

GeneChip[®] HT 3' IVT Express Kit

Abstract

The GeneChip[®] HT 3' IVT Express Kit represents the newest high-throughput target generation assay for 3'-based arrays. The kit includes the same reagent formulations as the manual 3' IVT Express Kit and provides the same performance and ease-of-use enhancements.

This configuration of the 3' IVT Express Kit is optimized for use with liquid-handling robotics and generates high-quality, array-ready target and requires minimal manual intervention. In this white paper, we describe the technical performance of the HT 3' IVT Express Kit in conjunction with automation scripts written for the GeneChip[®] Array Station (GCAS).

Introduction

The HT 3' IVT Express Kit is available in two convenient configurations: 1 x 96 reactions and 4 x 24 reactions (Table 1). The reagents can be loaded onto a robotic liquid-handling instrument and target can be generated with minimal intervention. We have also developed an automation script for GCAS that can run 24 or 96 samples at a time.

Table 1: The 3' IVT Express Kit is available in several convenient configurations.

Format	Part number	Configuration
Manual	901228	10 reactions
	901229	30 reactions
HT	901225	4 x 24 reactions
	901253	1 x 96 reactions

The HT 3' IVT Express Kit uses the same reagent formulations as the 10- and 30-reaction kits. We compared target generated manually to target prepared on GCAS and found very high average probe set and fold change correlations. We also examined the reproducibility of target made within the 96-well plate of a single run, between two runs on the same GCAS instrument, and between two runs on different GCAS instruments. In all cases, the resulting data from GeneChip[®] HG-U133 Plus 2.0 Arrays (in cartridge format) was highly reproducible. In addition, we compared the current HT One-Cycle Target Labeling Kit to the HT 3' IVT Express Kit and found very comparable array results.

Materials and methods

Target preparation

To prepare target, 50 ng of total MAQC A and B RNA was combined with poly-A RNA controls, diluted to a final volume of 5 μ L, and dispensed into a 96-well plate. 100 ng of total RNA was used for the tissue panel experiment. GCAS was used to generate target using the TP_0005 script and the 24- or 96-sample workflow. All IVT reactions were incubated for 16 hours. After purification, 2 μ L of the aRNA was diluted into 198 μ L and the UV absorbance was measured on a spectrophotometer to determine the aRNA concentration. The aRNA samples were then normalized to a single target concentration before fragmentation.

Hybridization, wash, stain, and scanning

For each sample (see Table 2), 250 μ L of hybridization cocktail was created manually using fragmented

aRNA in conjunction with the GeneChip[®] Hybridization, Wash, and Stain Kit. 200 μ L of the hybridization cocktail were manually added to HG-U133 Plus 2.0 Arrays and hybridized overnight for 16 hours. Following hybridization, the arrays were washed and stained on a GeneChip[®] Fluidics Station 450 using reagents from the Hybridization, Wash, and Stain Kit. Washed and stained arrays were scanned using a GeneChip[®] Scanner 3000.

Table 2: RNA samples used in this study.

Total RNA	Description	Vendor	Part No.
MAQC A	Universal Human Reference RNA	Stratagene	740000
MAQC B	Human Brain Reference RNA	Ambion	6050

Data processing and analysis

CEL files were processed in Expression Console[™] Software using robust multi-array analysis (RMA) for probe set summarization. Average probe set signal values are calculated from the replicate samples ($n = 3$). Pearson correlation coefficients (R) of probe set values and fold change were calculated using custom scripts.

Results

Average aRNA yield

Target was generated from 50 ng of total RNA input of the MAQC A and B samples on three GCAS instruments. One run each of 24 and 96 samples was carried out on each instrument. The MAQC A and B samples were distributed across the plate with 6 technical replicates each in the 24-sample run and 24 technical replicates in the 96-sample run. Average total aRNA yield is shown in Figure 1. The error bars represent one standard deviation from the mean. In addition, the average coefficient of variation (CV) for the yield was calculated across the three instruments. The results show consistent aRNA yields with minimal well-to-well variation.

