Ambion® WT Expression Kit

Improved Target Amplification

for Affymetrix® GeneChip® Human, Mouse, and Rat Exon and Gene 1.0 ST Arrays

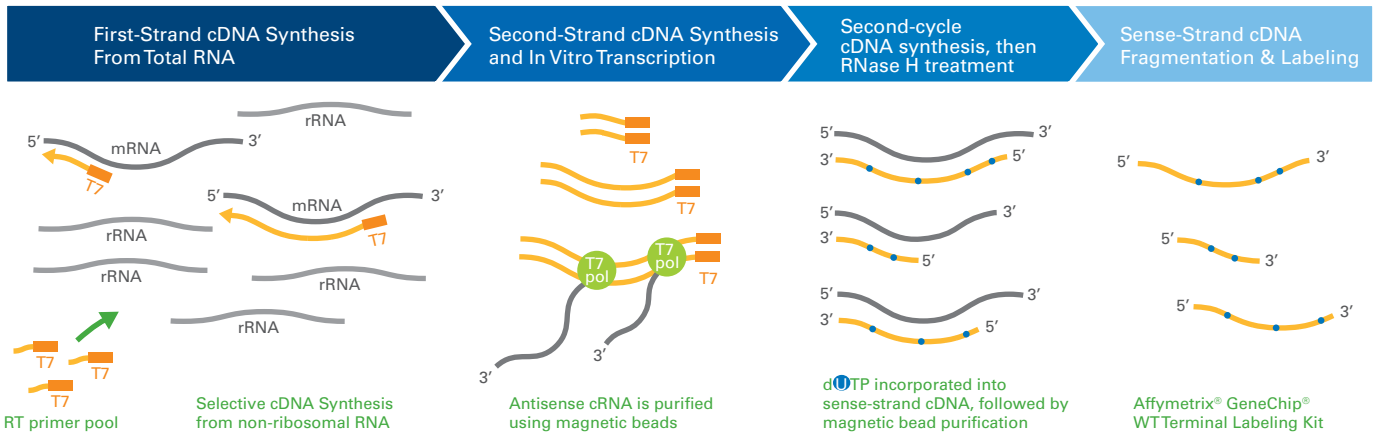

Figure 1. Ambion® WT Expression Kit Procedure Overview. The streamlined protocol requires as little as 50 ng sample input and selectively reverse-transcribes non-ribosomal RNA for complete and unbiased coverage of the transciptome. The procedure is compatible wth Affymetrix® GeneChip® Terminal Labeling Kit.

- Amplify total RNA directly—without a separate rRNA depletion step
- Start with as little as 50 ng of RNA
- Results show high correlation with those from Affymetrix® GeneChip® WT cDNA Synthesis and Amplification Kit
- Pre-formulated Master Mixes significantly reduce hands-on time
- Recommended by Affymetrix® for use with GeneChip® Human, Mouse, and Rat Exon and Gene 1.0 ST Arrays

Novel Priming Method: Eliminate rRNA Depletion

The WT Expression Kit is the latest RNA amplification innovation from Ambion. It enables faster, more sensitive, and highly reproducible results with Affymetrix® whole transcriptome microarrays. The kit is designed to generate amplified sense-strand cDNA ready for fragmentation and labeling using the Affymetrix® GeneChip® WT Terminal Labeling Kit (Figure 1). The Ambion® WT Expression Kit has been optimized specifically for use with Affymetrix® GeneChip® Human, Mouse, and Rat 1.0 ST Arrays, where "ST" stands for sense target, and the probes on the arrays are distributed throughout the entire length of each transcript.

The first-generation Affymetrix® amplification method requires depletion of ribosomal RNA (rRNA) from RNA samples for optimal exon-level analysis. Conversely, the Ambion® WT Expression Kit employs a novel, patent-pending, reverse transcription (RT) priming method that eliminates the need for a separate rRNA depletion step. The kit includes RT primers designed with a proprietary oligonucleotide matching algorithm that eliminates primer sequences with homology to known ribosomal RNA sequences. The result is complete and unbiased coverage of the transcriptome, with a significant reduction in rRNA amplification, compared to other methodologies.

