TechnicalNote

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GeneChip® 3′ IVT PLUS Reagent Kit (3′ IVT PLUS Kit) vs. GeneChip® 3′ IVT Express Reagent Kit (3′ IVT Express Kit) data comparison

Introduction

Affymetrix has recently launched **GeneChip® 3′ IVT PLUS Reagent Kit** (3′ IVT PLUS Kit), which is replacing **GeneChip® 3′ IVT Express Reagent Kit** (3′ IVT Express Kit).

Similar to 3′ IVT Express Kit, the new 3′ IVT PLUS Kit creates hybridization-ready target from total RNA samples for gene expression profiling analysis optimized for GeneChip® 3′ Expression Arrays. 3′ IVT PLUS Kit is designed for sample types such as fresh or frozen tissues, cell lines, and globin-reduced whole blood. It generates amplified and biotinylated complementary RNA (cRNA, also known as aRNA) from poly(A) RNA in a total RNA sample. RNA amplification is based upon linear amplification and employs T7 *in vitro* transcription (IVT) technology. 3′ IVT PLUS Kit does not require any up-front removal of ribosomal RNA due to the oligo-d(T) priming strategy, which primes the poly(A) tail, similar to 3′ IVT Express Kit. 3′ IVT PLUS Kit is comprised of reagents and a protocol for preparing hybridization-ready targets from 50 to 500 ng of total input RNA.

The experimental data presented herein is a comparison of 3′ IVT PLUS Kit (P/N 902416) to the existing 3′ IVT Express Kit (P/N 901229). We show performance concordance for cRNA yields, technical reproducibility, signal correlation, and fold change correlation using the most widely used GeneChip® Human Genome U133 (HG-U133) Plus 2.0 Arrays from Affymetrix.

Experimental methods, design, and results

Using 3′ IVT Express Kit and 3′ IVT PLUS Kit, the hybridization targets were prepared from the commercial total RNA samples listed in Table 1 with the manufacturer's recommended protocol.

Figure 1: Bioanalyzer image from different fresh frozen tissues and cell lines with their corresponding RINs.

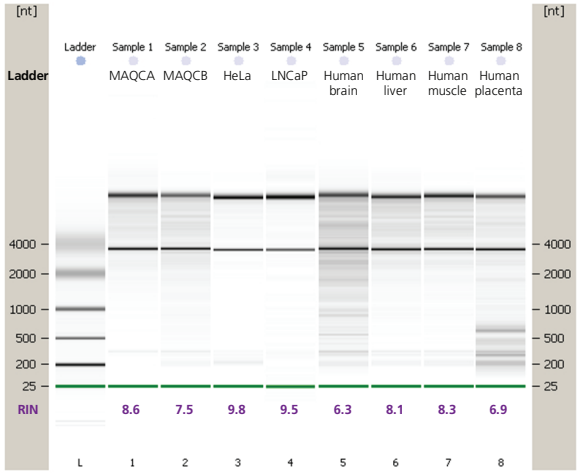
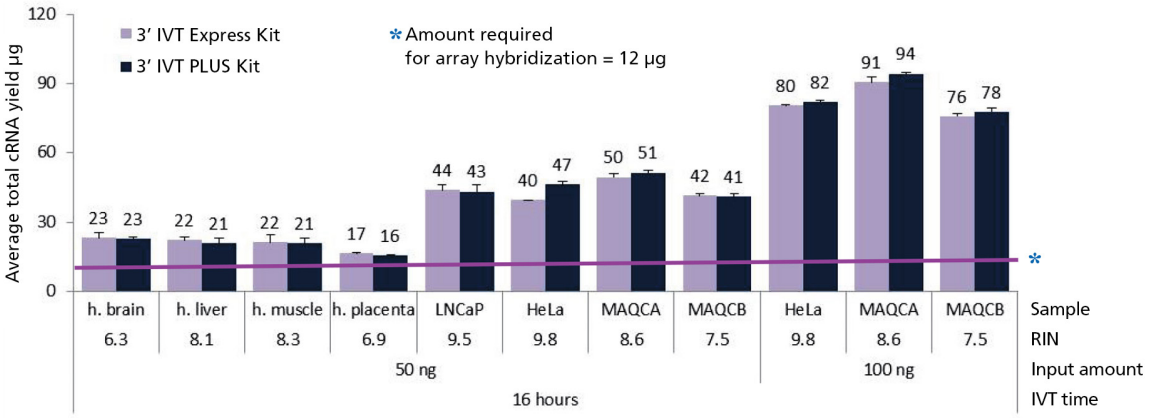


