Assessment of Sensitivity and Dynamic Range of New Generation Microarray Technology using the MAQC Samples

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Abstract

We have developed a new generation microarray platform with improved transcript detection sensitivity. This new platform provides expression measurements that more accurately reflect the range of gene activities in biological systems. First, by reducing feature size and spacing, the footprint of the microarray is reduced, while feature count and content remain constant. The reduction in area allows for a more concentrated target sample without increasing RNA input. Higher concentration samples result in higher signals, allowing for accurate detection of transcripts previously below the detection limit. An extended dynamic range scanner prevents saturation of bright features. This system, which also includes new tools for automated scanning and data extraction, increases the range of gene expression measurements to greater than five logs. To assess the resulting performance and data quality, we used RNA samples from the MicroArray Quality Control (MAQC) project, and compared the performance of Agilent's new 4 pack high-density microarrays to the previous generation Agilent microarrays. We demonstrate enhanced sensitivity, increased dynamic range and improved reproducibility, and confirm the utility of the MAQC samples for ongoing assessment of data quality in microarray experiments.

Introduction

Recently the MicroArray Quality Control (MAQC) consortium published the most comprehensive study to date assessing the performance and cross platform comparability of microarray data (MAQC Consortium. *Nat. Biotechnol.* 24, 1151–1161 (2006)). This study laid a foundation and framework for assessment of microarray performance, whether for proficiency testing, or for assessing changes to microarray platforms. The commercially available samples and the metrics used in the study, as well as the large reference dataset generated by the study allow for the relatively straightforward assessment of microarray performance.

As demonstrated in the MAQC study, microarray performance is generally a balance among sensitivity, accuracy and reproducibility. Platform design choices that emphasize one aspect tend to do so at the expense of the others. At Agilent Technologies, we have designed our microarray platform to achieve an appropriate balance among these different performance attributes with a strong emphasis on accuracy and sensitivity of detecting differential expression across a wide dynamic range. The results of these design choices can be seen in the data presented in the MAQC study.

Through technology enhancements that allow for 4 individual whole genome microarrays to be printed on a single glass slide, we have improved both the sensitivity and reproducibility of the platform without sacrificing the accuracy of differential expression calls. Due to the reduced surface area of the array, an increased concentration of sample can be hybridized without increasing sample input requirements. Signals that might otherwise have been lost due to scanner saturation are captured by scanning at high and low gain settings using the new eXtended Dynamic Range (XDR) feature of the Agilent microarray scanner. Combining the feature intensity data from the two different scans is accomplished automatically with the latest version of the Agilent Feature Extraction software (v9.1).

We have used the commercially available MAQC samples to evaluate the performance of Agilent's new high density multi-pack whole human genome arrays (4 pack). Using some of the analysis methods and metrics put forth in that study, we compare the performance of the new generation microarrays to the "legacy" whole genome products (44K).

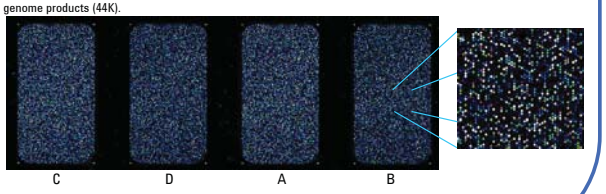

Figure 1: Image of an Agilent whole human genome 4 pack microarray slide hybridized with the four MAQC samples

Experimental Design

Microarray Design:

Control 44k microarrays (Whole Human Genome Oligo Microarray Kit, P/N G4112A) contain one microarray on each 1"x3" glass slide. The 41,000 non-control probes on this microarray are identical to those present on the new 4 pack microarrays (Whole Human Genome Oligo Microarray Kit, P/N G4112F). Each 4 pack microarray slide contains four individual whole human genome arrays.

