White Paper

Technical Performance of the Ambion® WT Expression Kit

Abstract

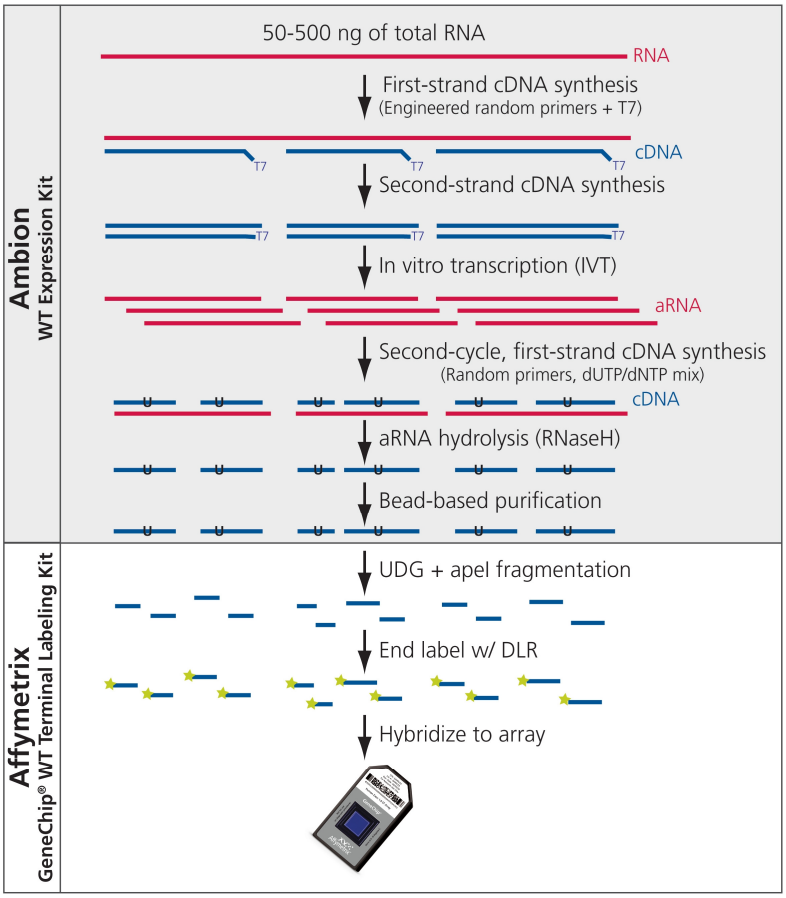
The Ambion® WT Expression Kit offers a simple and robust method for generating sense-stranded DNA target for hybridization to GeneChip® Exon and Gene 1.0 ST Arrays. The basic chemistry is the same as the Affymetrix® GeneChip® WT Sense Target Labeling and Control Reagents kit; however, the Ambion WT Expression Kit utilizes a streamlined protocol with master mixes, does not require an upfront rRNA-reduction step for optimal performance, and uses optimized reagents that enable lower total RNA input amounts. In this white paper, we present the technical performance of the Ambion WT Expression Kit and compare Exon 1.0 ST Array data generated using both kits.

Introduction

The Ambion WT Expression Kit uses a novel method for priming the reverse transcription step of the first-cycle, first-strand cDNA synthesis reaction. The kit employs an engineered set of primers that exclude sequences that match ribosomal RNA (rRNA). The result is a priming method that specifically primes non-ribosomal RNA from a total RNA sample (including poly-A and non-poly-A-containing mRNAs) and eliminates the need for an upfront rRNA reduction step for optimal exon-level performance. In addition, the enzymatic reactions have been optimized so lower total RNA input levels can be used to generate the same mass of target for hybridization to Exon and Gene 1.0 ST Arrays.

Figure 1 shows the workflow for the new WT assay. The Ambion WT Expression Kit generates purified sense-strand cDNA that has Uracil incorporated. That cDNA is then amenable to fragmentation and labeling using the Affymetrix WT Terminal Labeling Kit.

Figure 1: WT assay workflow.



