subtracted and normalized signals, p-values, statistically significant log ratios of Cy3 (or Af3) and Cy5 (or Af5) labeled transcripts, and a scatter plot of the processed data.

In data file package a probe layout file containing a complete list of probe positions and target sequences is included in the file directory of each chip.

## V. Suggestions for Data Analysis

In case you want to perform your own data analysis we have the following suggestions.

1. Background should be calculated from the median of 5% to 25% of low intensity cells. BKG0 and blank cells should be excluded for the background calculation.
2. All "Production_Use_Probes" (including BKG0, PUC2 …,) and blank cells which are listed in a supplied layout file, should be excluded during data normalization.