Figure 1 shows the Agilent® Bioanalyzer profiles and RNA Integrity Numbers (RIN) for each of the samples. The minimum input mass amount of 50 ng of total RNA and technical triplicates assays and arrays were performed. Following purification, cRNA yields were calculated based on concentration measurements from a NanoDrop spectrophotometer, and cRNA size distributions were analyzed using an Agilent 2100 Bioanalyzer RNA 6000 Nano Kit. HG-U133 Arrays were hybridized with 50 ng/µl labeled targets per recommendations from both protocols. The resulting CEL files were quantile-normalized as a group in Affymetrix' Expression Console™ Software 3.1 using the RMA algorithm for probe set summarization. The MAS 5.0 algorithm was used to calculate percent present (% P).

Table 1: Total RNA samples used in this study.

RNA	Description	Vendor	Part number
MAQC A	Universal human reference RNA	Agilent	740000
MAQC B	Human brain reference RNA	Life Technologies	AM6050
HeLa	HeLa cell line total RNA	Life Technologies	AM7852
LNCaP	LNCaP cell line total RNA	Jinfiniti Biosciences	LNCaP
h. brain	Human brain total RNA	Life Technologies	AM7962
h. liver	Human liver total RNA	Life Technologies	AM7960
h. muscle	Human muscle total RNA	Life Technologies	AM7982
h. placenta	Human placenta total RNA	Life Technologies	AM7950

Figure 2: cRNA yields. The labeled cRNA yield generated by 3' IVT PLUS Kit and 3' IVT Express Kit for different fresh frozen tissues and cell lines with the corresponding RIN values. Please note that only 12 µg of total RNA is required for the array hybridization step.



As shown in Figure 2, both kits generated similar cRNA yields from the 50 ng and 100 ng input amount of total RNA, which was sufficient for the 49-format array hybridization threshold for all samples. As seen in Table 2, the average technical reproducibility from the triplicates was greater than 0.97, and % median coefficient of variation (% CV) was less than 10%, which indicates excellent precision in the data. In addition, % Present (% P) differed by less than 1% between kits, which indicates similar detection sensitivity. Signal correlations plots for all probe sets are shown in Figure 3. The signal correlations for all samples were greater than 0.97, which indicates excellent agreement between the two assays.

Table 2: Technical reproducibility (replicate signal correlation), % P, and % CV.

	Pairwise replicate signal correlation (average ± stdev)		% P (average ± stdev)		% CV	
	3' IVT PLUS Kit	3' IVT Express Kit	3' IVT PLUS Kit	3' IVT Express Kit	3' IVT PLUS Kit	3' IVT Express Kit
MAQC A	0.985 ± 0.001	0.984 ± 0.000	52.1 ± 0.7	50.9 ± 0.4	6.43%	7.43%
MAQC B	0.984 ± 0.002	0.982 ± 0.001	51.2 ± 0.5	49.8 ± 0.5	7.29%	7.99%
HeLa	0.982 ± 0.004	0.988 ± 0.000	45.5 ± 0.2	45.4 ± 1.5	4.37%	5.95%
LNCaP	0.983 ± 0.001	0.986 ± 0.000	47.7 ± 0.8	47.2 ± 1.1	6.34%	7.37%
h. brain	0.981 ± 0.000	0.980 ± 0.002	49.9 ± 1.6	49.7 ± 1.4	8.55%	7.60%
h. liver	0.978 ± 0.002	0.984 ± 0.000	40.3 ± 0.7	39.0 ± 1.7	6.41%	8.51%
h. muscle	0.981 ± 0.003	0.983 ± 0.001	38.8 ± 0.2	38.8 ± 1.0	6.37%	8.36%
h. placenta	0.985 ± 0.001	0.986 ± 0.001	47.7 ± 0.8	47.2 ± 1.1	7.44%	7.02%

Correlation, % P, and % CV for probe set signal is calculated from N = 3 samples and all 58,675 probe sets.

Figure 3: Signal intensity correlation of all 58,675 probe sets. Each scatter plot shows the average RMA summarized probe set signal intensities (log_2), comparing the 50 ng input of 3' IVT PLUS Kit (y axis) to 3' IVT Express Kit (x axis). The signal correlations for all samples were greater than 97%, which indicates excellent agreement between the two assays.