Consistent Results with Less Input RNA

With the Ambion® WT Expression Kit, samples as small as 50 ng of total RNA can be analyzed on Affymetrix® GeneChip® Human, Mouse, and Rat Exon and Gene 1.0 ST Arrays. Previous methods required 1 µg of total RNA—an amount that may be

difficult or impossible to obtain from limited samples such as stem cells or small tissue samples, or may require significant time and expense spent culturing cells. The ability to use smaller amounts of RNA enables analysis of precious samples and provides more efficient and cost-effective experimentation for most sample types.

To demonstrate the performance of the new Ambion® WT Expression Kit, RNA from three sample types (HeLa cells, and Microarray Quality Control (MAQC) A and B samples) was prepared in triplicate using either the Affymetrix® GeneChip® WT cDNA Synthesis and Amplification Kit or the Ambion® WT Expression Kit, and analyzed on Human Exon 1.0 ST Arrays. Total RNA (1 µg) prepared by the Affymetrix® protocol underwent an rRNA-depletion step while just 50 ng total RNA (20-fold less) was prepared for microarray analysis using the Ambion® WT Expression Kit. Both sets of samples were handled according to the manufacturers' recommendations.

As shown in Figures 2 and 3, samples prepared using the Ambion® WT Expression Kit resulted in expression measurements that were highly concordant with those obtained from the same starting samples prepared using the Affymetrix® method. Direct correlation of $\log_2$ ratios for MAQC samples A and B is remarkably high, ($r > 0.94$) at both the exon and transcript levels (Figure 2). Correlation of $\log_2$ ratios with gene expression data obtained using TaqMan® Gene Expression Assays for real-time PCR is also high for both kits ($r > 0.92$, Figure 3). This high correlation with real-time PCR results indicates that relative accuracy, as measured with an alternative gene expression platform, is very high for the new kit.

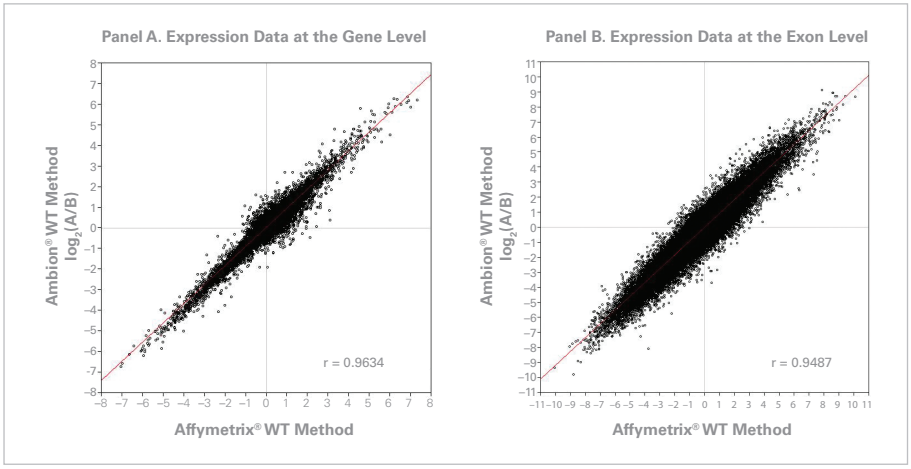

Figure 2. Equivalent Performance of Ambion® WT Expression Kit and Affymetrix® WT Sense Target Labeling Assay. The scatter plots compare the $\log_2$(A/B) ratios (as outlined by the MAQC guidelines [1]) for the Affymetrix® WT Sense Target Labeling Assay and the new Ambion® WT Expression Kit. Input RNA (1 µg before rRNA depletion for the Affymetrix® method, or 50 ng for the Ambion® method) was amplified according to each manufacturer's instructions. cDNA was then fragmented and labeled using the Affymetrix® GeneChip® WT Terminal Labeling Kit and hybridized to GeneChip® Exon and Gene 1.0 ST Arrays. **Panel A.** Correlation of $\log_2$ ratios for the core subset of genes found on the exon array (n = 21,980). **Panel B.** Correlation of ratios for the core subset of exons (n = 232,448). No filtering based on call data was applied. Correlation between the two methods is high at both the gene and exon levels, indicating high concordance.

