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Biology for a better world

RNA-Seq misses

Delivers!

Volume 1

RNA-Seq misses what HTA Delivers!

RNA-Seq

Don't believe the hype. RNA-Seq promises the discovery of new expression events, but it doesn't deliver.

Did you know that RNA-Seq misses important biology? Typical read depths of 40 million reads miss changes of low- and medium-abundance genes as well as exon-level changes and alternative splicing events.

Does your RNA-Seq overestimate discovery of novel events? Many claims of discovering novel isoforms are overinflated. Library prep artifacts and variable analysis choices create noise. Comparisons to oversimplified gene models prevent comprehensive analysis of transcript isoforms.

Is it taking too long to get meaningful data from RNA-Seq? Investigators are spending months to generate and interpret their sequence data, devoting more time and resources to informatics and less to biology.

Are your RNA-Seq experiments limited by sample type? Protocols to analyze precious clinical samples, such as FFPE and blood samples, are unproven, difficult, and give inconsistent results.

HTA

Affymetrix delivers the complete solution! GeneChip® Human Transcriptome Array 2.0 (HTA), analysis software, and assays for tissue, blood, and FFPE.

Discover more: The deepest view of exons and splice junctions with consistent performance across high-, medium-, and low-abundance transcripts. Complete and comprehensive analysis based on coding and non-coding gene models derived from all major public databases.

Reliable performance: Precision and accuracy equivalent to 2 full lanes of RNA-Seq data per sample.

Fast and convenient: From RNA to biology within a week. Workflows accommodating small to large numbers of samples. Simple, easy-to-use data analysis designed for the investigator.

Validated on multiple sample types: Proven performance from clinical samples, including blood and FFPE, with as little as 50 ng.

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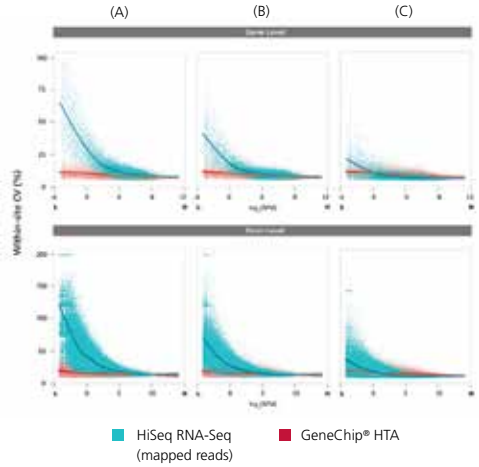

Figure 1. For RNA-Seq, both the depth of transcriptome sequencing as well as expression level influence precision. GeneChip® Human Transcriptome Array 2.0 (HTA) generates precise gene expression data regardless of transcript abundance.

The median gene or exon expression level [x-axis, $\log_2$ reads per million (RPM)] for every measured transcript in relation to the coefficient of variation [y-axis] for repeated measurements is shown for HTA (red points and best-fit as red line) and sequencing using the Illumina® HiSeq™ 2000 System (RNA-Seq, 100+100 bp read-pairs, blue points and best-fit as blue line). RNA-Seq data generated by all but the deepest sequencing contains significant noise, thereby missing important differential expression.

(A) = 1/8 HiSeq lane, 30 million mapped reads
(B) = 1/2 HiSeq lane, 120 million mapped reads
(C) = 2 HiSeq lanes, 480 million mapped reads

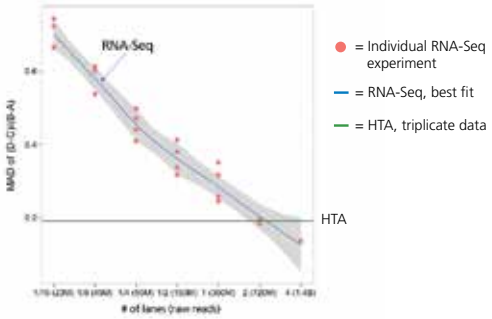

Figure 2. Accuracy for RNA-Seq is dependent upon read depth. GeneChip® Human Transcriptome Array 2.0 (HTA) delivers accurate results equivalent to 2 full lanes of RNA-Seq.

By evaluating a linear tissue mixture model in which RNA from 2 samples are mixed in known proportions, the accuracy of expression was evaluated across all measured exons. The y-axis is the calculated error or MAD (median(|x - median(x)|) of (D-C)/(B-A)) where A = Universal Human Reference RNA (UHRR), Agilent Technologies, Inc.; B = Human Brain Reference RNA (HBRR), Life Technologies, Inc.; C = 75%A/25%B; and D = 25%A/75%B. HTA delivers the equivalent accuracy in gene expression measurements throughout the transcriptome as sequencing a sample across 2 full lanes on a HiSeq™ 2000 System.

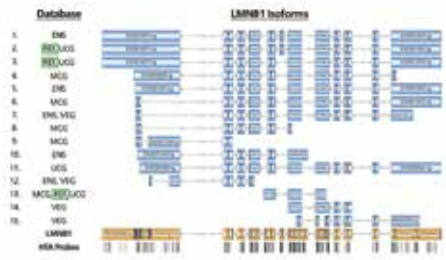

Figure 3. GeneChip® Human Transcriptome Array 2.0 (HTA) delivers the most comprehensive view of the transcriptome. A comprehensive analysis of a gene requires combining transcript diversity from multiple data sources.

A typical gene (LMNB1, shown here) contains many isoforms, and measuring changes in the relative abundance of each isoform provides new insights into disease and biology. HTA measures expression changes at the exon and sub-exon level, taking into account the diversity of transcript isoforms derived from alternative splicing. Ten probes at each unique exonic region provide independent measurements of every transcript isoform from each gene. In addition, HTA contains 4 unique probes for every known exon-exon junction (not shown). On average, 140 probes per gene are measured. Because a typical RNA-Seq experiment utilizes RefSeq database for interpretation, in this example, only 3 isoforms for LMNB1 would be analyzed (#2, 3, and 13). HTA, with content from multiple databases, allows analysis of all 15 transcript isoforms independently.

RNA-Seq misses what

HTA

Delivers!

See how HTA can deliver for you!

Contact your local Affymetrix Account Manager or sales@affymetrix.com for more information.

Ordering information

Part number	Description
902309	Kit, GeneChip® Human Transcriptome Array 2.0 and GeneChip® WT PLUS Reagent Kit, 10 samples
902310	Kit, GeneChip® Human Transcriptome Array 2.0 and GeneChip® WT PLUS Reagent Kit, 30 samples
902311	Kit, GeneChip® Human Transcriptome Array 2.0 and SensationPlus™ FFPE Amplification and WT Labeling Kit, 12 samples
902312	Kit, GeneChip® Human Transcriptome Array 2.0 and SensationPlus™ FFPE Amplification and WT Labeling Kit, 24 samples

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