

New GeneChip Hybridization, Wash, and Stain Kit reagents provide equivalent array performance to reagents from former supplier

Affymetrix has replaced the GeneChip® Hybridization, Wash, and Stain Kit (P/N 900720) with our own high-quality reagents. Configurations and formulations have not changed.

This white paper compares the performance of the kit manufactured at Affymetrix with the former supplier's kit and shows that the two have an equivalent level of performance.

Materials and methods

Three lots of the former Hybridization, Wash, and Stain (HWS) Kit and three lots of the HWS Kit manufactured by Affymetrix were tested using GeneChip® HG-U133 Plus 2.0 Arrays and GeneChip® Human Exon 1.0 ST Arrays.

Targets were prepared with 1 µg of Microarray Quality Control (MAQC) A and B total RNAs:

- GeneChip® One-Cycle Target Labeling and Control Reagents, P/N 900493 (for specific protocol, see the *GeneChip® Expression Analysis Technical Manual*, P/N 702232, revision 2)
- GeneChip® WT Sense Target Labeling and Control Reagents, P/N 900652 (for specific protocol, see the *GeneChip® Whole Transcript (WT) Sense Target Labeling Assay Manual*, P/N 701880, revision 4)

A pool of fragmented and labeled cRNA (for HG-U133 Plus 2.0 Arrays) or single-stranded cDNA (for Human Exon 1.0 ST Arrays) was used to test all of the HWS Kit lots in triplicate for each total RNA.

CEL files from the HG-U133 Plus 2.0 Arrays were quantile normalized in Expression Console™ Software using the RMA algorithm for probe set summarization. The MAS 5.0 algorithm was used to calculate percent present calls. Exon- and gene-level analyses were performed on the Human Exon 1.0 ST Arrays. The CEL files were analyzed using Affymetrix Power Tools (APT) release apt-1.8.6. For the gene-level analysis, multiple probes on different exons were summarized into a single, expression-level data point that represents all transcripts derived from each gene (for more details, see the Application Note, *Whole-transcript Expression Analysis*, available at http://www.affymetrix.com/support/technical/appnotes/wt_appnote.pdf).

To perform signal intensity correlation and fold change correlation analysis, the data was filtered before determining the correlation coefficients to remove nonresponsive probe sets. To be included in the signal intensity correlation analysis, probe sets had to be called present using a detection call in which 50 percent or more of the replicates in both test conditions have a DABG threshold *p*-value less than or equal to 0.01.

To perform fold change correlation analysis, probe sets not detected for both MAQC A and MAQC B were not plotted. Probe sets had to be called present in at least half of the three replicates for only one tissue. A unique list of present probe sets was then created from the union of the two tested conditions and was then used to plot each individual fold change correlation plot.

Results and discussion

Table 1 shows the average percent present calls for the HG-U133 Plus 2.0 Arrays using each lot. The difference in percent present calls, when compared to the average of the three former supplier lots with the three Affymetrix lots, is 0.2 percent for MAQC A and 1 percent for MAQC B.

Table 1: Average percent present calls.

RNA	Former supplier % present calls \pm St. Dev	Affymetrix % present calls \pm St. Dev
MAQC A	55.5% \pm 0.71%	55.3% \pm 1.06%
MAQC B	54.0% \pm 0.66%	53.0% \pm 0.96%

The signal intensity correlation for the intra-lot (former versus former or Affymetrix versus Affymetrix) and inter-lot comparison (former supplier versus Affymetrix) was calculated to determine whether the different manufacturing sites produced any bias in the array signal.

Table 2 shows the Pearson correlation coefficient (R) of average RMA-summarized probe set intensities among lots. For the HG-U133 Plus 2.0 Arrays and Human Exon 1.0 ST Arrays, the R values were higher than 0.99 for inter-signal intensity correlations.

Table 2: Pearson correlation coefficient (R) for the signal intensity correlation for HG-U133 Plus 2.0 Arrays and Human Exon 1.0 ST Arrays.