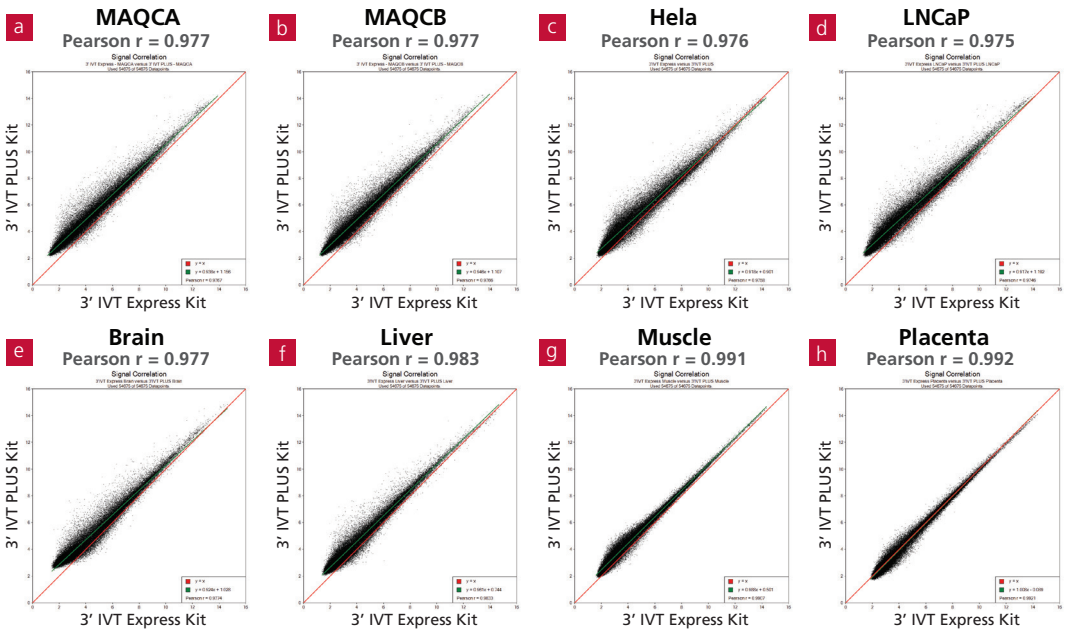
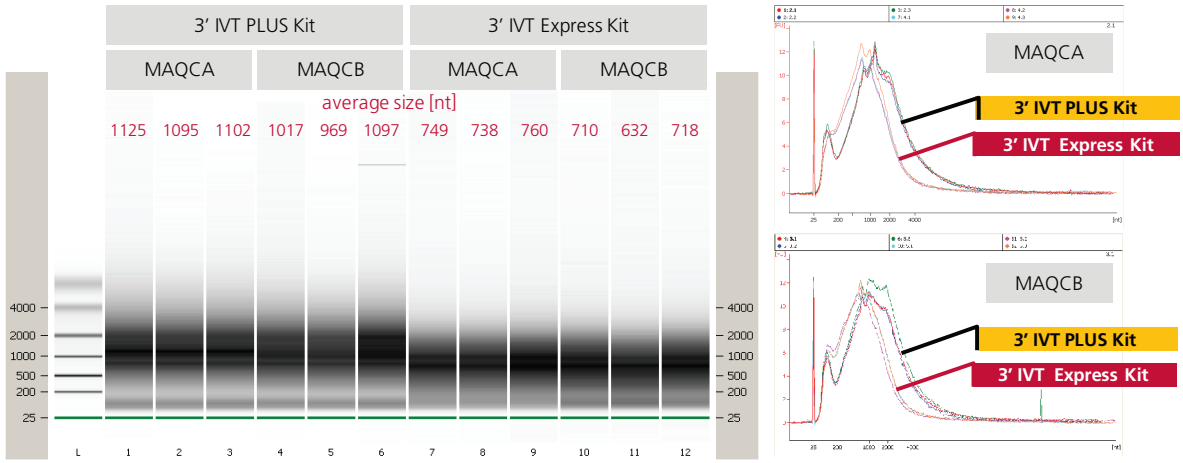


Figure 4: Assay-derived cRNA profiles from MAQC A and B samples. Average size nucleotide [nt] was determined from 50% of the total area with region start size at 60 nt.