Materials and Methods

aRNA and cDNA yield study

Five amounts of HeLa total RNA were used as input for the Ambion WT Expression Kit, with six technical replicates each. In addition, two input amounts were tested on the MAQC A and B samples, with three technical replicates each. In each case, the aRNA concentration was measured using a NanoDrop™ Spectrophotometer and the total aRNA yield was calculated by multiplying the concentration by the elution volume. 10 µg of aRNA was used as input for the second-cycle cDNA synthesis reaction. Following purification, the single-stranded cDNA yield was calculated using the same approach as the aRNA.

Assay comparison – MAQC A/B study

Table 1: RNA samples used.

Total RNA	Description	Vendor	Part No.
HeLa	Cervical Adenocarcinoma (HeLa-S3)	Ambion	7852
MAQC A	Universal Human Reference RNA	Stratagene	740000
MAQC B	Human Brain Reference RNA	Ambion	6050

Three technical replicate target preparations from each of the two RNA samples (MAQC A and B) were prepared using either the Ambion WT Expression Kit or the Affymetrix WT Sense Target Labeling Kit. For the Ambion kit, 50 ng of total RNA was used as input. For the Affymetrix kit, 1 µg of total RNA was processed through the RiboMinus™ rRNA reduction kit prior to first-cycle cDNA synthesis. Sense-strand cDNA generated by both kit types was fragmented and end-labeled using the Affymetrix WT Terminal Labeling Kit. In each case, 5.5 µg of single-stranded cDNA was fragmented and 5.0 µg of labeled target was hybridized to each array. Targets were generated separately, but hybridized as a group to GeneChip® Human Exon 1.0 ST Arrays in cartridge format.

Hybridization, washing, staining, and scanning

Human Exon 1.0 ST Arrays were hybridized at 45°C for 16 hours in a rotating hybridization oven. Arrays were washed and stained on a GeneChip® Fluidics Station 450 using standard protocols for Exon 1.0 ST Arrays. Washed and stained arrays were scanned on a GeneChip® Scanner 3000 (GCS 3000).

Data analysis

Resulting CEL files were processed as a group using the Affymetrix Power Tools (APT). The array data was sketch quantile normalized and robust multi-chip analysis (RMA) was used for gene and exon-level probe set summarization. The core gene-level content was used for subsequent analyses.

Results

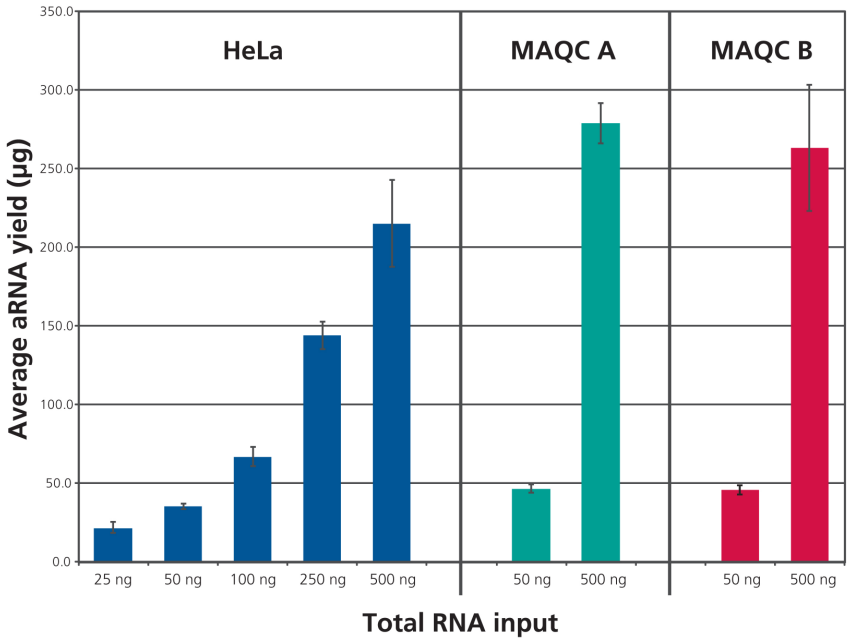
aRNA yield

Average aRNA yield was calculated for various inputs of three total RNA samples and is displayed in Figure 2 (p. 3). A minimum of 10 µg of aRNA is required for the second-cycle cDNA synthesis. Total yields of approximately 20 µg (in a 40 µL elution) are necessary for continuing the assay without requiring concentration of the aRNA via vacuum centrifugation. With HeLa total RNA, we were able to generate sufficient aRNA with inputs as low as 25 ng. Because HeLa total RNA generally provides better yields than the average RNA, an input of 50 to 100 ng should be sufficient for most high-quality RNA samples.

