Data Reproducibility and Concordance Between Agilent SurePrint G3 Human miRNA Microarrays

Application Note

Authors

Nilanjan Guha and Petula D'Andrade
Agilent Technologies, Inc.
Santa Clara, CA USA

Introduction

Agilent SurePrint G3 Human miRNA microarrays generate complete miRNA profiles, providing broad insight into human gene expression and regulation. The content is regularly updated from the miRBase database in order to ensure that generated results are current. The aim of this study is to demonstrate concordance in the results achieved with identical oligonucleotide probes on two versions of Agilent miRNA microarrays [(AMADID 021827, release 12.0) and (AMADID 031181, release 16.0)], based on different revisions of the miRBase database and provided on different microarray formats. Since probes for a given microarray will remain constant for subsequent versions of Agilent SurePrint G3 Human miRNA microarrays, barring any miRBase sequence changes, it is necessary to confirm that the TotalGeneSignal achieved is consistent. The data show Agilent's strength in probe reproducibility, a crucial factor for identifying miRNA of differential expression.

Agilent Technologies

Experimental

Agilent SurePrint Human miRNA microarrays [(AMADID 021827, release 12.0) and (AMADID 031181, release 16.0)] were tested using a range of Agilent RNA samples: Universal Human miRNA Reference RNA (p/n: 750700); MVP Total RNA, Human Placenta (p/n: 540025); MVP Total RNA, Human Brain (p/n: 540005); and MVP Total RNA, Human HeLa S3 Cells (p/n: 540087).

The microarrays were processed according to Agilent's miRNA microarray protocol, version 2.4. Two slides were processed per array design. On each 8X slide, the RNA samples were processed in duplicate using the array layout pattern shown in Figure 1. Following hybridization, the slides were scanned using the Agilent High Resolution C-scanner.

Generating correlation plots across microarrays

The feature extracted .txt files for four replicates of each sample (total 16 files) were loaded into GeneSpring as two separate experiments, in order to allow for the comparison between microarray versions. No normalization and baseline transformation was performed.

The following steps were performed to complete the analysis:

- Based on the PCA plot, the three best replicates of each sample were retained.
- In the **All Entities** list, a separate entity list was made for only biological probes.
- Each sample type was then analyzed separately by removing the other RNA sample types using the **Add/Remove** samples function.
- Only **Detected** probes for each RNA sample type were **filtered by flags**, while entities present in all three replicates were retained.
- The common entities (only for biological probes, after filtering by flags) list between the two miRNA versions were exported and joined by **SystematicName**, so that the **SystematicName** is present in both files.
- A table was created with the **SystematicName** and results for each design version in a separate column.
- Results were transferred to SpotFire for visualization and analysis of identical samples.

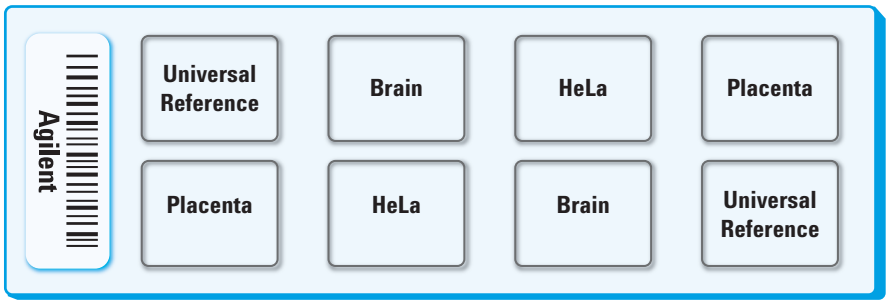

Figure 1. Slide layout for miRNA microarray comparison.

Results and Discussion

The SurePrint G3 Human miRNA microarray (Release 16.0, 8X60K) and SurePrint G3 Human miRNA microarray (Release 12.0, 8X15K) demonstrated tight correlation between technical replicates for each of the RNA samples tested (Figure 2). The correlation plots between the different AMADID versions for each sample revealed high concordance of signal intensities for identical oligonucleotide probes present in each of the versions (Figure 3).

Conclusions

A comparison between two versions of SurePrint G3 Human miRNA Microarrays, based on different revisions of the miRBase database and provided on different microarray formats, (AMADID 031181, Release 16.0, 8X60K) and Human miRNA Microarray, V3 (AMADID 021827, Release 12.0, 8X15K), shows tight data reproducibility. In addition, there is a good concordance of the probes' signal between AMADID 021827, (Release 12.0) and the newer version AMADID 031181(Release 16.0).