Comparison	HG-U133 Plus 2.0 Arrays		Exon 1.0 ST Arrays (exon-level analysis)		Exon 1.0 ST Arrays (gene-level analysis)	
	MAQC A	MAQC B	MAQC A	MAQC B	MAQC A	MAQC B
Average Affymetrix lot-to-lot correlation	0.998 \pm 0.001	0.998 \pm 0.001	0.985 \pm 0.008	0.986 \pm 0.005	0.995 \pm 0.003	0.992 \pm 0.004
Average former supplier lot-to-lot correlation	0.999 \pm 0.000	0.999 \pm 0.001	0.992 \pm 0.002	0.984 \pm 0.007	0.997 \pm 0.002	0.993 \pm 0.001
Affymetrix vs. former supplier lot correlation	0.999	0.998	0.996	0.991	0.999	0.996

Figure 1 shows the graphs for the signal correlation of all former supplier versus all Affymetrix lots for MAQC A total RNA (1A) and MAQC B total RNA (1B) for the HG-U133 Plus 2.0 Arrays. Figures 2 and 3 show the graphs for the signal correlation of all former supplier versus all Affymetrix lots for MAQC A total RNA (A) and MAQC B total RNA (B) for the exon-level analysis and gene-level analysis, respectively. No significant bias in array data was detected.

Figure 1: HG-U133 Plus 2.0 Arrays—gene-level probe set signal intensity correlation for all former supplier lots versus all Affymetrix lots.

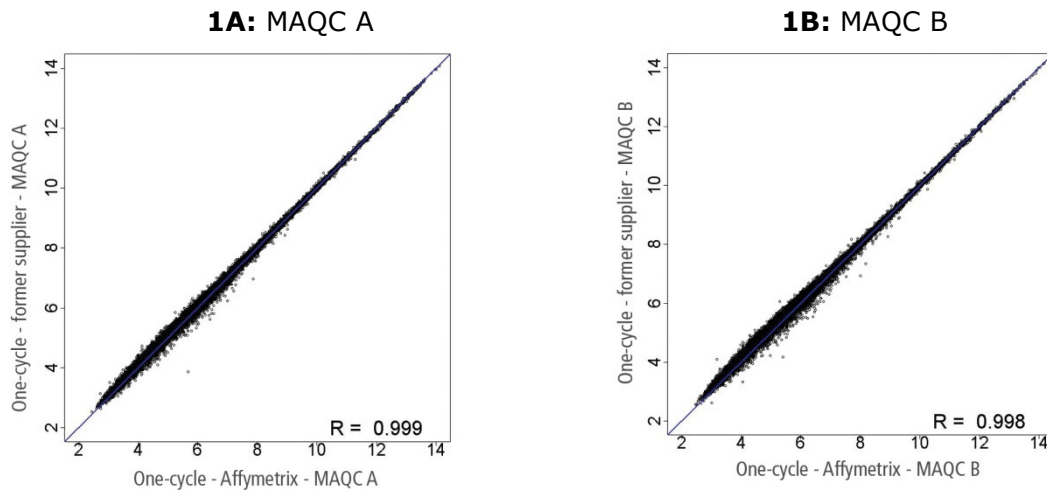


Figure 2: Human Exon 1.0 ST Arrays—exon-level probe set signal intensity correlation for all former supplier lots versus all Affymetrix lots.