Table 2: Average aRNA yield.

RNA	Input	Avg. aRNA yield (µg)	Std. dev.
HeLA	25 ng	21.6	3.5
	50 ng	35.4	1.9
	100 ng	67	6.3
	250 ng	14.2	8.7
	500 ng	215.4	27.7
MAQC A	50 ng	46.6	2.8
	500 ng	278.9	12.9
MAQC B	50 ng	45.7	2.7
	500 ng	263.1	40.1

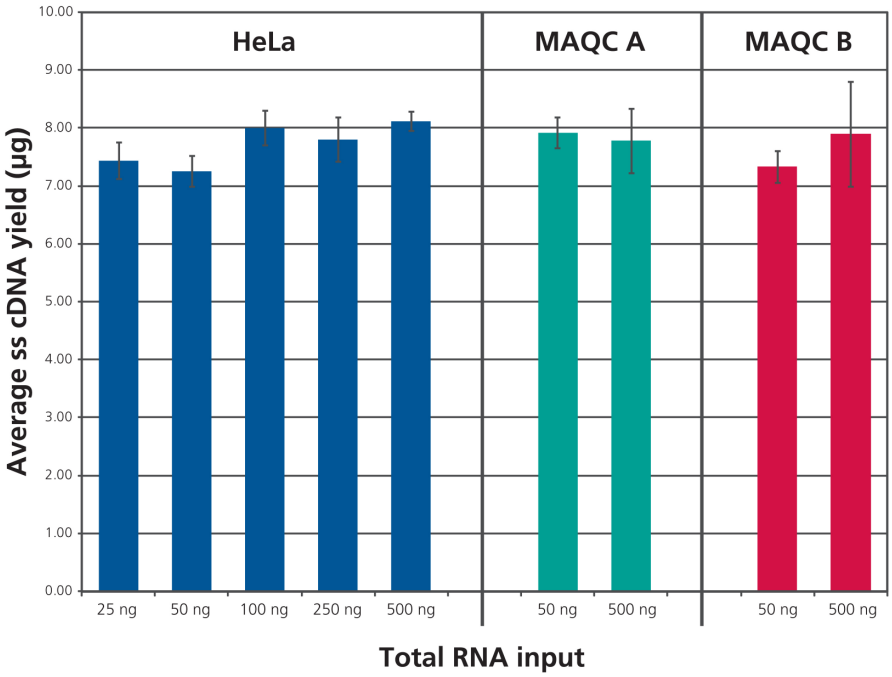
Figure 2: Average aRNA yield.



cDNA yield (10 µg cRNA input)

A constant mass of 10 µg of aRNA was used as input into the second-cycle cDNA synthesis reaction. Thus, it is not surprising that each of the original total RNA input amounts have roughly the same average single-stranded cDNA yield during the second-cycle cDNA synthesis step (Figure 3). The median of all samples was 7.70 µg. 5.5 µg of sense-strand cDNA was used as input into the fragmentation and labeling step.

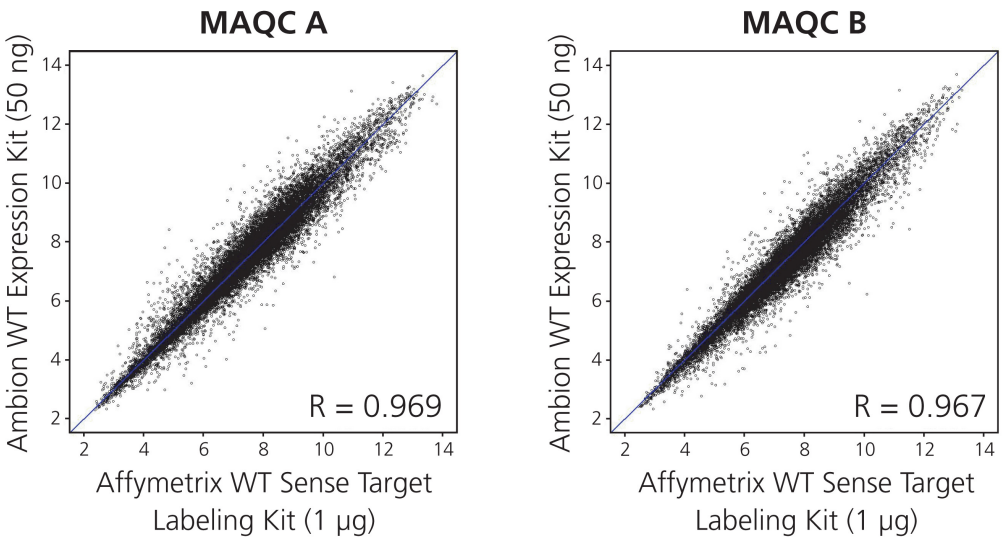
Figure 3: Average single-stranded cDNA yield (10 µg of aRNA was used for second-cycle cDNA synthesis reaction).



