Sequencing of Whole Blood Cell RNA: Duplex Specific Nuclease Treatment for Removal of Abundant RNA Species

Meeting:
ASHG 2011

Session Title: Complex Traits: Theory and Methods

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Abstract:
Background: RNA sequencing using Next Generation Sequencing methods (NGS) is rapidly replacing microarrays to measure changes in gene expression. However, sequencing of whole blood RNA can be confounded by the high abundance of RNAs such as hemoglobin. We describe the use of Duplex-Specific Nuclease (DSN) to remove high abundance RNAs, and compare RNA sequence data from treated RNA to that obtained by microarray and RT-qPCR.

Methods: RNA was purified from whole blood collected in Paxgene tubes from consented donors. RNA from multiple individuals was combined into 5 independent pools. Total RNA was fragmented, converted to cDNA and then subjected to adaptor ligation and PCR. Amplified cDNA from the first pool was either treated with 2 units of DSN for 5 hours, or left untreated; the four remaining pools were treated with DSN as described above. cDNA was then sequenced on the Illumina GAII platform using standard Illumina protocols.

Results: Sequencing of untreated cDNA resulted in 257,709 reads per kilobase of exon model (RPKM) for HBA1 sequence, 25% of the total reads sequenced. Treatment with DSN reduced this to 926 RPKM for HBA1, effectively reducing the amount of HBA1 by 99.6%. Comparable results were seen for HBA2 and HBB (99.7 and 99.8% reductions respectively). Comparison of the four treated pools to microarray and RT-qPCR data derived from the same samples showed good concordance (median r2 value of 0.547, 17,069 genes; median r2 value of 0.841, 176 genes respectively). DSN treatment also reduced the levels of other high abundance genes such as histone, ribosomal and ubiquitin components as compared to microarray and sequence data by linear regression.

Conclusions: Treatment of whole blood total RNA with DSN appears to be an effective way to reduce high abundance transcripts, prior to RNA sequencing, improving the depth of the transcriptome revealed. In addition, better correlation was observed between RNA sequencing and RT-qPCR data than between microarray and RT-qPCR data.