SEED • Biosciences Alithea Genomics

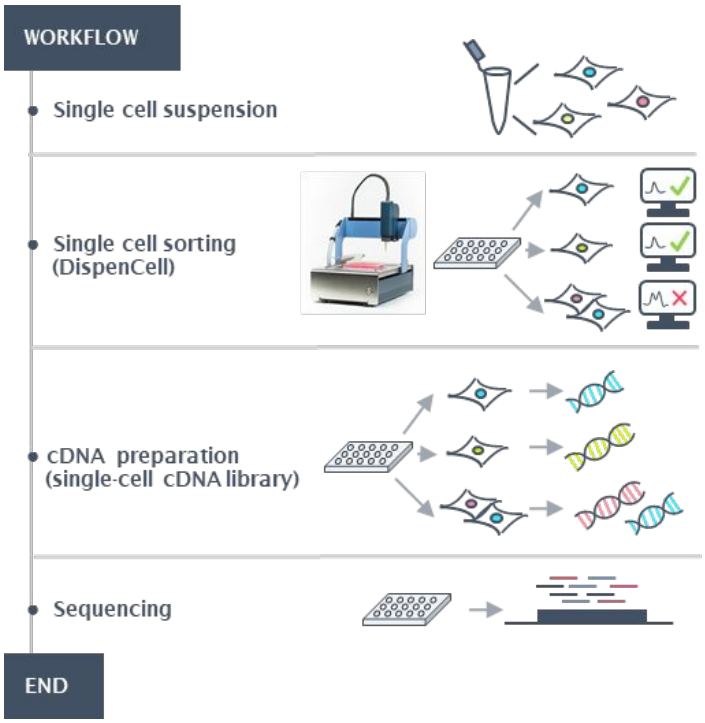
DispenCell for highly-sensitive and affordable single-cell RNA sequencing with Alithea Genomics

Introduction

• Single-cell RNA sequencing (scRNA-seq) enables unprecedented and unbiased gene expression analysis in complex samples. Still, **scRNA-seq remains technically challenging and often cost-prohibitive**.

• Current solutions enable efficient analysis of a fraction of cells but starting from >10,000s cells. **A streamlined protocol for scRNA-seq library preparation of rare cell populations is still missing**.

We present here **a novel, affordable and highly sensitive plate-based assay, combining the DispenCell-S1 (SEED Biosciences) with the MERCURIUS™ High-sensitivity BRB-seq kit (Alithea Genomics) for single-cell isolation, library preparation and sequencing from rare cell populations (Fig.1).**



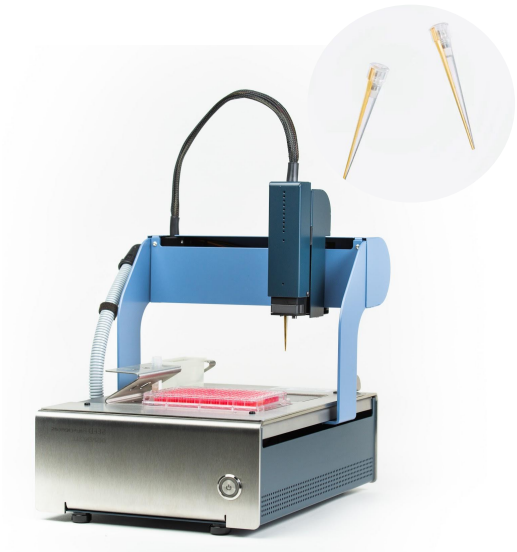


Figure 1: *Novel workflow for single-cell RNA sequencing, combining DISPENCELL (SEED Biosciences) and MERCURIUS™ High-sensitivity BRB-seq kits (Alithea Genomics).*

Single-cell dispensing

• Lymphoblastoid cells were dispensed individually into a 384-well plate pre-loaded **with the lysis buffer required for the library preparation** (HS BRB-seq kit, Alithea Genomics).

• The software DispenSoft (SEED Biosciences) enables **a single-cell quality control post-processing, in a simple, rapid and fully traceable process**: in green, the wells with 1 cell, and in red, the wells with 0 or >1 cell. Each impedance profile can be examined manually; with a single sharp peak being the signature of a single cell while multiple peaks result from multiple cells

• To discriminate between debris and cells, a threshold was set by gating on the size-based histogram. Only the peaks higher than the threshold were counted as cells. We obtained 77 wells with 1 cell, for a plate-filling rate of 80% (Fig.2).