Since MAQC A and B samples represent a wide range of both signal and fold change differences, we show a more in-depth look at the data. As seen in Figure 4, cRNA lengths from the new 3' IVT PLUS Kit were slightly longer than 3' IVT Express Kit. This gives confidence in the data integrity since 3' IVT arrays have probe sets which are, by design, closer to the 3'-end of transcripts.

Table 3: Expression Console™ Software analysis summary.

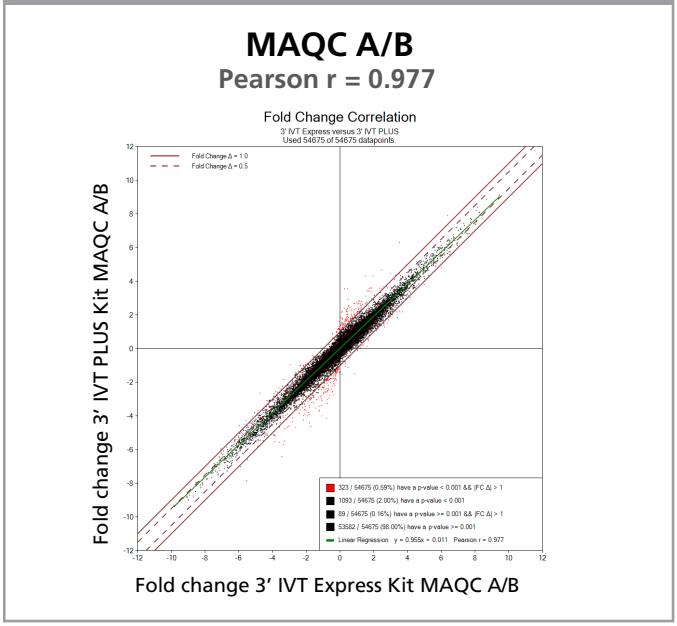
Kit	3′ IVT PLUS Kit		3′ IVT Express Kit	
RNA input	50 ng MAQCA	50 ng MAQCB	50 ng MAQCA	50 ng MAQCB
	average ± stdev	average ± stdev	average ± stdev	average ± stdev
% median CV	6.43%	7.29%	7.43%	7.99%
% Present (MAS 5.0)	52.10 ± 0.73	51.19 ± 0.45	50.91 ± 0.43	49.76 ± 0.50
Actin 3′/5′ ratio	1.30 ± 0.03	1.98 ± 0.08	6.41 ± 0.53	15.42 ± 0.70
GAPDH 3′/5′ ratio	0.97 ± 0.02	1.10 ± 0.02	1.44 ± 0.06	2.11 ± 0.08
Poly-A 3′ detection	all "P"	all "P"	all "P"	all "P"
Poly-A ratio r^2*	0.994	0.999	0.998	0.993

*3′ signal intensities vs. relative ratio for each of the poly-A controls

A summary of the metrics derived from Expression Console Software analysis is shown in Table 3. Overall, % P and % CV metrics were clearly comparable between the two assays, while the Actin 3′/5′ bias ratio is higher for 3′ IVT Express Kit, in agreement with the Agilent Bioanalyzer profiles.

Figure 5 shows the scatter plot of average fold change values [log(MAQC A/B)], comparing the 50 ng input of 3′ IVT PLUS Kit (y axis) to 50 ng input of 3′ IVT Express Kit (x axis). The Pearson correlation coefficient (r) calculated using all probe sets without filtering is 97.7%. The fold change discordance (significant p-value <0.001 and fold change >1) is 0.59%. This demonstrates that the absolute signal intensities from both assays are in good agreement (Figure 3), as well as the ability to detect meaningful gene-level differences between two samples remains very similar.

Figure 5: MAQC A/B fold change correlation.



Conclusion:

All of the data presented above clearly demonstrates that 3′ IVT Express Kit and 3′ IVT PLUS Kit are very similar, and current 3′ IVT Express Kit users can expect very concordant performance from the new 3′ IVT PLUS Kit on a wide variety of important array specification metrics. From an empirical perspective, both the kits have an extremely similar assay workflow. This allows a user to seamlessly switch from 3′ IVT Express Kit to 3′ IVT PLUS Reagent Kit.

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P/N EMI03428 Rev. 1