ABSTRACT

Next Generation RNA-Seq (RNA-Seq) is a methodology for comprehensive in-transcriptome cDNA sequencing at a ScanTools accuracy and precision never seen with previous technologies. We conducted a comparative RNA-Seq study of basic cell growth conditions to understand and improve high quality therapeutic protein production in CHO cells. In this study, we examined global changes in gene expression in CHO cells across different cell subtype and media-specific parameters. Eight CHO RNA samples from different cultures were sequenced on two full slides of a SOLiD™ System resulting in approximately 760 million, 50 base pair (bp) reads per sample. These reads were mapped to multiple reference sequences including CHO ESTs, mRNAs, and whole human genomes with associated gene sets. From this, we report estimates of transcript expression levels and use known annotation to infer functional differences that can be associated with changing basic bioproduction growth conditions. These findings may uncover novel genetic mechanisms that could be optimized for improved bioproduction. This database set represents the characterization of the CHO transcriptome at an unprecedented depth.

RESULTS

Figure 6. MA fold change clog transcript abundance graphs for genes significantly differentially expressed between RNA-Seq data sets. The figure plots the log2 fold change for a gene between two conditions, followed by P-value at the bottom of each graph. The differentially expressed genes were identified as those with a fold change of 2 or greater and a p-value of 0.05 or less. The restraint on the vertical axis is to highlight those genes which are upregulated in a given condition.

Figure 7. MA fold change clog transcript abundance graphs for genes significantly differentially expressed between RNA-Seq data sets. The figure plots the log2 fold change for a gene between two conditions, followed by P-value at the bottom of each graph. The differentially expressed genes were identified as those with a fold change of 2 or greater and a p-value of 0.05 or less. The restraint on the vertical axis is to highlight those genes which are upregulated in a given condition.

Figure 8. Visualizing exon coverage and differential expression. Screen shot from the University of California Santa Cruz Genome Browser depicting coverage of individual DG44 reads aligning to mouse exons. (A) Reads mapping in high abundance on to mouse exons on chromosome 14. After using DESeq to scale the reads from each sample, it is evident that at high IgG producing cells (blue peak) over express this gene relative to low IgG producing cells (green peak) by approximately 10 fold. (B) Zooming in on exons 23 and 24, we can see in detail where reads align to each exon. Areas of no coverage suggest that there is either a sequence specific bias at this location making it difficult to sequence or homology between hamster and mouse is much lower in this region preventing reads from mapping.

Figure 9. General, over represented functional classes of top differentially expressed genes across DG44 and RevO cell lines. Gene ontology and pathway enrichment for DEGs was determined using the R packages, GSEA® and NCBI Entrez Gene annotation.

CONCLUSIONS

With the use of RNA-Seq technology and cognate computational approaches, we have successfully established RNA-Seq an essential tool in CHO cells under several conditions using the mouse genome to derive meaningful biological information. Normalized expression values were used to identify significantly differentially expressed genes for orthogonal mouse genes. From these, functional information may be gleaned which will be used to identify gene targets associated with cell subtype and media conditions for optimizing bioproduction activities. Future studies will involve mapping to the CHO genome once available and alternative platform validation of putative targets.

REFERENCES


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Comparative Analysis of CHO Cell Transcriptional Dynamics under Different Cell Culture Conditions using Next Generation RNA-Seq Sequencing

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