Low Input RNA-Seq—Transcription Profiling of Low Input Samples with Success

Introduction

RNA-Seq is a powerful tool to profile gene expression and to identify new transcripts and structure variations. However, the assays generally require a considerable amount of RNA or tissues/cells to achieve consistent results with high fidelity. This white paper outlines the fidelity, reproducibility, and sensitivity of RNA-Seq performed by Otogenetics using limited starting materials from 10 to 1000 cells. The RNA-Seq assays optimized by Otogenetics support the use of low input samples for RNA-Seq without sacrificing data quality or reproducibility.

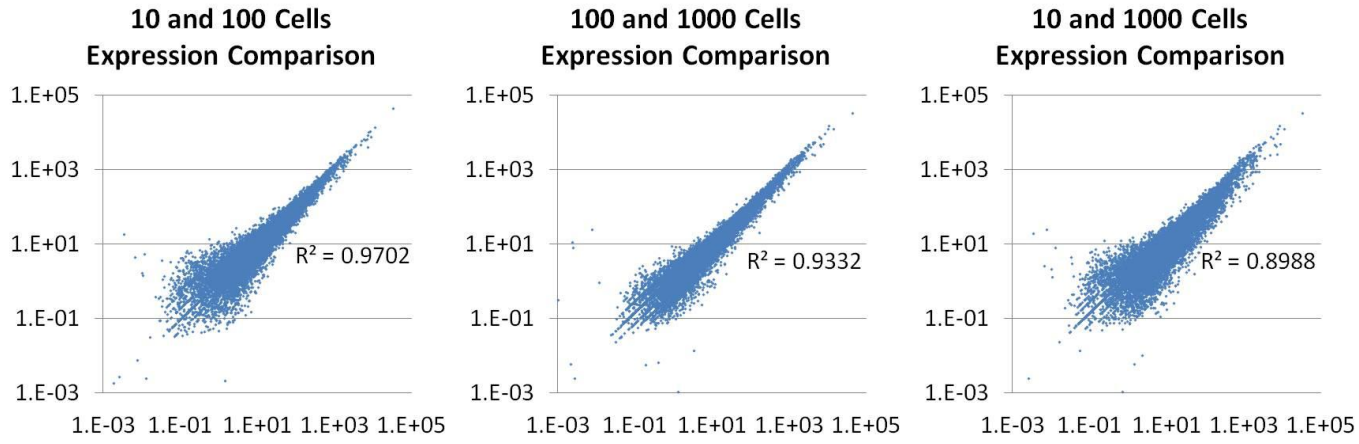
Starting Material Requirements

The quality of the starting material is the number one consideration for RNA-Seq. Special care should be taken when handling samples for any RNA-Seq Assay. For the example data shown here, 10, 100, or 1000 human HeLa cells were directly lyzed for RNA-Seq without RNA isolation. Please contact Otogenetics for instructions of sample handling, preparation, and shipping for RNA-Seq specific to your sample type and volume (sales@otogenetics.com).

Evaluation of Sequencing Data

As shown in the Table at right, RNA-Seq using 10, 100, or 1000 cells as the starting material yielded similar sequencing data metrics, including percent of mapped reads or duplication rate. Furthermore, gene expression was compared between samples with varying cell inputs using FPKM, Fragments Per Kilobase of transcript per Million mapped reads, to evaluate the fidelity and detection sensitivity of the assay with varying limited input. As shown in the figure below, the three samples generated similar gene expression profiles. The expression levels of <0.3% genes are profiled differentially for the same sample with three input levels, supporting the fidelity of the assays with as little as 10 cells. In addition, the higher cell inputs detect more low abundant genes (<5% of the transcriptome), suggesting input levels correlate with detection sensitivity. The latter contributed to the differential profiles with varying cell inputs, suggesting that low abundant transcripts account for a large portion of the differential transcripts among three inputs.

No. of cells	Sequencing Data					
	Mapped Percent	Duplicates	Intergenic In Mapped	Actual Size Bp	Reads	Q30 Pct
10	86.83	9.86	16.00	1,285,073,748	10,319,080	92.7; 92.8
100	90.10	9.07	15.60	1,300,204,080	10,319,080	94.2; 93.7
1000	88.00	8.01	16.50	941,895,612	7,475,362	93.4; 93.9



Summary

- As little as 10 cells can be used to generate high fidelity gene expression profiles using RNA-Seq assays optimized by Otogenetics.
- The sensitivity to detect low abundant transcripts (<5% of the transcriptome) could be improved by increasing inputs.

References

http://www.illumina.com/techniques/sequencing/rna-sequencing/ultra-low-input-single-cell-rna-seq.html
http://www.clontech.com/US/Products/cDNA_Synthesis_and_Library_Construction/Next_Gen_Sequencing_Kits/Single_cell_RNA_Seq_Kits_for_mRNA_seq/Overview?sitex=10020:22372:US