Signal correlation

To compare the performance of the Ambion® WT Expression Kit to the Affymetrix WT Sense Target Labeling Kit, we generated gene-level scatter plots (using core content) and signal correlation for both RNA types. Average probe set signal (RMA) of the three replicates was used for the scatter plots shown in Figure 4. The Ambion WT Expression Kit produced Exon 1.0 ST Array data with very high signal correlation to the Affymetrix WT Sense Target Labeling Kit (Pearson correlation coefficients greater than 0.967).

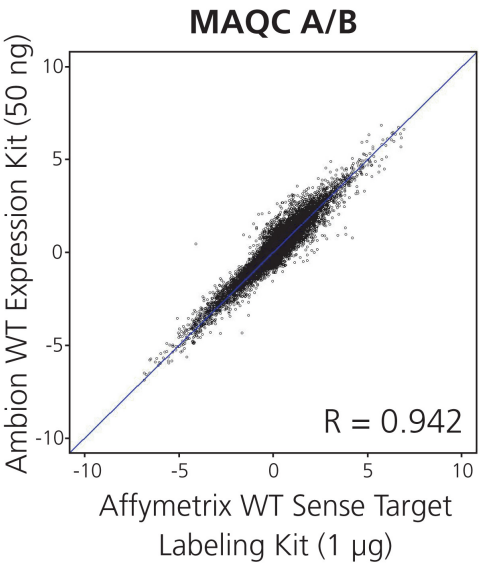
Figure 4: Average signal correlation.



Fold change correlation

Similarly, we generated fold change correlations (MAQC A/B) of the Ambion WT Expression Kit relative to the Affymetrix WT Sense Target Labeling Kit. In this case, gene-level fold change values (RMA, core) were used and are plotted in Figure 5. The Ambion kit showed very high fold change correlations when compared to the Affymetrix WT Sense Target Labeling Kit.

Figure 5: Fold change correlation.



Conclusion

Our testing demonstrates excellent performance of the Ambion® WT Expression Kit and shows that the kit generates array data with very high correlation to the Affymetrix WT Sense Target Labeling Kit. The new Ambion kit enables the use of lower total RNA input amounts without the need for upfront rRNA reduction. In addition, the simplicity of the protocol and the incorporation of bead-based purification steps makes the assay highly amenable to automation on liquid-handling systems.

Because of the nature of the selective priming during the first-cycle, first-strand cDNA synthesis reaction, the assay is currently optimized for use with human, mouse, and rat samples. The kit can be used to generate target from any organism; however, organisms with rRNA sequences that are significantly different from human may see a smaller reduction of cDNA transcripts being made from rRNA.

Ordering information

From Ambion

Name	P/N	Size
Ambion® WT Expression Kit	4411973	10 reactions
Ambion® WT Expression Kit	4411974	30 reactions

From Affymetrix

Name	P/N	Size
GeneChip® WT Terminal Labeling and Controls Kit Contains: ▪ 900670 GeneChip® WT Terminal Labeling Kit (10 reactions) ▪ 900433 GeneChip® Poly-A Control Kit ▪ 900454 GeneChip® Hybridization Control Kit	901525	10 reactions
GeneChip® WT Terminal Labeling and Controls Kit Contains: ▪ 900671 GeneChip® WT Terminal Labeling Kit (30 reactions) ▪ 900433 GeneChip® Poly-A Control Kit ▪ 900454 GeneChip® Hybridization Control Kit	901524	30 reactions
GeneChip® Hybridization, Wash, and Stain Kit	900720	30 reactions