TECHNICAL REPORT

Performance of the Ovation® PicoSL WTA System V2 on Affymetrix Human Gene 1.1 ST Array Plates

Introduction

The Ovation PicoSL WTA System V2 is based on proprietary Ribo-SPIA® technology—a rapid, simple and sensitive amplification process for nucleic acids. The Ribo-SPIA process generates microgram quantities of double-stranded cDNA which can be directly analyzed by qPCR, or further processed for use in microarray experiments or next-generation sequencing (NGS) analyses. The ds-cDNA produced can be used with either antisense strand (3'-based) or sense strand (ST) array designs. An increasing number of Affymetrix® microarrays are available in cartridge format as well as "array plate" or "array strip" formats. These latter array types are used for automated, high throughput processing of samples on the Affymetrix GeneTitan® and GeneAtlas™ instruments, respectively.

The Ovation PicoSL WTA System V2 (Part No. 3312) is a time-efficient and cost-effective solution for cDNA target preparation from as little as 500 pg of total RNA. This system generates double-stranded cDNA (ds-cDNA) that is ready for labeling using the Encore® Biotin Module (Part No. 4200) before hybridization to mini-size (169 format) Affymetrix GeneChip cartridge arrays or to array plates.

This technical report describes the performance of the Ovation PicoSL WTA System V2 with Affymetrix Human Gene 1.1 ST Array Plates processed on the GeneTitan instrument. RNA reference samples, MAQC-A (UHRR, Agilent) and MAQC-B (Brain, Life Technologies), were used to assess the performance of the system. The entire process from total RNA to a hybridization cocktail ready for array hybridization took about seven hours to complete.

TABLE 1. **Assay and array performance.**

	Assay performance		Array performance
Sample	**Input RNA [ng]**	**Yield [µg]**	**Pos_vs_neg_auc**
MAQC-A	0.5	2.6	0.862
	5	4.7	0.896
	10	5.0	0.896
	20	5.4	0.897
	40	5.7	0.904
MAQC-B	0.5	3.4	0.872
	2	4.4	0.883
	20	4.7	0.894

Total RNA input and associated yields of ds-cDNA after amplification.

Methods

Total RNA samples (MAQC-A and MAQC-B) were processed using the Ovation PicoSL WTA System V2 at defined total RNA inputs. Triplicate reactions were performed at each input level for a total of 24 reactions. The resulting cDNA was quantified by spectrophotometry and the size distribution of the cDNA product was characterized using an Agilent 2100 Bioanalyzer. The cDNA was then processed using the Encore Biotin Module in preparation for array hybridization. Hybridization cocktails were constructed for each sample using the Affymetrix GeneTitan Hybridization, Wash and Stain Kit for WT Array Plates (Affymetrix part number 901622), as outlined in the Ovation PicoSL WTA System V2 user guide. The samples were then hybridized to an Affymetrix Human Gene 1.1 ST Array Plate using the GeneTitan instru-

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ment and following the manufacturer's instructions.

Assay Performance

The cDNA yield from each reaction was assessed by spectrophotometry and the results are given in **Table 1**. Increasing amounts of input RNA led to slightly higher yield of ds-cDNA. In all cases, yield was at least 2.5 µg, the minimum quantity required for array hybridization.

The ds-cDNA was analyzed using an Agilent 2100 Bioanalyzer RNA 6000 Nano LabChip® using the total RNA program (**Figure 1**). The traces shown are typical, but may vary with sample type.

Array Performance

The pos_vs_neg_auc (AUC) value is a QC metric correlating with overall array performance. It reflects the specificity of the array where 0.5 indicates no specificity and 1.0 indicates 100% specificity. Typically, a value above 0.8 is considered acceptable. The AUC values in this experiment range from 0.86 to 0.90 (**Table 1**), indicating that the NuGEN target has a high level of specificity. It has been observed that the AUC value is slightly lower when using less than 1 ng of input RNA, but the value stabilizes with higher amounts of input RNA.