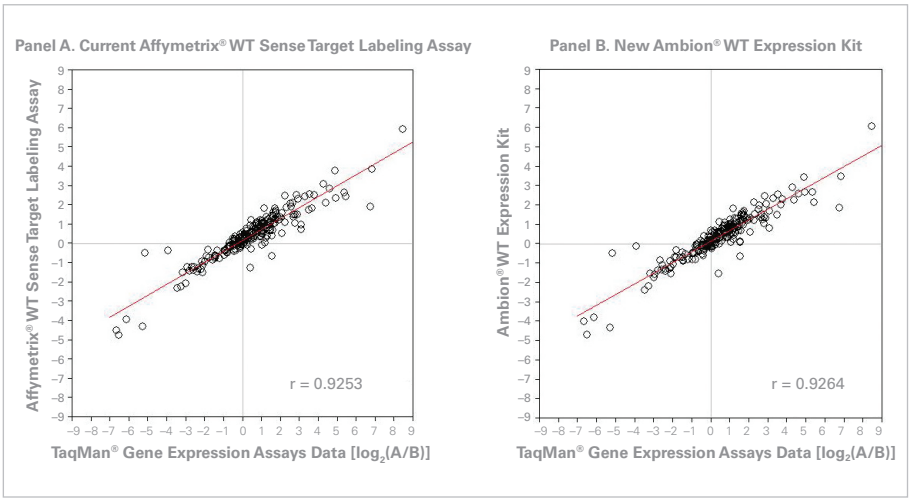

Figure 3. High Correlation Between Microarray and TaqMan® Real-Time PCR Data Using Ambion® and Affymetrix® Microarray Sample Preparation Kits. The scatter plots compare the $\log_2$(A/B) ratios (as outlined by the MAQC guidelines [1]) of microarray and TaqMan® real-time PCR data. Each point represents a gene that was called generally present by real-time PCR for sites 1–3 of the MAQC reference dataset, and that could be mapped to the exon array (n = 230). Input RNA (1 µg total RNA that was then processed to deplete rRNA for the Affymetrix kit, or 50 ng total RNA for the Ambion® kit) was amplified according to each manufacturer's instructions. cDNA was then fragmented and labeled using the Affymetrix® GeneChip® WT Terminal Labeling Kit, and hybridized to Affymetrix® GeneChip® Human 1.0 ST Arrays. Panels A and B compare the Affymetrix® and Ambion WT® protocols with the MAQC real-time PCR data, respectively. The correlation of data from array analysis with those from real-time PCR is very similar regardless of which kit was used to prepare samples for array analysis, indicating that the relative accuracy of results using the two methods is similar.

Sensitive Expression Profiling

In addition to the lower input RNA requirement and high concordance between the Affymetrix® method and TaqMan® real-time PCR data, the Ambion® WT Expression Kit provides a significant increase in sensitivity (Figure 4). A greater number of probe sets detected above background was obtained at the exon level with the Ambion® WT Expression Kit as a result of an increased signal-to-noise ratio. The signal-to-noise ratio was calculated by comparing the relative contribution of perfect-match signal with background probe signal.

Lastly, the increased sensitivity conferred by the new Ambion® WT Expression Kit was examined by comparing differential expression analysis results from the two methods. While differentially expressed genes and exons of MAQC A and MAQC B samples were similar between the two kits (78% and 89%, respectively, Figure 5), significantly more differential expression was observed on arrays hybridized with samples prepared using the Ambion® WT Expression Kit. As seen in Figure 5, 499 more genes and 6,850 more exons were found to be differentially expressed in samples prepared using the Ambion® kit, compared to samples prepared with the Affymetrix® kit. This increase in detection of differentially expressed exons and genes is likely due to increased sensitivity and reproducibility afforded with the Ambion® WT Expression Kit.

The Ambion® WT Expression Kit eliminates the need for rRNA depletion, delivering shortened processing time. Additionally, the selective priming method avoids amplification of targets with homology to known ribosomal RNA sequences, leading to increased signal-to-noise and probe set detection above background, higher array sensitivity, and increased detection of differential gene expression.

