High viability cell sorting and 3’ RNA-Seq for gene expression from single cells

Jose Morachis[1], Jonathan Shaffer[2], Huailu Chen[1], Nicole Jagnandan[1], Will Alaynick[1], Sam Rulli[2]
[1]NanoCellect Biomedical, Inc., San Diego, CA, [2]QIAGEN, Frederick, MD

Abstract

Biological insights continue to be further dissected with the increasing availability of microfluidic and genomic tools that can resolve information at the single cell level. More importantly, the ability to achieve single-cell RNA sequencing enables transcriptomic analysis of an individual cell and provides information on prevalence, heterogeneity, and gene expression at high biological resolution. However, most labs lack the tools to properly isolate single cells into 96- or 384-well plates and do not have the capacity to develop a complex RNA-Seq protocol. By combining two technologies, the WOLF® Cell Sorter (NanoCellect) and QIAseq UPX 3’ RNAseq kits (QIAGEN), we provide a complete workflow solution that allows for greater simplicity, improved single-cell RNA-Seq detection, and experimental flexibility.

Here, we present an overview of the complete workflow: from cell detection, single cell sorting, and the experimental design of single-cell RNA-Seq experiments using QIAseq UPX kits. Furthermore, we provide an example of the typical data analysis workflow; from handling of the FCS flow data to RNA-Seq data analysis using GeneGlobe NGS Analysis Center’s integrated cloud-based RNA-Seq data analysis.

Microfluidic Cell Sorting Technology

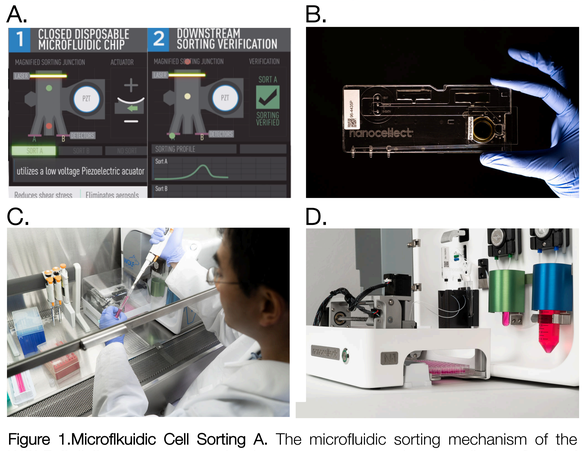

Figure 1.Microfluidic Cell Sorting **A.** The microfluidic sorting mechanism of the WOLF Cell Sorter uses a gentle piezo actuator to gently sort cells at <2 psi of pressure. **B.** The microfluidic-based single-use cartridge for the WOLF sorter. **C.** The WOLF Cell Sorter and N1 Single-Cell dispenser, in a TC hood, is an easy-to-use, aerosol-free, sterile and disposable system for selection and sorting of cells in bulk, or directly into 96- or 384-well plates. **D.** The WOLF Cell Sorter and the N1 Single-Cell module.

Workflow

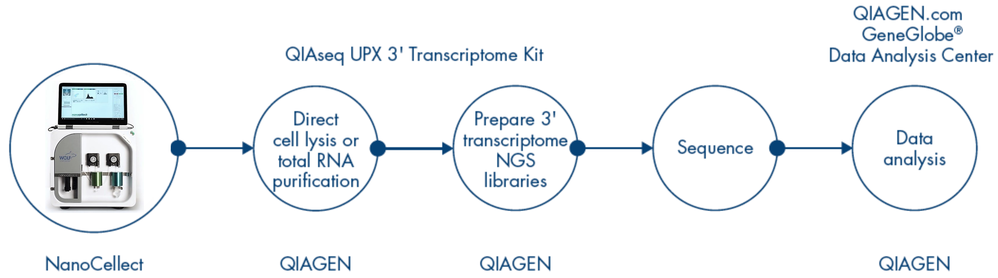

Figure 2. Single-cell analysis workflow. Starting with a mixture of suspended cells, the sample is loaded onto the WOLF Cell Sorter. Target cells are selected (based on 5 optical flow cytometry parameters) and then dispensed into 96-well QIAseq plates, which are pre-loaded with cell lysis buffer. Single-cell RNA is first reverse transcribed and each RNA molecule is given a Unique Molecular Index (UMI) and assigned well-specific Cell IDs (up to 384-wells). Following reverse transcription with integrated template switching, all cDNAs are combined, enabling simplified, single-tube library construction. Together, the WOLF and the QIAseq UPX 3' Transcriptome Kit enable high-throughput next-generation sequencing (NGS) of polyadenylated RNAs from single cells with Illumina® sequencing.

Single Cell Dispensing (Sample Prep)

