

POSTER PRESENTATION

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# Transcriptome profiling from formalin-fixed, paraffin-embedded tumor specimens by RNA-seq

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*From* Beyond the Genome: The true gene count, human evolution and disease genomics  
Boston, MA, USA. 11-13 October 2010

Molecular analysis of archival tumor specimens has accelerated the discovery of clinically useful biomarkers. RNA-seq of RNA extracted from formalin fixed, paraffin-embedded (FFPE) specimens, a potentially valuable source of biomarkers, is hampered by the presence of highly abundant ribosomal RNA (rRNA), which is difficult to remove due to extensive RNA degradation. Here we report RNA-seq results from human FFPE samples.

RNA was extracted from estrogen receptor positive (ER+) and negative (ER-) FFPE breast tumors 7-8 years after fixation and embedding. Sequencing libraries were prepared using a proprietary protocol with known strand direction. On average, 14 million reads were obtained from each lane of the Illumina GAI. After employing a proprietary rRNA depletion method, 18S and 28S rRNA are reduced to less than 5% of total mapped reads. Approximately 25 000 unique Ref-seq gene transcripts were detected in each library, demonstrating detection of most rare and intermediate transcripts as well as abundantly expressed transcripts. The relative abundance of the estrogen receptor gene *ESR1* and co-expressed transcripts in the *ESR1+* and *ESR1-* tumors correlated with expression differences measured by qPCR.

Directional analysis indicated that 95% of the reads mapped to known genes had the correct direction, whereas remaining 5% with antisense direction, which possibly serve as regulatory functions. Among the uniquely mapped reads, ~ 25% were within known exons, 41% in introns, and 34% in intergenic regions. 'Valleys' and 'hills' were commonly observed across the three mapped regions. A proprietary region analysis method was developed to identify any region (not limited to known Ref-seqs) exhibiting differential expression. Using this method, we identified 34 regions that

were highly differentially expressed between the *ESR1+* and *ESR1-* tumor specimens. These regions represent exons, introns and intergenic areas. Our findings were confirmed by qPCR.

These preliminary studies demonstrate the feasibility of using RNA-seq with FFPE tumor specimens. We anticipate that RNA-seq will provide a new perspective about transcriptional changes that underlie the phenotypes of individual tumors.

Published: 11 October 2010

doi:10.1186/gb-2010-11-S1-P31

Cite this article as: Qu *et al.*: Transcriptome profiling from formalin-fixed, paraffin-embedded tumor specimens by RNA-seq. *Genome Biology* 2010 11(Suppl 1):P31.

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