UCSF/Fluidigm Paper Proves Shallow Sequencing of Related Single Cells Sufficient to Harvest Meaningful Biological Information

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New Method Decreases the Cost of Single-Cell Sequencing; Frees Up Resources to Analyze More Cells

SOUTH SAN FRANCISCO, Calif.--(BUSINESS WIRE)--In a paper published in *Nature Biotechnology* this week, UC San Francisco and Fluidigm Corporation (NASDAQ:FLDM) scientists have demonstrated that shallow single-cell mRNA sequencing (approximately 50,000 reads per cell) is sufficient for unbiased classification of cell identities.

The study shows shallow sequencing of single cells provides enough data to distinguish cells with similar attributes. The shallower depth reduced the sequencing required by two orders-of-magnitude (dropping from 5 million to 50,000 reads), thus dramatically lowering the proportional cost of sequencing. Researchers found that increasing the number of cells studied, instead of sequencing to greater depth, provided a better understanding of the diversity and range of expression in total cell populations.

“This paper articulates a conceptual shift in how we view RNAseq analysis in single cells,” said Alex Pollen, PhD, lead author on the paper and postdoctoral scholar in Arnold Kriegstein's laboratory at the Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research at UCSF. “In addition to exploring the consequences of low-depth analysis, the paper includes a pilot study in the brain that reveals some new biology about nervous system development. We are studying a community of different, but related, cell types in the brain. We are breaking that community down into the different populations of cells with the goal of understanding their functional parts and components so we can accurately predict how they will develop.”

The study demonstrated that the Fluidigm C_1^{TM} Single-Cell Auto Prep System could be used to distinguish and identify similar cell types — such as NPC (neural progenitor cells), primary neurons and radial glia (which were isolated from different regions of the brain) — from one another.

“Biologically, same cell types have unique transcriptional activities when they are in different micro environments. The paper shows the value of looking at the relationship between classification, spatial, and functional dynamics of the cells,” said Pollen.

The paper establishes a new method for unbiased analysis and comparison of cell populations from heterogeneous tissue by single-cell capture and low-coverage sequencing of many cells. Surveying large numbers of single cells can reveal rare cell populations, developmental intermediates and biomarkers that are often hidden in bulk tissue studies. The ability to profile gene expression of single cells is essential to advancing our understanding of molecular complexity of living cells, tissues and organisms.

The paper analyzed the transcriptomes of 301 cells from 11 distinct populations across the brain. In developing human cortex, the researchers identified multiple diverse cell types, including radial glia, intermediate progenitors and immature neuronal subtypes. Pollen et al. identified EGR1 and FOS as novel candidate targets of Notch signaling in human, but not found in mouse, radial glia.

“Advanced cellular barcoding strategies allowed parallel sequencing of large numbers of single cells at ultra-low depths,” explained Joe Shuga, PhD, co-author on the paper and a senior scientist at Fluidigm Corporation. “We created the C_1 Single-Cell Auto Prep System to routinely capture single cells and perform reverse transcription and cDNA amplification in nanoliter reaction volumes, which increases the effective concentration of reactants and improves the accuracy of mRNA sequencing.”

The research was supported by the California Institute for Regenerative Medicine, a Damon Runyon Cancer Research Foundation postdoctoral fellowship, and by National Institute of Neurological Disorders and Stroke awards R01NS075998 and R01NS072630. The study was jointly conceived, designed, performed and analyzed by researchers at UCSF and Fluidigm.

For access to the complete paper — Low-coverage single-cell mRNA sequencing reveals cellular heterogeneity and activated signaling pathways in developing cerebral cortex, Nature Biotechnology.2014 Aug 3;509(7505):363-9 — please visithttp://info.fluidigm.com/Pollen_Et_Al.html.

For complete access to the full dataset used in this paper, please visit: http://info.fluidigm.com/Pollen-Data-Set.html.

Technology

The Fluidigm C_1 Single-Cell Auto Prep System is based on the company’s innovative microfluidic technology that enables researchers to rapidly and reliably isolate, process, and profile individual cells for genomic analysis. Scientists can extract, reverse transcribe, amplify, and ultimately detect and analyze cell activity using one technology thereby reducing the variability caused by multi-platform technical errors.

About Fluidigm

Fluidigm (NASDAQ:FLDM) develops, manufactures, and markets life science analytical and preparatory systems for growth markets such as single-cell biology and production genomics. We sell to leading academic institutions, clinical laboratories, and pharmaceutical, biotechnology, and

agricultural biotechnology companies worldwide. Our systems are based on proprietary microfluidics and multi-parameter mass cytometry technology, and are designed to significantly simplify experimental workflow, increase throughput, and reduce costs, while providing excellent data quality. Fluidigm products are provided for Research Use Only. Not for use in diagnostic procedures.

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