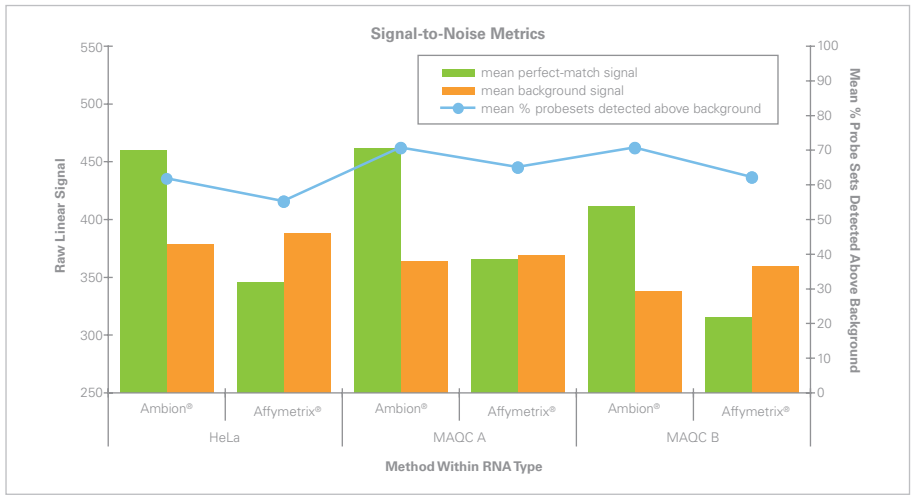

Figure 4. Comparison of Signal-to-Noise Metrics Between the Ambion® and Affymetrix® Kits. Probe sets detected above background (exon level) and raw signal values for both perfect-match and background probes are shown. The Ambion® WT Expression Kit shows significantly more probeset detection above background than the Affymetrix® kit. This increase appears to be due to increased perfect-match signal and relatively low background. The increase in sensitivity results in more genes and exons identified as differentially expressed (See Figure 5) and more reliable signal quantitation. Affymetrix® Expression Console™ Software v1.1 was used to calculate signal and probeset detection above background (n = 287,329 probesets) with the core content only.

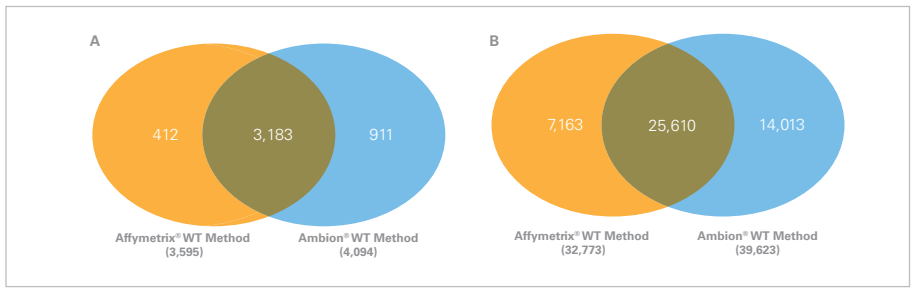

Figure 5. Concordance of Differential Expression Between Ambion® and Affymetrix® RNA Amplification Methods. Differentially expressed genes (Panel A) or exons (Panel B) between sample A and sample B were defined as having a fold change ≥|2| and p-value <.001. The Ambion® method encompasses 88.5% or 78.1% of the differential expression detected with the Affymetrix® method for genes or exons, respectively. At both the gene and exon levels, the Ambion® WT method detected more differentially expressed genes. This result is likely due to an overall increase in sensitivity and reproducibility with the Ambion® WT method.

ORDERING INFORMATION

Description	Size	P/N
Ambion® WT Expression Kit	10 rxns	4411973
Ambion® WT Expression Kit	30 rxns	4411974
Affymetrix® Products		
GeneChip® Human Exon 1.0 ST Array	30 arrays	900651
GeneChip® Human Gene 1.0 ST Array	30 arrays	901087
GeneChip® WT Terminal Labeling Kit	30 rxns	900671
GeneChip® Poly-A RNA Control Kit	100 rxns	900433
GeneChip® Hybridization Control Kit	30 rxns	900454

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NOTES

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