Qu et al. Genome Biology 2010, 11(Suppl 1):P31
http://genomebiology.com/2010/11/S1/P31

Genome Biology

POSTER PRESENTATION Open Access

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

Kunbin Qu*, John Morlan, Jim Stephans, Xitong Li, Joffre Baker, Dominick Sinicropi

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 GAII. 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.

Genomic Health, Inc., Redwood City, CA 94063, USA