Reproducibility

Target generated by each of the GCAS instruments was hybridized to HG-U133 Plus 2.0 Arrays. The average fold change (MAQC A/B) values were calculated using RMA for probe set summarization. Figure 2 shows highly reproducible results across sample workflows and across GCAS instruments. In Panel A, the average fold change value is plotted for the two runs (24- and 96-sample workflows) on a single GCAS instrument. Panel B shows the correlation of fold change between two GCAS instruments. Finally, in Panel C, the average fold change is taken from a single 96-sample run, and the correlation is shown for samples on the extreme right side of the 96-well plate compared to samples from the left side. In all cases, the average fold change correlation is greater than or equal to 0.99.

Figure 1: Average total aRNA yield.

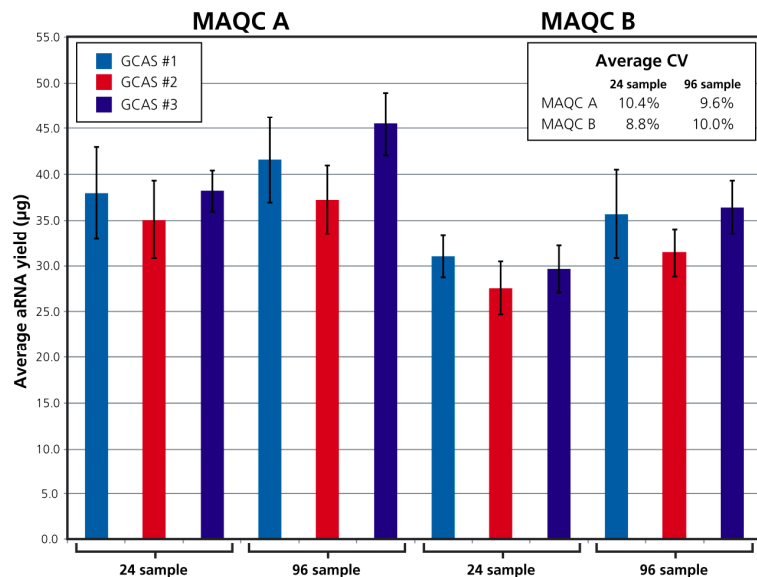
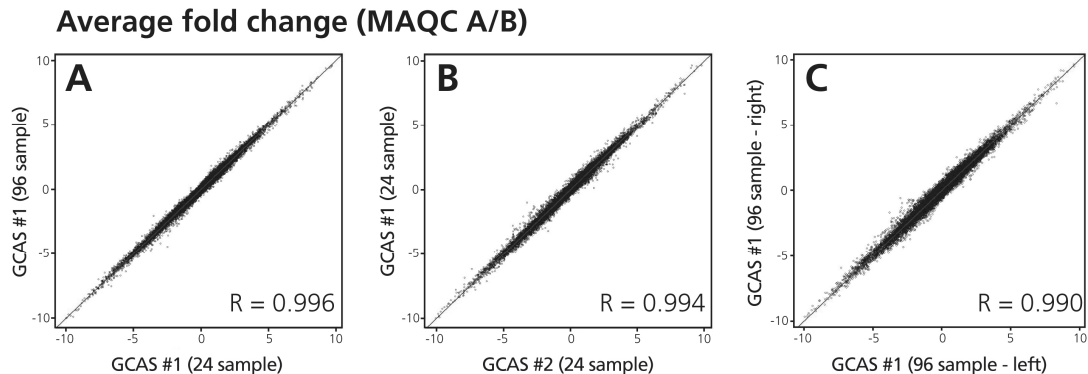


