AFFYMETRIX®

Technical Note

Comparison between the GeneChip® 3′ IVT Express Kit and GeneChip® One-Cycle Target Labeling and Control Reagents

The GeneChip® 3′ IVT Express Kit is the latest technology in RNA target preparation for Affymetrix microarray experiments. This Technical Note shows that the 3′ IVT Express Kit demonstrates the same level of performance as the GeneChip® One-Cycle Target Labeling and Control Reagents.

Using only 50 ng of input total RNA, the 3′ IVT Express Kit produced comparable aRNA yield and present calls as the One-Cycle Target Labeling and Control Reagents using 1 µg of input total RNA. Average signal correlation between the two labeling methods was greater than or equal to 0.97 and the correlation of fold change between two diverse RNA samples was 0.98.

GeneChip One-Cycle and Two-Cycle reagents will be discontinued September 1, 2009.

Introduction

The GeneChip® 3' IVT Express Kit is Affymetrix' next generation of expression reagents. Using a streamlined protocol, you can use as little as 50 ng starting material for a single round of amplification and decrease target labeling time to a single day with appropriate total RNA inputs. The kit includes all necessary labeling and control reagents for 3' expression studies.

This Technical Note compares the performance of the 3' IVT Express Kit and GeneChip® One-Cycle Target Labeling and Control Reagents and shows that the two kits demonstrate the same level of performance. To avoid potential impact on array data, however, Affymetrix recommends against switching reagent kits mid-study.

Overview

Targets were prepared from Microarray Quality Control (MAQC) A and B total RNA (see Table 1) using the One-Cycle Target Labeling and Control Reagents or the 3' IVT Express Kit. Total RNA input amounts and IVT incubation times are outlined in Table 2. Following purification, aRNA (cRNA) yield was calculated based on concentration measurements from a spectrophotometer. Average aRNA yield is shown in Figure 1.

Labeled target was hybridized to the GeneChip® HG-U133 Plus 2.0 Array. CEL files were quantile normalized in Expression Console™ using the RMA algorithm for probe set summarization. The MAS5.0 algorithm was used to calculate percent

Table 1: Total RNA samples used.

RNA	Description	Vendor	P/N
MAQC A	Universal Human Reference RNA	Stratagene	740000
MAQC B	Human Brain Reference RNA	Ambion	6050

Table 2: Sample preparation conditions.

Product	RNA	Input amt.	IVT incubation time	Replicates
One-Cycle Reagents	MAQC A MAQC B	1 µg	16 hours	3 3
3' IVT Express Kit	MAQC A MAQC B	50 ng	16 hours	3 3
3' IVT Express Kit	MAQC A MAQC B	500 ng	4 hours	3 3

present (%P). Data from 50 ng input prepared with the 3' IVT Express Kit was compared to data from 1 µg input prepared with One-Cycle Reagents (Figures 2 and 3).

Conclusion

Customers currently using One-Cycle Target Labeling and Control Reagents can expect the same level of performance from the 3' IVT Express Kit. Although the kits are comparable in performance (all Pearson Coefficients ≥ 0.97) Affymetrix recommends against switching reagents mid-study to avoid potential impact on array data.

Figure 1: Average aRNA yield and percent present (%P).
Total aRNA yield (in µg) for each sample was calculated by multiplying the measured aRNA concentration by the elution volume. The average total yield is displayed in the bar graph. Percent present calls (%P) were calculated for each sample using the MAS5.0 algorithm in Affymetrix® Expression Console™. The average %P is displayed as diamonds. For all data, light teal represents One-Cycle Reagents and blue represents the 3' IVT Express Kit.