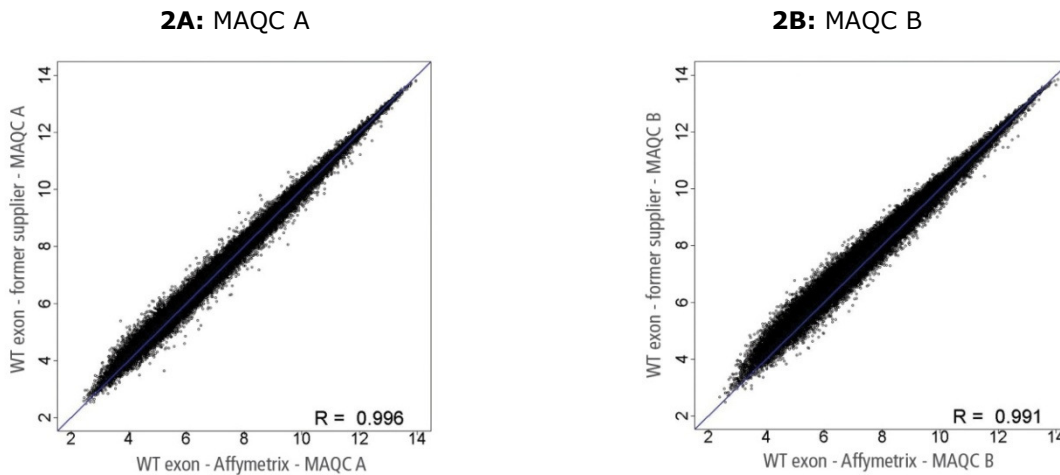
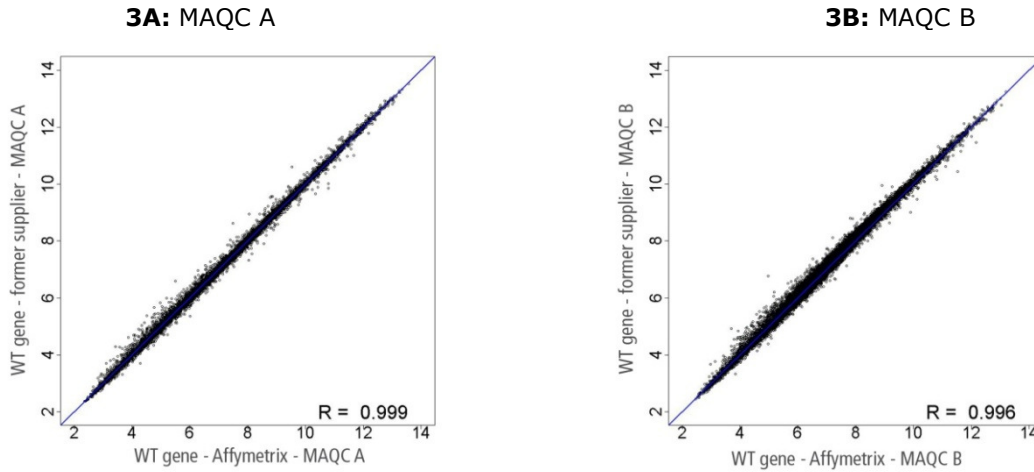


Figure 3: Human Exon 1.0 ST Arrays—gene-level probe set signal intensity correlation for all former supplier lots versus all Affymetrix lots.



The MAQC A/B fold change correlations were also calculated for the intra- and inter-lot comparison. Table 3 shows the Pearson correlation coefficient (R) of average fold change values [$\log(\text{MAQC A/B})$] among lots. R values were higher than 0.98 for inter-lot comparisons on the HG-U133 Plus 2.0 Arrays and Exon 1.0 ST Arrays.

Table 3: MAQC A/B fold change correlation.

Comparison	HG-U133 Plus 2.0 Arrays	Exon 1.0 ST Arrays (exon-level analysis)	Exon 1.0 ST Arrays (gene-level analysis)
Average Affymetrix lot-to-lot correlation	0.992 ± 0.002	0.937 ± 0.027	0.950 ± 0.024
Average former supplier lot-to-lot correlation	0.993 ± 0.001	0.952 ± 0.017	0.965 ± 0.013
Affymetrix vs. former supplier lot correlation	0.996	0.986	0.991

Figure 4A shows the graphs for the fold change correlation of all former supplier versus all Affymetrix lots for the HG-U133 Plus 2.0 Arrays.

Figures 4B and 4C show the graphs for the fold change correlation of all former supplier versus all Affymetrix lots for the exon-level analysis and gene-level analysis, respectively, of the Human Exon 1.0 ST Arrays.