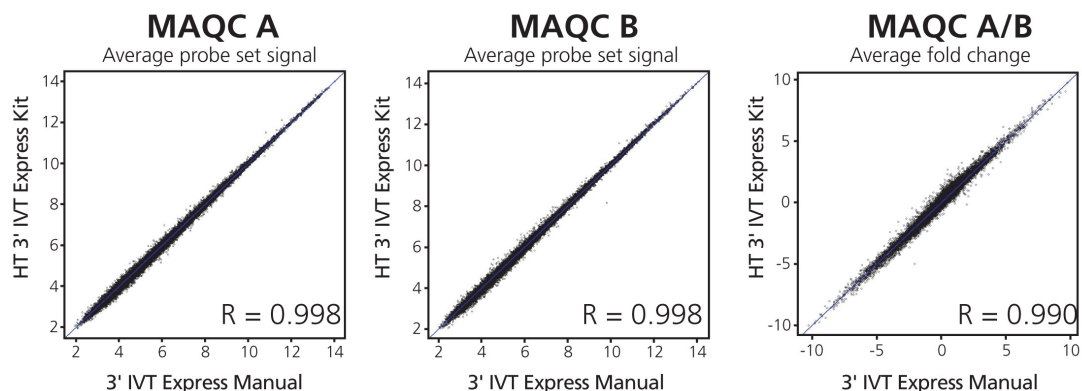
Figure 2:
Fold change correlation across or within GCAS runs.



Comparison to manual 3' IVT Express Kit

To compare the performance of manually generated target versus target prepared using automated methods, we selected three technical replicates each of MAQC A and B target prepared either manually or on the GCAS instrument (96-sample workflow). The target was hybridized to HG-U133 Plus 2.0 Arrays. Figure 3 shows the scatter plots of MAQC A and B average probe set signal, as well as average fold change (MAQC A/B) values. The raw signal and fold change demonstrate a high correlation to array data from manually and GCAS prepared target.

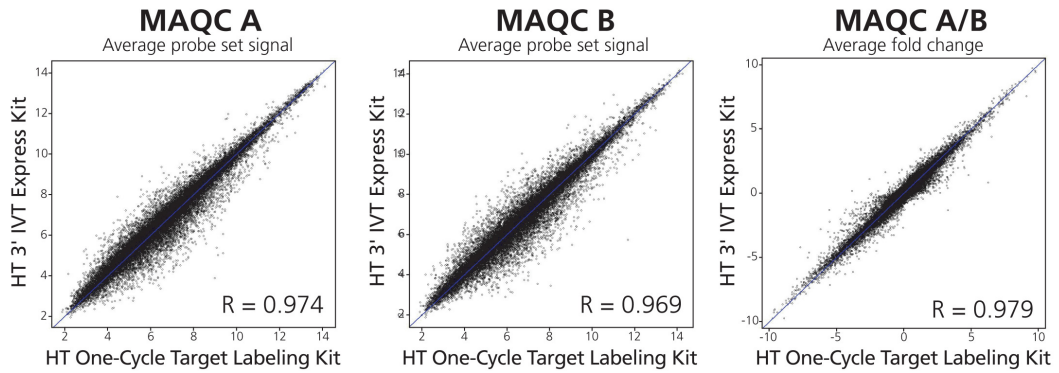
Figure 3:
Correlation of manual and automated target preparations.



Comparison to HT One-Cycle Target Labeling Kit

Target generated by the HT 3' IVT Express Kit was also compared to target generated by the HT One-Cycle Target Labeling Kit. Target was prepared from the MAQC A and B total RNA samples on two GCAS instruments using the respective 24-sample workflow automation scripts written for each of the kits. 50 ng of total RNA was used as input for the HT 3' IVT Express Kit and 1 μ g was used for the HT One-Cycle Target Labeling Kit. Fragmented and labeled target was hybridized on the same day to HG-U133 Plus 2.0 Arrays. Figure 4 shows scatter plots of the average probe set signal for both the MAQC A and B samples, as well as average fold change (MAQC A/B). The high Pearson correlation coefficients (R) demonstrate that the two kits produce highly comparable array results.