The principal component analysis (PCA) plot (**Figure 2**) clearly shows the separation of the samples by type in the first dimension. It also illustrates that, with decreasing levels of RNA, the samples are separated in the second and third dimension. These data reflect an expected slight increase in noise with very low input of total RNA.

Figure 3 shows a heat map of the Pearson correlation coefficients (CC) of the three replicates at each input level. Decreased noise in the data is observed as sample size increases, as shown by the increasing color intensity. **Table 2** presents the lowest Pearson

FIGURE 1. **Typical Bioanalyzer traces for cDNA generated by the Ovation PicoSL WTA System V2.**

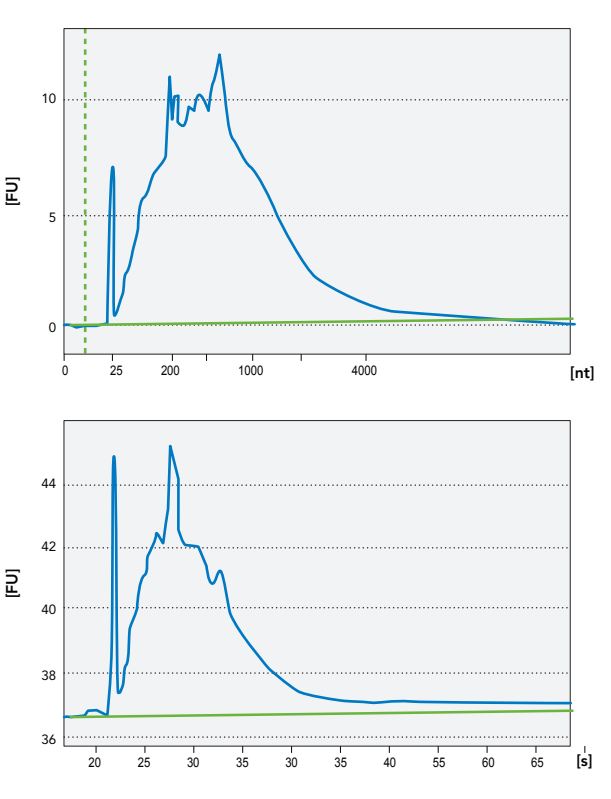
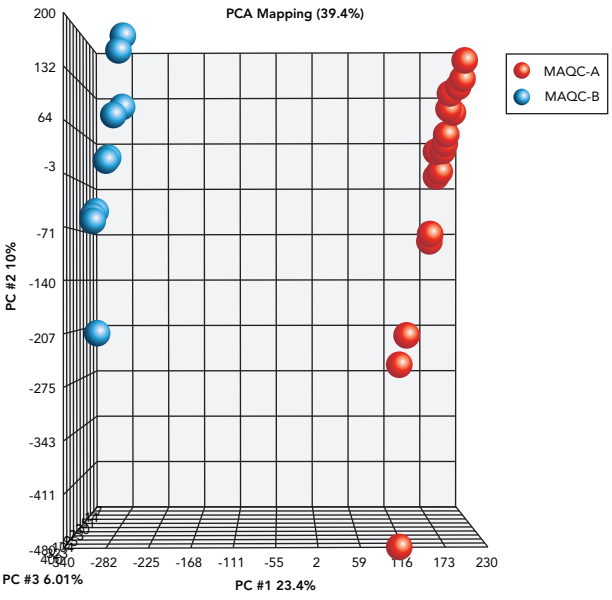


FIGURE 2. **Principal Component Analysis (PCA) based on probe signal intensities.**



correlation coefficient for each group of replicates. Although these correlation coefficients are high, it is evident that increasing amounts of total RNA are associated with reduced noise in the data, which further improves the correlation among replicates.

Data Validation

The MAQC reference samples used in these experiments are well characterized. A list of 882 differentially expressed genes, which have been determined by qPCR, is publicly available[1]. These qPCR data are based on analysis of sample RNA without any prior modification. In order to validate the array data obtained in this experiment, it was compared to the published qPCR data.