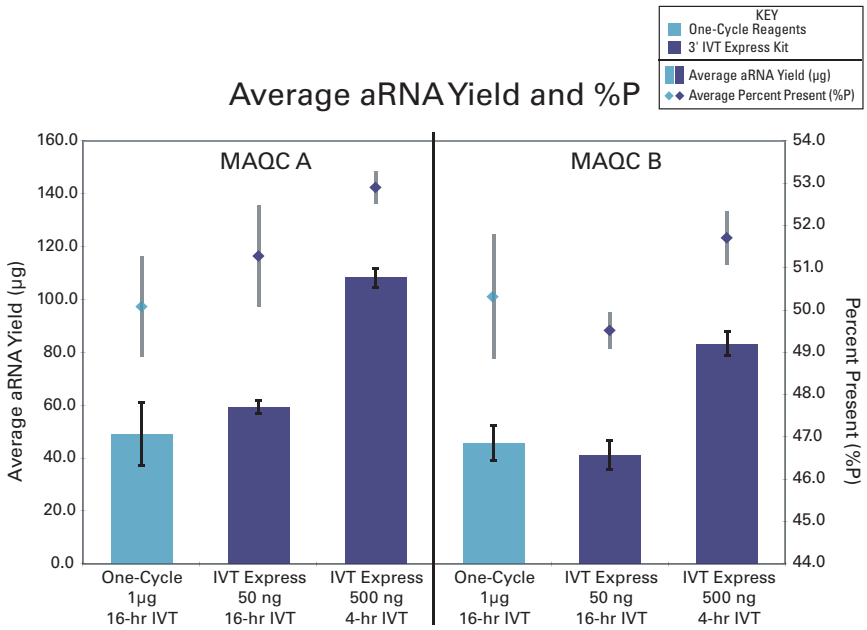


Figure 2: Signal intensity correlation.
Scatter plots of average RMA summarized probe set intensities comparing 50 ng input from 3' IVT Express Kit to 1 µg input from One-Cycle Reagents. The Pearson Correlation Coefficient (R) is calculated for each comparison.

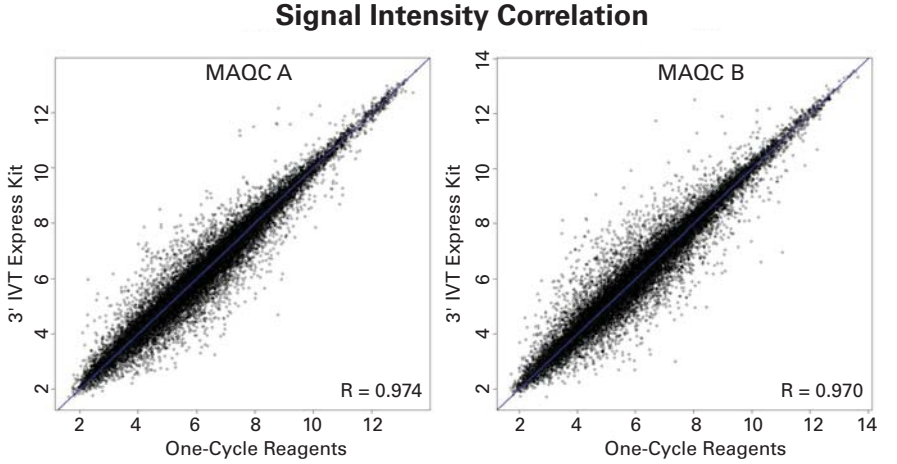
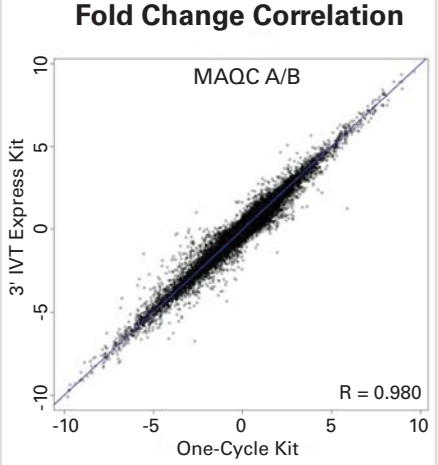


Figure 3: MAQC A/B fold change correlation.
Scatter plots of average fold change values [log(MAQC A/B)] comparing 50 ng input from 3' IVT Express Kit to 1 µg input from One-Cycle Reagents. The Pearson Correlation Coefficient (R) is calculated for the comparison.



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