Experimental design:

Total RNAs obtained from Stratagene (Universal Human Reference RNA, "A") or Ambion (Human Brain Reference RNA, "B") were used as the starting material for labeling and hybridization to the microarrays. Sample mixtures of "A" and "B" were generated as for the MAQC study using a 3:1 (C) or 1:3 (D) volumetric mixture of A and B samples, respectively, in one batch. All samples were labeled in quadruplicate by each of three users, and included the Agilent One-Color RNA Spike-In for quality control and integrated measurement of dynamic range. Each of the four labeling reactions per user for samples A and B was split, with a portion hybridized to a 44K control array, and a portion hybridized to a 4 pack array. Mixed samples C and D were hybridized to the 4 pack arrays only. All RNA labeling reactions and hybridizations were carried out using either the standard One Color processing protocol for 44k control arrays (G4140-90040 V.1.0.1, February 2006) or the new One Color processing protocol (G4140-90040 V.5.0.1, 9/2006) for the 4 pack arrays. The 44K arrays were scanned at a gain of 100% while the 4 pack arrays were scanned using the new XDR function of the Agilent DNA Microarray Scanner using gain settings of 10% and 100%.

Data Analysis:

Data were extracted using Agilent Feature Extraction, version 9.1.3, using the recommended protocol for each format. Following feature extraction, data were loaded into GeneSpringGX v 7.3 for normalization. For both formats the same standard normalization method was applied. All data was divided by the 75th percentile of the signals on the array. This percentile was calculated using all of the data on the array. For calculation of B/A ratios, all "B" data points were divided by the median of the "A" values for that probe.

Sensitivity and Dynamic Range

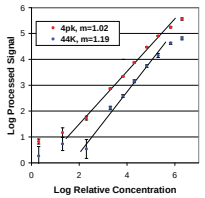

Figure 2: Agilent Spike-In concentration response

RNA Spike-In control results for two different format microarrays hybridized with sample B are shown in Figure 2. These Spike-In controls are included in the labeling reaction and span six orders of magnitude in concentration. The graphs shown in Figure 2 demonstrate the 4 pack arrays (shown in red) have a greatly expanded dynamic range as compared to the 44K arrays. Scanner saturation of the probe representing the highest concentration transcript is eliminated by use of the XDR scanning capabilities, and lower concentration transcripts are now detected due to increased sample concentration as well as improvements in hybridization conditions.

The response of biological probes is also improved using the new 4 pack format. Figure 3 illustrates the detected signals for the same sample hybridized on the two formats. As can be seen in the figure, overall signal correlation is good between the formats, with higher probe intensities for the 4 pack array. In addition, 5600 probes that were either below the detection limit (three times the measured background noise) or saturated on the 44K array are now detectable on the 4 pack array. The arrays represented here are the same as those with spike-in data shown in Figure 2, and the 4 pack array is from the slide shown in Figure 1.

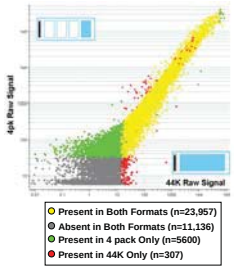

Figure 3: Signal comparison of biological probes between 4 pack and 44K

Differential Expression Detection

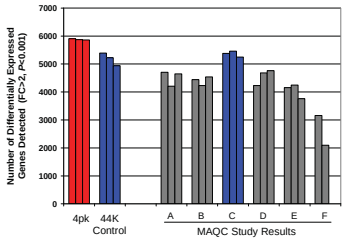

Figure 4: Number of differentially expressed genes detected

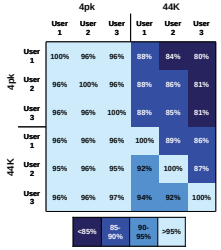

Figure 5: Gene list agreement among users for 4 pack and 44K

The primary goal of gene expression microarray experiments is to detect changes in gene expression levels between samples. In this analysis, we calculated the number of genes differentially expressed between two MAQC samples (A, Stratagene Universal Human Reference, and B, Ambion Human Brian Reference) and looked at the agreement of those genes between the two formats. To facilitate comparison to data presented in the MAQC study, we focused on the set of 12091 commonly mapped genes as a basis for comparison to those data. For the 4 pack and 44K experiments performed here, differential expression was determined using the same method as described in the MAQC study.