For the array data, a t-test was done on the 20 ng input groups and fold changes of differentially expressed genes were calculated. **Figure 4** shows a plot of log-2 transformed qPCR results versus fold-changes from the array data for genes determined to be differentially expressed with a p-value of <0.01. qPCR data are available for 352 genes that were found differentially expressed at this p-value cutoff, and the Pearson correlation coefficient is 0.919. Significantly, only 4 genes are discordant between the two analyses. **Table 3** shows the results of varying the p-value cutoff. With increasing stringency, the correlation between technologies increases.

Conclusion

This report describes the performance of the Ovation PicoSL WTA System V2 on Gene 1.1 ST Array Plates processed on the Affymetrix GeneTitan instrument. Similar performance was observed using the Affymetrix GeneAtlas instrument and using cartridge based GeneChip Gene 1.0 ST Arrays. The entire workflow from total RNA to a hybridization cocktail ready for array hybridization was completed within a single day. The assay is fully automatable and

FIGURE 3. **Pearson correlation coefficient matrix based on signal intensities. Boxes show the high degree of concordance among replicate samples at the same input level.**

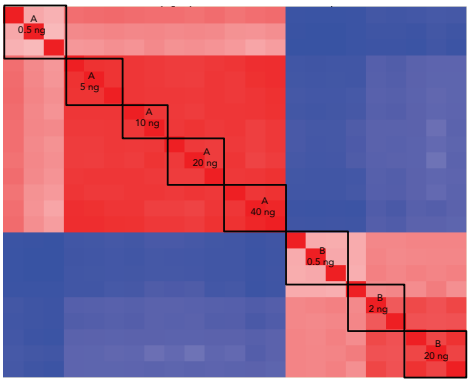
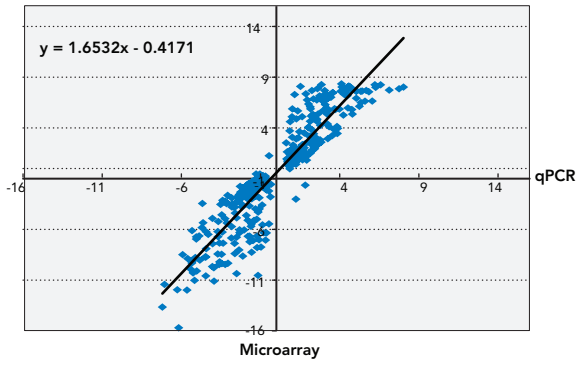


TABLE 2. **Minimal Pearson correlation coefficients within groups of replicates.**

Sample	Input [ng]	CC
MAQC-A	0.5	0.939
	5	0.985
	10	0.984
	20	0.982
	40	0.984
MAQC-B	0.5	0.943
	2	0.955
	20	0.978

FIGURE 4. **Concordance plot of 352 pre-filtered, differentially expressed genes.**



Filtering for a p-value ≤ 0.01 leaves 352 genes to be plotted.

can easily be run in high throughput format, if desired.

The increasing number of Affymetrix arrays available in array plate and array strip format offers a great opportunity for the research community to study a wide range of biological questions. As researchers use such technologies as laser capture microdissection or flow sorting it is imperative to be able to access these small tissue samples to answer increasingly specific biological questions. The Ovation PicoSL WTA System V2 offers an efficient, cost effective sample preparation system enabling analysis of valuable specimens where limited material is available.

References

1 Shi, L et al. the MicroArray Quality Control (MAQC) project shows inter- and intra-platform reproducibility of gene expression measurements. *Nature Biotechnology.* 2006 Sep;24(9): 1151–1161.

TABLE 3. **Concordance analysis of q-PCR data versus array data.**

Significance (p-value)	# Genes Plotted	# Genes Discordant	Pearson CC
None	882	126	0.860
0.01	352	4	0.919
0.001	165	0	0.943
0.0001	68	0	0.953

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M01261 v1.3