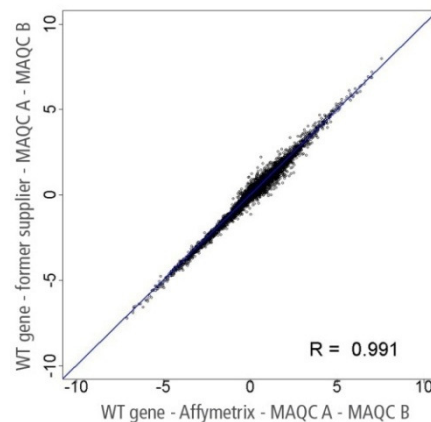
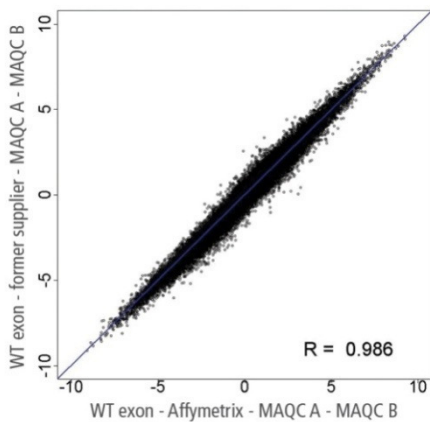
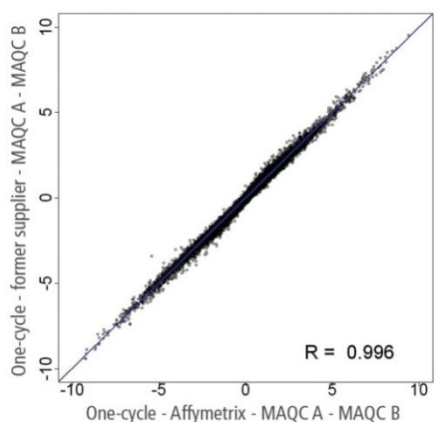
Similar performance was observed for the lots produced at the two different manufacturing sites.

Figure 4A-C: Fold change correlation for all former supplier lots versus all Affymetrix lots.

4A: HG-U133 Plus 2.0 Arrays

4B: Human Exon 1.0 ST Arrays (exon-level analysis)

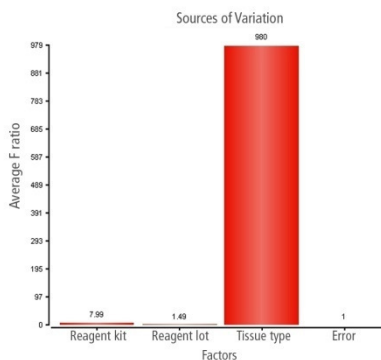
4C: Human Exon 1.0 ST Arrays (gene-level analysis)



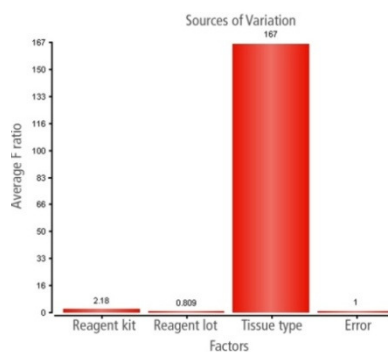
Using the data from each lot at both manufacturing sites, a multi-factor ANOVA (Partek® Genomics Suite™, version 6.4, build 6.09.0129, ©2009 Partek, Inc.) was performed to explore the contributions of each of the parameters (reagent site, reagent lot, tissue type). The sources of variation are graphically represented in Figure 5A for the HG-U133 Plus 2.0 Arrays, 5B for exon-level analysis using the Human Exon 1.0 ST Arrays, and 5C for gene-level analysis using the Human Exon 1.0 ST Arrays. As expected, the RNA sample type was by far the largest contributor to variation in the data set. Variation from the manufacturing site and lot-to-lot variation were negligible relative to real biological differences in the data.

Figure 5A-C: ANOVA sources of variation.

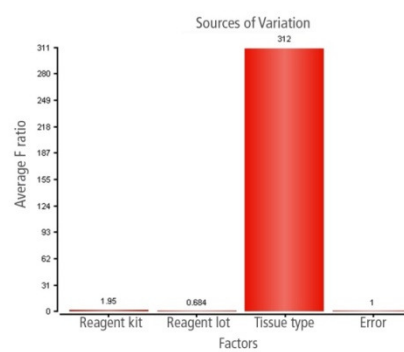
5A: HG-U133 Plus 2.0 Arrays



5B: Human Exon 1.0 ST Arrays (exon-level analysis)



5C: Human Exon 1.0 ST Arrays (gene-level analysis)



Conclusion

The new GeneChip® Hybridization, Wash, and Stain Kit manufactured by Affymetrix demonstrates the same level of performance as the kit from former supplier. As shown by the data in this white paper, customers can therefore use the new kit in current experiments.