The number of differentially expressed genes for each user and array format is shown in the left of Figure 4. On the right are the numbers reported for the different test sites and platforms in the MAQC study. The values plotted are provided in supplemental table S7 from the main MAQC paper. The data on the right highlighted in blue represent the values for the Agilent platform in the MAQC study.

Figure 5 illustrates the gene list agreement between the 4 pack and 44K formats and among the users. The numbers shown are the percentage of genes detected by the user and format shown in the row which are also detected by the user and format shown in the column. In all cases, >95% of genes detected by any format and user were also detected by each user with the 4 pack format.

Comparison To TaqMan®

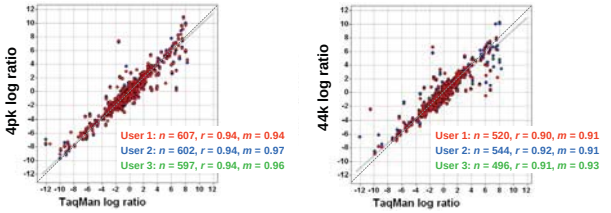

Figure 6: Correlation between microarray and TaqMan® data

Relative accuracy of microarray platforms can be assessed by comparison to gene expression measurements collected by alternative platforms. Figure 6 presents scatter plots of the 4 pack and 44K $\log_2$(B/A) ratio data with the TaqMan® data presented in the MAQC study. Shown are data where at least 3 replicates were detected in both samples by both the microarray and TaqMan®. The number of data points included for each user as well as the correlation and slope of the individual orthogonal fits are shown in the inset. Dotted lines represent the 45 degree line, while solid lines represent the orthogonal fit of all the data.

Figure 7 presents the average slopes (solid bars) and correlations (hatched bars) for these data (left) as well as the data presented in the MAQC study (right, values from supplemental tables S12 and S13). The data on the right highlighted in blue represent the values for the Agilent platform in the MAQC study.

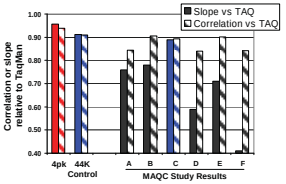

Figure 7: Correlation and slope of microarray to TaqMan® data

Reproducibility

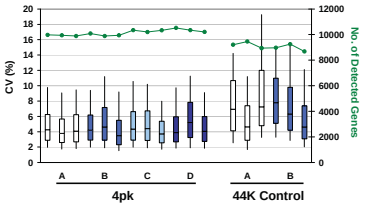

Figure 8: Signal variation within user

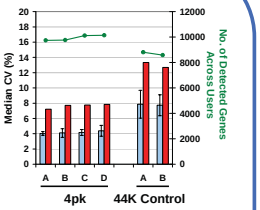

Figure 9: Signal variation among users

Reproducibility is represented as the coefficient of variation within or among users for normalized expression signals from those probes that were generally detected. Figure 8 shows the distributions of the within user CV as box and whisker plots for each sample, user, and format. Shown in green are the numbers of genes detected in at least three replicates for each sample per user; these genes were used to calculate the CVs. Figure 9 shows the median CVs both within user (blue), and across user (red). Shown in green are the number of genes detected in at least three replicates for all three users; these genes were used to calculate the CVs.

Both formats demonstrate good reproducibility with median within user CVs lower than 10%. The 4 pack microarrays show generally improved reproducibility as compared to the 44K microarrays, due in part to the increased concentration of hybridization.

Conclusions

We have used the MAQC samples to evaluate performance of a new generation microarray technology, the Agilent 4 pack microarray, and compared performance to that of the previous generation.

- While our previous generation microarrays demonstrated outstanding sensitivity in the MAQC study, the new 4 pack microarray extends our leadership in sensitivity and dynamic range of differential expression detection.
- Agilent's already excellent accuracy, as reflected by concordance with TaqMan®, is further improved in the new 4 pack format.
- Reproducibility of signal shows significant improvement, with median interarray CVs less than 5%

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