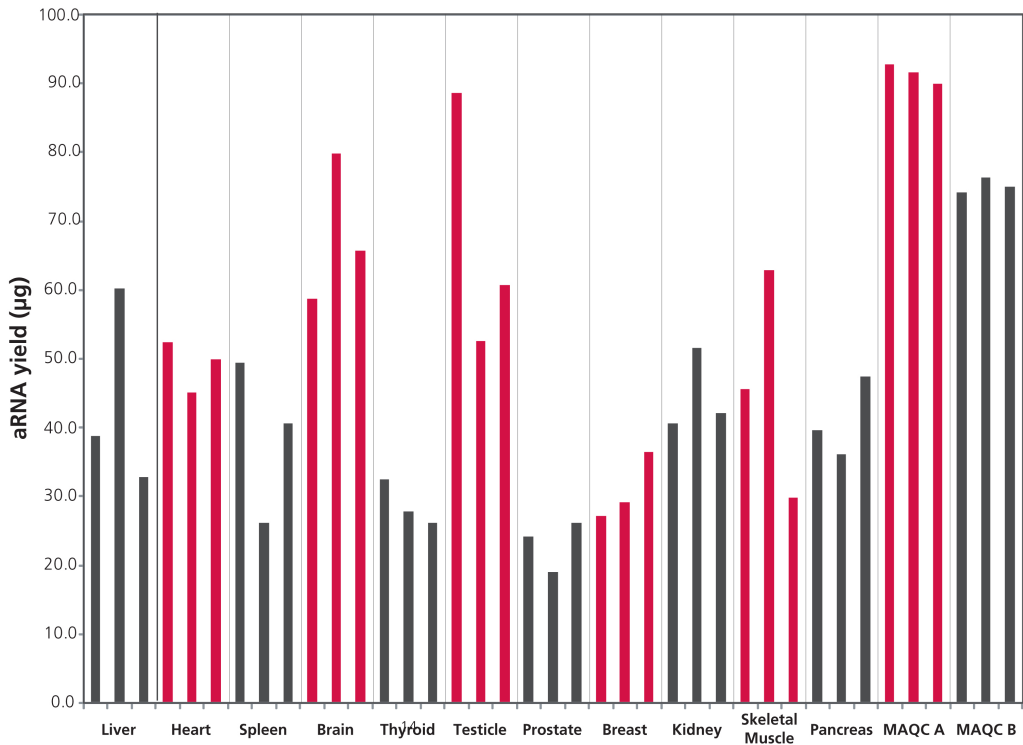
Figure 4: Correlation of HT 3' IVT Express and HT One-Cycle Target Labeling Kit preparations.



Tissue panel

To demonstrate the ability of the HT 3' IVT Express Kit to function across a broad range of total RNA sources, we generated target from total RNA samples of a diverse set of human tissues. Three biological replicate samples from 11 human tissues (plus MAQC A and B) were used in this study. For each sample, 100 ng of total RNA was used as input. Target was generated from all samples in a single 96-sample run on GCAS. The average aRNA yield for each sample is shown in Figure 5. With the exception of the MAQC A and B samples, each bar represents a different total RNA sample (biological replicates). Because quality can vary from sample to sample, it is not unexpected to see variability in the average aRNA yields within a single tissue. The HT 3' IVT Express Kit was able to generate sufficient aRNA quantities for hybridization from each of the samples profiled in this experiment.

Figure 5: Average aRNA yield using 100 ng input across a variety of human tissue samples.



Discussion

The HT 3' IVT Express Kit represent the newest technology in high-throughput, automated RNA target preparation. Target generated on a liquid-handling robot such as GCAS can be hybridized to 3' expression arrays in the cartridge or plate format. The kit comes in two convenient configurations—1 x 96 or 4 x 24 reaction formats—and the scripts written for GCAS allow for robust target preparation with minimal human intervention.

Using the HT 3' IVT Express Kit, GCAS produces cRNA yields that are consistent and comparable to manual processing. The reproducibility within a single run, across runs on a single instrument, and between two GCAS instruments is excellent, with fold change/Pearson correlation coefficients of greater than or equal to 0.90. Array data shows equivalent performance of target generated in a manual or automated fashion. And the new HT 3' IVT Express Kits produce data that is similar to that of the previous-generation HT One-Cycle Target Labeling Kit.

The HT 3' IVT Express Kit is also compatible with other automation providers. Please visit www.affymetrix.com to learn more.