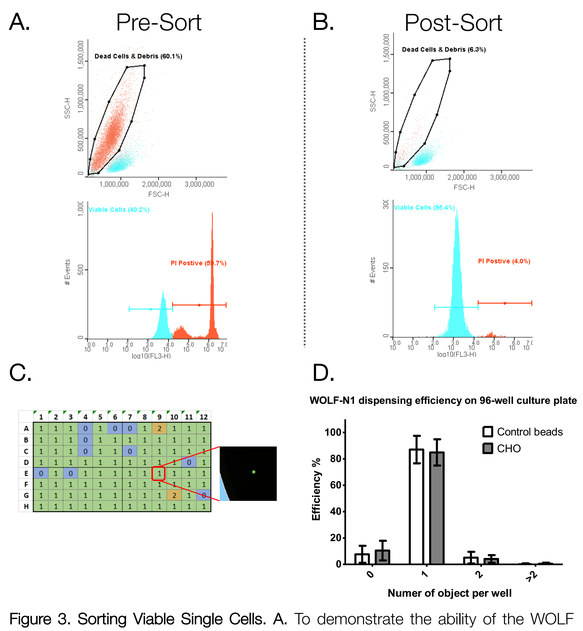

Figure 3. Sorting Viable Single Cells. **A.** To demonstrate the ability of the WOLF Cell Sorter in sorting viable cells from dead cells and debris, we started with a 50:50 mix of heat treated (dead) and healthy CHO cells. Propidium Iodide (PI) was used to label dead cells. **B.** Sorting of PI-negative cells showed nearly complete removal of dead cells. **C.** Example of evaluating the ‘object number per well’ and a representative bead image on a 96-well culture plate read by Synentec® NyOne imager. **D.** Dispensing efficiency of control beads or CHO cells shown as objects number per well on the 96-well plate. N= 3 cartridges, triplicate replicates.

QIAseq UPX 3' Transcriptome Kit

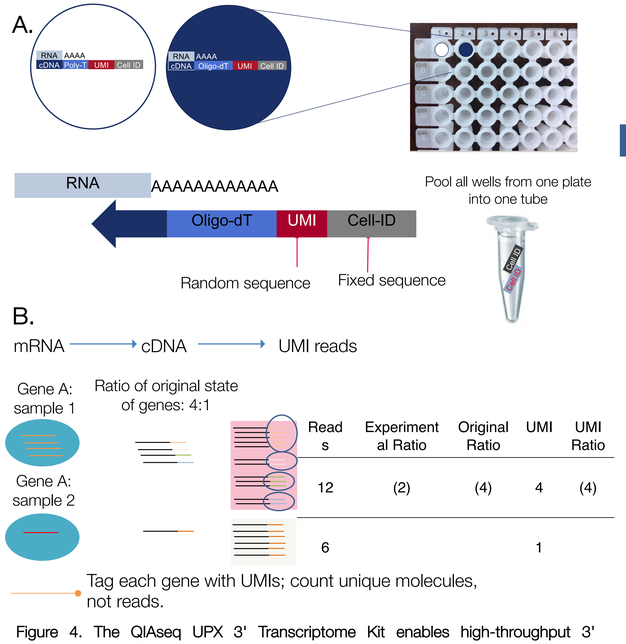

Figure 4. The QIAseq UPX 3' Transcriptome Kit enables high-throughput 3' transcriptome NGS from ultralow amounts of RNA. **A.** The combination of cell IDs and sample IDs enables up to 18,432 libraries to be sequenced together. Starting with 1–1000 cells or 10 pg to 10 ng of isolated RNA, the kit uses LNA-enhanced chemistry for increased accuracy, specificity and sensitivity **B.** Unique molecular indices (UMIs) eliminate library amplification bias for accurate gene expression by using cloud-based read alignment and single-cell analysis.

Directly dispense into PCR plates

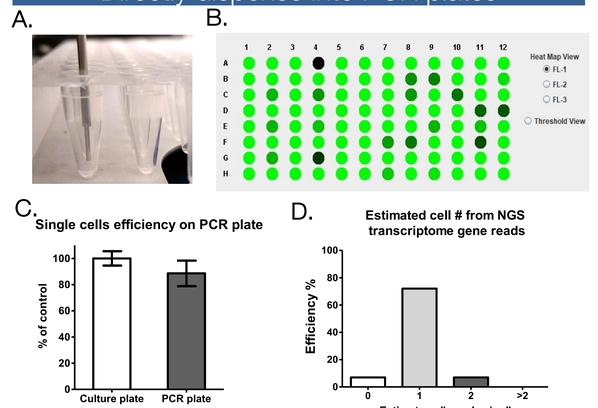

Figure 5. Sorting directly into QPCR plates. **A.** Representative Image of single-cell dispensing into a PCR plate using the N1 Single Cell module **B.** The fluorescent intensity of dispensed single cells analyzed by the WOLFViewer software. **C.** The single-cell dispensing efficiency comparison between the standard culture plates and 96-well PCR plates. **D.** Single-cell deposition rate estimated by the 3’ transcriptome profile. The cell number in each well was estimated by the number of gene reads.

Neuron clusters using scRNA-Seq

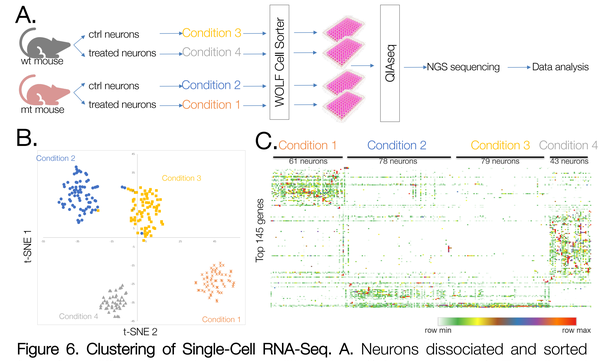

Figure 6. Clustering of Single-Cell RNA-Seq. **A.** Neurons dissociated and sorted from four different experimental conditions into QIAseq plates. **B.** T-distributed Stochastic Neighbor Embedding (t-SNE) analysis of single neuron transcriptomes from four experimental conditions. **C.** Unsupervised hierarchical clustering of gene expression levels in single neurons. Both cells (columns) and genes (rows) were clustered using average linking method after z-score adjustment of each row.

Acknowledgement

The work in NanoCellect has been supported by SBIR programs from NIH-NIGMs. We acknowledge UCSD and other collaborators for sharing their insights.