Array-SeQ: An open array platform that simultaneously profiles genotype and phenotype of single cells

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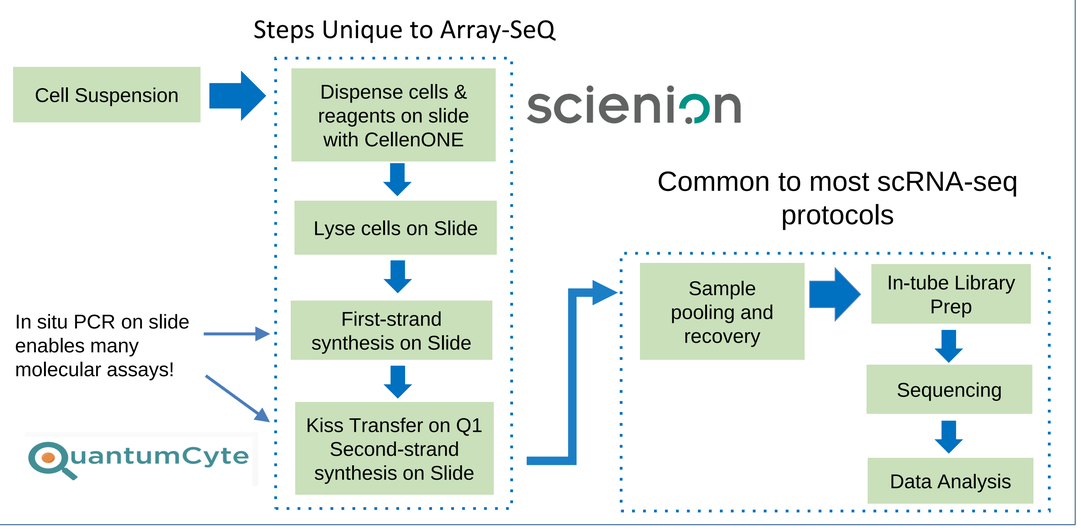
Abstract

Single-cell analysis can provide deep insights into disease, drug resistance, and tissue heterogeneity. A number of methods have been developed for transcriptomic analysis of single cells; however, all currently available methods are limited by their inability to correlate cellular phenotype with the transcriptomic signature. QuantumCyte's unique surface tension based, open array platform is capable of analyzing transcriptomic signatures of thousands of single cells while simultaneously profiling cellular phenotypic characteristics. In this poster, we present early data derived from single-cell 3' RNA-Sequencing performed using our Array-SeQ platform. We show that the platform is capable of providing high sensitivity and high specificity transcriptomic and microscopy cell phenotypes. While we only demonstrate two assays, the open platform allows for arbitrary implementation of molecular, cellular, and histochemical assays at the single-cell level.

Optimized Assay Workflow



Array-SeQ System

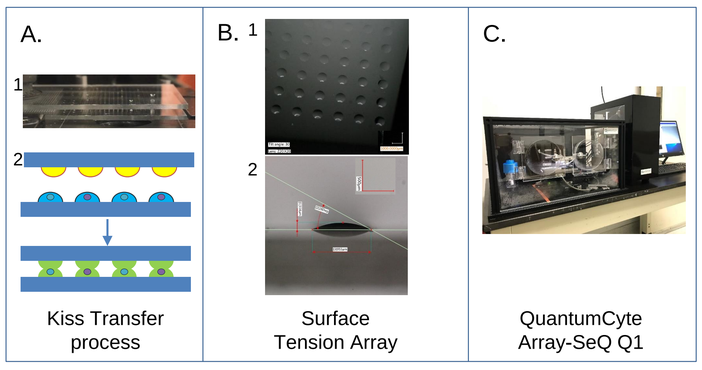


Fig-1 Kiss-transfer technology. The Kiss-transfer process brings cells and reagents together. Cells and reagents are deposited in Array-SeQ features with the CellenONE from Scienion. (A) A photograph {1}, and schematic of the kiss-transfer process {2}. (B) An image of the surface tension array {1}, and dimensions of a 1.3 nl reagent dome in an Array-SeQ feature {2}. (C) QuantumCyte's Array-SeQ Q1 tool.

Materials and Methods

PC3 cells (human prostate cancer cell line) and CT26 cells (mouse colon cancer cell line) were suspended in PBS and deposited into individual features of an Array-SeQ slide containing pre-spotted barcodes. Cells were deposited using Scienion's CellenONE single cell deposition tool as per the barcode map. Microscopic scan confirmed each feature contained single cell. Cells were lysed and mRNA was barcoded during first strand synthesis in individual features on the slide. Second strand reagents were delivered into individual features using our proprietary kiss-transfer process on our Q1 tool. Libraries for each cell were compartmentalized through second-strand cDNA synthesis. Following second-strand synthesis, cDNA from all the features was pooled and processed as a single library in a tube. Concentration of library was determined using Qubit HS-dsDNA assay and average fragment size the library was determined using Agilent TapeStation 4200. Library was sequenced on Illumina MiSeq. Bioinformatic analysis was performed using our proprietary bioinformatics pipeline.