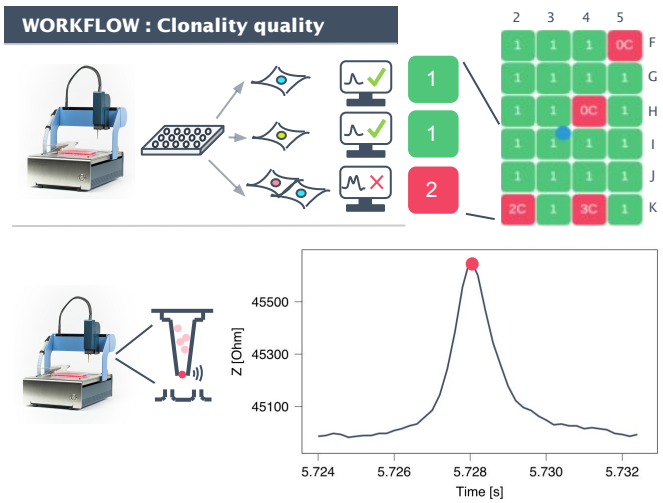


Figure 2: *Single-cell quality control. a) Map of the plate; b) Example of a single-cell signature.*

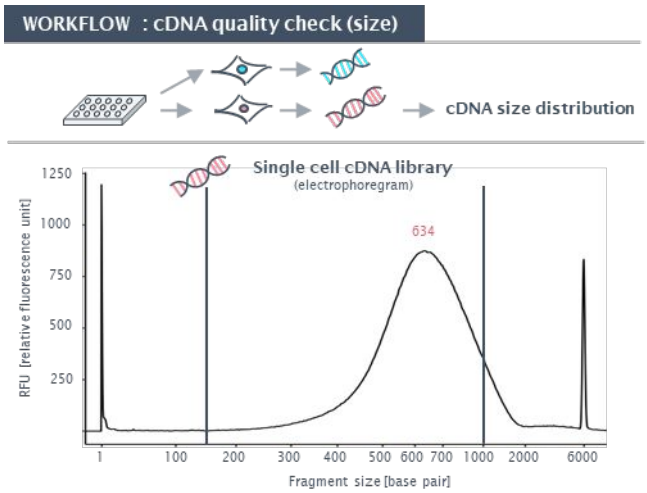


Figure 3: *Fragment size distribution of a single cell library.*

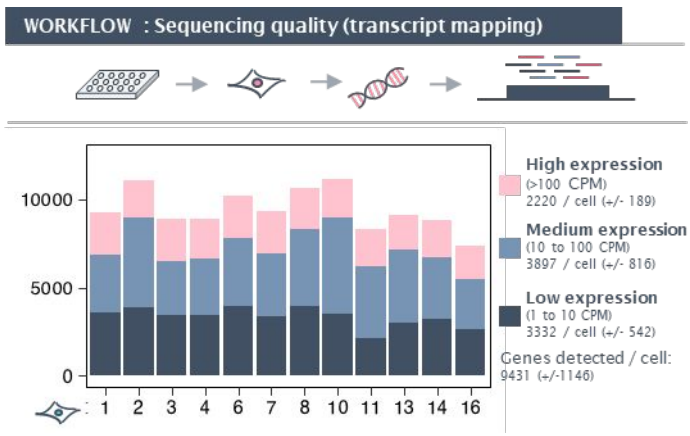


Figure 4: *Number of detected genes for each single-cell library sequenced.*

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cDNA amplification and sequencing

- The sequencing libraries were produced (Alithea). 1 ng of double-stranded cDNA was tagmented and a second PCR amplification performed (12 cycles, Illumina Nextera). The final libraries were purified using AMPure beads and run on a Fragment Analyzer.

- All profiles demonstrated similar fragment size distribution, in the expected range, with a single peak around 600 bp, showing good library quality and good reproducibility (Fig.3).

- The libraries were sequenced using the NextSeq instrument at 0.5 Mio reads per library. The sequencing reads were aligned to the human genome (GRCh38.102) using STAR (2.7.9a).

- All the single-cell libraries allowed for the detection of around 10'000 genes, with a good proportion of poorly expressed genes (⅓ of the genes detected) (Fig.4).

- **Those results confirm high data quality and high reproducibility across the wells with single cell samples.**

The workflow validated here, combining the DispenCell from Seed Biosciences and the MERCURIUS™ High-sensitivity BRB-seq kits from Alithea Genomics, provides high quality sequencing data, enabling a robust end-to-end simple and affordable library preparation and sequencing from single cells.

References

- *Bonzon, D. et al. (2020) 'Impedance-Based Single-Cell Pipetting', SLAS TECHNOLOGY, 25(3), pp. 222–233.*
- *Muller, G. et al. (2020) 'Traceable Impedance-Based Dispensing and Cloning of Living Single Cells', SLAS TECHNOLOGY, 25(3), pp. 215–221.*
- *Hannart, H. et al. (2021) 'Traceable Impedance-based single cell pipetting: from a research set-up to a robust and fast automated robot', SLAS TECHNOLOGY: Translating Life Sciences Innovation. In Press.*