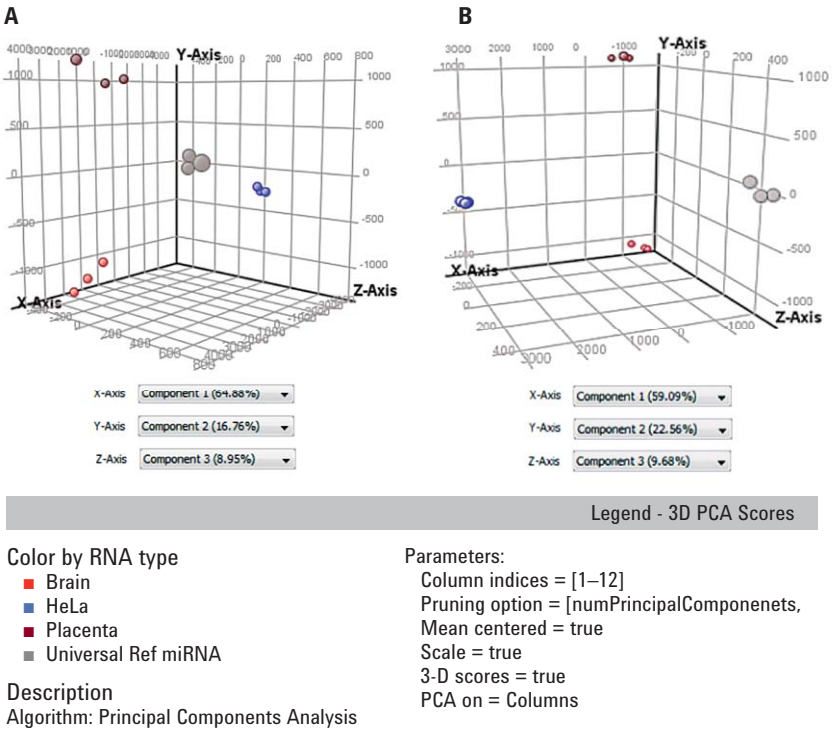

Figure 2. PCA plot for three replicates of each sample: AMADID 031181, Release 16.0, 8X60K (A) and AMADID 021827, Release 12.0, 8X15K (B).

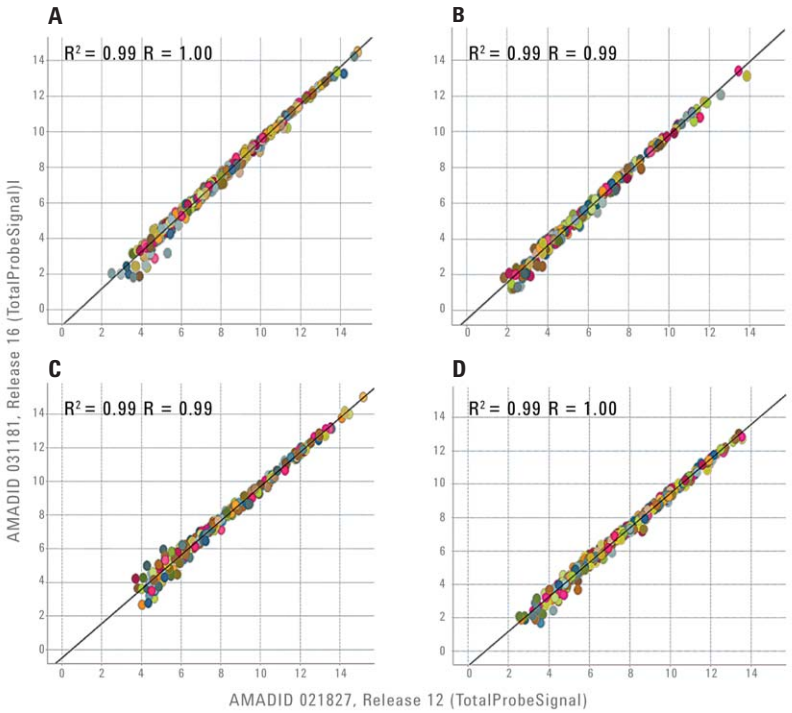

Figure 3. Correlation plots of AMADID 031181 (Release 16.0, 8X60K) versus AMADID 021827 (Release 12.0, 8X15K) MVP Total RNA, Human Brain (A), MVP Total RNA, Human HeLa S3 Cells (B), MVP Total RNA, Human Placenta (C), Universal Human miRNA Reference RNA (D).

www.agilent.com/genomics/microarrays

Published in the USA, March 30, 2012
5991-0045EN

Agilent Technologies