Microscopy profiling of cell viability

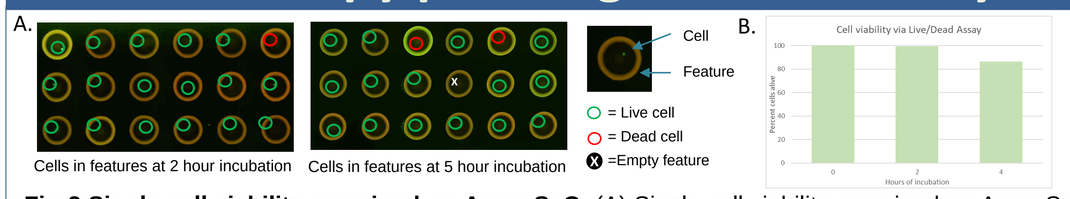


Fig-2 Single-cell viability examined on Array-SeQ. (A) Single cell viability examined on Array-SeQ by microscopy as a proof of concept of cellular phenotype. Single cells could then be assayed by 3' RNA-seq and correlated to viability phenotype. (A) Microscopy images of single cells in Array-SeQ features at 10x magnification. The cells are fluorescently labelled with live/dead stain. (B) Bar chart representing viability of the cells printed on Array-SeQ at 0h, 2h, and 4h. Data shows high viability of cells up to 4h in instrument.

3' RNA-Seq using Array-SeQ generates high quality data

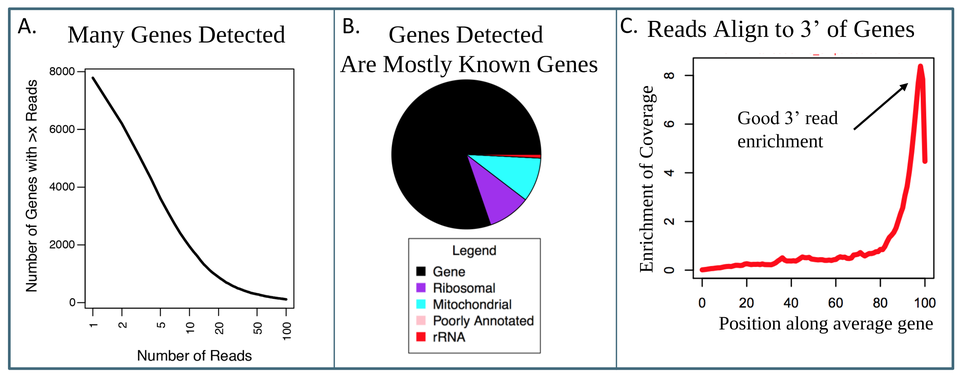


Fig-3 3' RNA-Seq data on Array-SeQ. (A) Data showing robust detection of genes from cells processed on our platform using 3' RNA-Seq workflow. (B) Functional annotation pie chart of the detected genes showing that majority of the detected genes are well annotated with negligible contamination from rRNA. (C) Read alignment analysis showing enrichment of mapped reads at the 3' end of the detected transcripts in accordance with 3' RNA-seq workflow.

High single-cell specificity on Array-SeQ

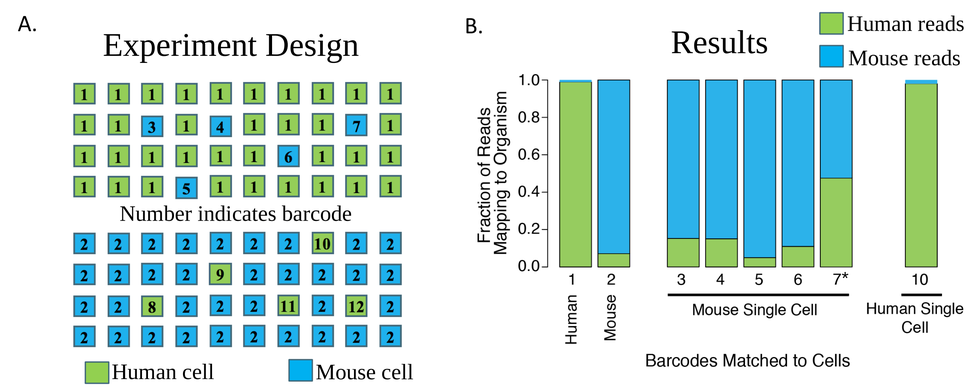


Fig-4 Sequencing of human and mouse single cells show high specificity on the Array-SeQ platform. (A) Barcoding schematic of the deposited cells. Human cells are labeled with barcodes 1, 8, 9, 10, 11, 12 and mouse cells are labeled with barcodes 2, 3, 4, 5, 6, 7. (B) Reads mapping to human reference genome hg19 (green) or mouse reference genome mm10 (blue) match the barcode-labeled organism. *Barcode 7 mixed with one barcode 1 feature.

Conclusions and Discussion

We have demonstrated Array-SeQ's platform's application for simultaneous profiling of cellular phenotype and 3' mRNA sequencing at a single cell level. Because of Array-SeQ's unique capability of genotype and phenotype analysis for >90% of the deposited cells, combined with >95% cell capture rate from CellenOne, Array-SeQ offers a significant advantage over traditional single-cell analysis technologies. This advantage is compounded for rare and precious samples. In addition to cellular phenotyping and gene expression analysis, we are developing molecular single-cell assays not currently feasible in any other platform. Array-SeQ is a powerful platform with exciting opportunities in single-cell gene expression profiling, biomarker and drug discovery, and tissue profiling space.

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