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Targeted single-cell RNA expression profiling for biomarker discovery

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New rapid and robust transcriptome-based methods for cellular characterization of the tumor microenvironment and biomarker discovery are required to improve prognosis and treatment of cancer and other diseases. However, challenges with current approaches for the above applications include high sample requirements, poor sensitivity, low dynamic range, and limited throughput. To address these limitations, we have developed the DriverMap™ targeted RNA expression profiling assay using a genome-wide set of 19,000 validated primer pairs that leverages the sensitivity of multiplex RT-PCR with the throughput and digital readout depth of next-generation sequencing (NGS). Starting from just 10pg (single-cell) to 100ng (10,000 cells) of total RNA is sufficient to quantify over 5 orders of magnitude variation in gene expression levels with performance similar to conventional qRT-PCR. Further, the use of gene-specific primers enables direct analysis of total RNA isolate and obviates the need for globin and rRNA depletion from whole blood samples. And, using a subset of primers empirically selected from the DriverMap™ assay, we have developed a novel single-cell targeted RNA expression (scTRex) profiling assay compatible with conventional oligo dT-molecular indexing, single- cell barcoding strategies. In this study, we present the performance of the assay to analyze the level of immune cell infiltration in tumor samples, and identify active pathways in tumor, xenograft samples and cell lines treated with small molecules. Preliminary studies demonstrate the assay's unparalleled specificity and sensitivity resulting in better detection of low abundance mRNA transcripts as well as an improved cost-effectiveness for high-throughput clinical applications.

Biography

Alex Chenchik is the President and Scientific Director of Cellecta Inc., Mountain View, California, USA. His work focuses on "Development and application of next generation functional genomic technologies for discovery biomarkers, drug targets and development novel drugs". He worked for Systems Biosciences, LLC as Vice-President of R/D and developed genetic screen technology with pooled lentiviral shRNA libraries in combination with a wide range of reporter cell lines. He also worked for BDB Clontech as Director of GCA Department and participated in the development of microarrays and disease-profiling arrays for expression profiling, PCR-based technologies for gene cloning, and subtraction-based approach for discovery of differentially expressed genes. He has done PhD in Molecular Biology (1982) from Institute of Molecular Biology, Moscow, Russia. He did MS in Chemistry (1978) at Institute of Fine Chemical Technology, Moscow, Russia.

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