Development of a novel, instrument-free, single-cell RNA sequencing technology (PIPseq) and its application to drug pathway discovery in lung cancer

Genomics

Ram Santhanam (rsanthanam@fluentbio.com), Fluent BioSciences, **Iain Clark**, UC Berkeley, **Christopher D'Amato**, Fluent BioSciences, **Ahmad Osman**, Fluent BioSciences, **Sruti Pandey**, Fluent BioSciences, **Yi Xue**, Fluent BioSciences, **Aaron May-Zhang**, Fluent BioSciences, **Adam Abate**, University of California, San Francisco, **Robert Meltzer**, Fluent BioSciences, **Sepehr Kiani**, Fluent BioSciences, **Kristina Fontanez**, Fluent BioSciences

Introduction: Single-cell RNA sequencing (scRNA-Seq) has enabled unprecedented insight into the biology of individual cells and tissues across a broad range of discovery and disease -relevant applications. Traditional scRNA-Seq workflows have included single cell sorting into wells, co-capture of cells with barcoded beads using microfluidic droplet encapsulation, or in-cell combinatorial indexing. Fluent BioSciences has developed a novel approach that relies on Pre-templated Instant Partitions (PIPs) to simultaneously segregate complex cell mixtures into partitions with barcoded template particles that can be easily processed for scRNA-seq (PIPseq). This approach eliminates the need for complex instrumentation and microfluidic consumables. Here, we use PIPseq to bioinformatically discriminate the transcriptomes of Gefitinib resistant and sensitive cell lines after drug treatment.

Materials and Methods: In order to evaluate tyrosine kinase inhibitor effects on adenocarcinoma cellular transcriptomics, PC9 cells, H1975 cells, or a mixed population (9:1) of PC9 and H1975, cells were treated with Gefitinib and processed with PIPseq. UMAP analyses of the transcriptome were performed and overlapped for each experiment.

Results: We demonstrate drug-treatment dependent gene expression changes in lung adenocarcinoma cells treated with the tyrosine kinase inhibitor Gefitinib. The resulting cell expression profiles clearly segregate by treatment condition in UMAP projections indicating PIPseq's ability to faithfully detect responses to drug exposure.

Conclusion: These studies establish the efficacy of PIPseq for single-cell transcriptomic analysis across multiple drug treatment conditions. The simple PIPseq workflow is optimized for comparing multiple sample treatment conditions in a single controlled experiment. With minimal upfront cost of implementation and footprint, PIPseq can be easily implemented in a core facility or laboratory, and democratizes the accessibility of scRNAseq across many applications.