ApplicationForum

Concordance of Affymetrix GeneChip® Human Transcriptome Array 2.0 and real-time PCR results using USB® VeriQuest® qPCR master mixes

Global gene expression analysis using Affymetrix GeneChip Human Transcriptome Array (HTA) 2.0, combined with Affymetrix Transcriptome Analysis Console (TAC) software, empowers scientists not only to gain information about RNA expression at the gene-level, but also about RNA alternative splicing at the exon-level in different biological samples.

Real-time PCR is routinely used to verify microarray results due to its high sensitivity and wide dynamic range. Our R&D team validated GeneChip HTA 2.0 gene-level fold change and alternative splicing data by using the USB First-Strand cDNA Synthesis Kit [PN 75780] followed by real-time PCR with USB VeriQuest Probe or SYBR® Green qPCR master mixes [PN 75650/75600]. We provide guidelines for this validation and show excellent inter-platform concordance between the microarray and the real-time PCR data.

Gene-level fold change validation

Gene expression in the MAQC A and MAQC B RNA samples from the MicroArray Quality Control Consortium¹ was measured using the Affymetrix GeneChip WT PLUS Reagent Kit followed by hybridization to Affymetrix GeneChip HTA 2.0. Microarray data was validated using VeriQuest Probe qPCR Master Mix.

- Gene-level fold change analysis of MAQC A vs MAQC B was performed using a modification of TAC software
- 84 genes were selected to span a wide range of expression levels and fold changes between RNA samples (Figure 1)
- Ready-made IDT PrimeTime® qPCR primers and primers designed using IDT's Custom qPCR Design Tool were used:
 - 1 primer set at an exon-exon junction towards the 5'-end of the gene
 - 1 primer set at an exon-exon junction towards the 3'-end of the gene

Affymetrix GeneChip HTA 2.0 microarray data showed excellent gene-level fold change correlation with USB VeriQuest Probe qPCR data (Figure 1). The location of the real-time PCR primers for each gene had minimal effect on the ability of VeriQuest qPCR master mix to validate Affymetrix gene-level microarray data (Figure 1).

Fig. 1. Excellent fold-change correlations and slopes achieved using VeriQuest Probe real-time PCR master mix.

qPCR Fold Change Correlation vs. Affymetrix HTA 2.0: Scatter plots compare the differential expression values (MAQC A vs. MAQC B) obtained by USB VeriQuest qPCR Probe master mix with either A) primers mostly towards the 5'-end or B) primers mostly towards the 3'-end of the genes. The line shown is the linear best-fit of the data. Correlation coefficient (R) and Slope are listed for each plot.

Interrogating alternative splicing events with VeriQuest qPCR master mix

Alternative splicing data using the Affymetrix GeneChip WT PLUS Reagent Kit and Affymetrix GeneChip HTA 2.0 for liver, muscle, and brain RNA were validated using VeriQuest SYBR Green qPCR Master Mix.

- Alt-splicing analysis was performed using TAC software (liver vs muscle, liver vs brain, muscle vs brain) with a p value <0.001.
- From the coding content, 5 genes were selected per tissue-pair for comparison (15 events in total), showing alternative splicing of an internal exon using the following criteria:
 - o Gene-level fold change was not significant: FC (linear) <2 or >2
 - o Splicing Index of internal exon was significant: SI (linear) >2 or <-2
- Alt-spliced (A) and constitutive (C) exons were identified in TAC software, and real-time PCR primer sets were designed on the website <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>

HTA 2.0 alternative splicing data showed very good qualitative correlation with VeriQuest SYBR Green qPCR data (Table 2). All internal exon alt-splicing events selected from HTA 2.0 data were confirmed by real-time PCR.

Table 2. All alternative splicing events selected for validation were confirmed by USB VeriQuest SYBR Green real-time PCR master mix.

Comparison	Gene Symbol	Transcript Cluster ID	Gene Fold Change (linear)		Probe Selection Region	Splicing Index (linear)	
			HTA 2.0	qPCR		HTA 2.0	qPCR
Liver vs Muscle	OXA1L	TC14000131.hg.1	-1.6	-1.8	PSR14001081.hg.1	4.7	2.4
	ABLIM3	TC05000816.hg.1	1.9	2.6	PSR05011626.hg.1	-7.0	-20.1
	HNRNPA1	TC12000461.hg.1	1.1	1.0	PSR12005965.hg.1	-3.6	-6.1
	ABLIM1	TC10001676.hg.1	-1.3	-1.5	PSR10022069.hg.1	-4.3	-9.7
	ARHGEF12	TC11001102.hg.1	1.1	-1.5	PSR11013970.hg.1	-7.0	-2.1
Liver vs Brain	TCF7L2	TC10000820.hg.1	1.2	1.5	PSR10009841.hg.1	6.5	5.0
	REPS1	TC06002164.hg.1	1.0	1.0	PSR06027253.hg.1	-3.0	-3.8
					PSR06027254.hg.1	-6.3	-6.7
	CARM1	TC19000188.hg.1	-1.2	-1.3	PSR19002877.hg.1	-3.5	-4.6
	CLTB	TC05002093.hg.1	-1.4	-1.8	PSR05028726.hg.1	-6.1	-27.5
Muscle vs Brain	STAU1	TC20000923.hg.1	1.4	1.3	PSR20012699.hg.1	-5.9	-5.3
	FGFR1OP	TC06004105.hg.1	-1.9	-2.6	PSR06014001.hg.1	5.2	3.0
	AGFG1	TC02001363.hg.1	1.0	-1.1	PSR02021327.hg.1	3.7	15.7
	FOXN3	TC14002331.hg.1	1.2	1.1	PSR14015818.hg.1	4.0	10.1
	PARP6	TC15002777.hg.1	1.4	1.2	PSR15015704.hg.1	-4.5	-14.4
	BICD2	TC09001340.hg.1	1.5	1.0	PSR09016810.hg.1	-5.3	-11.6

Gene-level fold change and exon-level Splicing Index from HTA 2.0 and VeriQuest SYBR Green real-time PCR master mix are compared. HTA 2.0 data is as reported by TAC software. Fold-change from real-time PCR data is the average fold-change of the constitutive amplicons. Splicing Index from real-time PCR data is the fold-change of the alt-spliced amplicon normalized to the average fold-change of constitutive amplicons. qPCR Splicing Index reported in the table is the average splicing indices of alt-spliced amplicons.

Summary

HTA 2.0 gene-level expression data for the classic MAQC A and MAQC B samples was validated with commercially available primers and design software from IDT. Excellent fold-change correlation of microarray and real-time PCR data was observed using VeriQuest Probe real-time PCR master mix (R=0.96, slope=0.70).

HTA 2.0 internal exon alternative splicing events for RNA samples from liver, brain, and muscle were validated using custom primer designs and VeriQuest SYBR Green qPCR Master Mix. To learn more, please visit www.usb.affymetrix.com/veriquest.

Reference: 1. MAQC consortium. (2006) *Nature Biotechnology* **24**, 1151-1161.

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