

Recent Advances in Single Cell Analysis

Jane Ru Choi*

Department of Mechanical Engineering, University of British Columbia, Vancouver, Canada

*Corresponding author: Jane Ru Choi, Department of Mechanical Engineering, University of British Columbia, Vancouver, Canada, Tel: +86-021-66135182; E-mail: janeruchoi@gmail.com

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Editorial

The nature of biology is diverse and heterogeneity always exists in different organisms, organs or tissues and even different cells. However, the heterogeneity of samples is often overlooked in most biological or clinical studies, which results in the loss of critical clinical information [1]. The majority of experimental results from cell or tissue cultures are based on the assumption that all cells in a culture are homogeneous. Given the fact that each cell holds a “unique barcode” that represents the DNA, RNA, and protein activities, it is essential to conduct omics studies at the single cell level [2].

Traditional bulk analytical methods (e.g., microscopy or flow cytometry) measure the average profile of the entire cell population, which have limitations in characterizing complex diseases such as cancer [3]. Recent advances in single-cell sequencing have led to paradigms shift in the field of genomics, away from bulk analysis and toward comprehensive studies of individual cells [4]. For instance, in cancer research, single-cell sequencing provides means to characterize intra-tumor heterogeneity in a large population of tumor cells, resolve cell-to-cell variations and identify rare cells, which opens up opportunity to determine key molecular properties that influences clinical outcomes [5]. Development of technologies for single-cell isolation, whole transcriptome amplification (WTA) [6] or whole-genome amplification (WGA) [7] along with next-generation sequencing provides the foundation that has enabled comprehensive single cell analysis. Generation of single-cell sequencing data from human cells can be described through three key steps: (1) isolation of single cells, (2) single cell sequencing and (3) bioinformatics and statistical analyses [8].

Isolating single cells from a tissue mass or from cell suspension is the first key step. The current methods of isolating single cells from abundant cell populations include manual picking, fluorescence-activated cell sorting, laser capture microdissection and microfluidics [9]. Manual picking of single cells has been employed in many protocols, such as in Smart-Seq, Smart-Seq2 and Cel-Seq library preparation. Normally, a micropipette is used to select a target single cell under a microscope [10,11]. However, compared to other single cell isolation methods, this method has poor sensitivity and is time consuming [1,12,13]. Fluorescence-activated cell

sorting has been broadly applied in many single-cell transcriptome studies [14]. Using uniquely tagged fluorophores, cell subpopulations of interest are sorted into a well plate within minutes for library preparation [15]. However, low numbers of cells (<one million) (e.g., circulating tumor cells) are not easily detected and isolated by aforementioned method. Additionally, flow cytometry is unable to process small volume of cells (i.e., several microliters) [16]. Laser capture microdissection is able to harvest cells of interest or isolate specific cells by removing unwanted tissues in either formalin-fixed paraffin-embedded (FFPE), cryostat sections using ultraviolet (UV) or infrared (IR)-coupled microscopy. A 7.5- μm spot-sized laser is pulse-fired at FFPE sections or frozen sections with the aid of a target beam to effectively isolate the single cells [17]. However, noise reduction and precision have yet to be improved during the UV/IR dissection [18].

Recent advances in microfluidic technologies (e.g., droplet microfluidics) have made it possible to sequentially process, manipulate small volume of sample and isolate single cells from bulk populations [19]. Recently, label-free cell sorting technologies including actuated cell sorting and passive cell sorting are employed to sort cells based upon their physical properties including shape, size, elasticity, polarizability, density etc. using microfluidic devices [20]. Actuated cell sorting relies upon optical, electrical or magnetic field-induced stimuli to sort cells across fluid streamlines. Passive cell sorting enables cell sorting based upon adhesion, filtration and inertial hydrodynamic forces. These technologies have enabled the high throughput sorting and capturing of single cells for downstream processes [21].

There are multiple methods available for the preparation of single cell sequencing libraries (i.e., DNA and RNA sequencing) following the process of single cell isolation. The sequencing libraries preparation process involves amplification of the genomic DNA or complementary DNA (in the case of RNA sequencing) [22]. WGA is necessary for single-cell DNA sequencing. Ideally, the procedure of WGA should have minimal sequence errors and biases. Multiple methods for WGA have been introduced such as degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR), multiple displacement amplification (MDA) and multiple annealing and looping-based amplification cycles (MALBAC).

They have their own advantages and limitations in respect to genome coverage and uniformity. Single-cell RNA sequencing requires amplification of the RNA transcripts before sequencing. Various methods of single-cell RNA sequencing have been substantially reviewed [23,24]. Recently, novel methods for single cell sequencing have been introduced, including accessible DNA regions (Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq)[25], simultaneous DNA sequencing and methylation [26] and simultaneous sequencing of DNA and RNA [27].

As for the bioinformatic and statistical analyses, single cell molecular subtyping, rare cell type detection, mutation detection, analyses of intra-tumour heterogeneity and copy number variations profiling are the most common analyses for cancer research. The data of single cell RNA sequencing have distinctly different distributional properties (e.g, zero-inflated expression distribution) compared to conventional bulk average RNA sequencing data probably due to the cell cycle effects [28]. As for the DNA sequencing, WGA typically produces data with limited genome coverage, and allelic dropout that results in the loss of one or more alleles during amplification. To date, a range of online resources and tools are available to ease the process of analyzing the data of single cell assay [29]. However, some analytical tasks still remain challenging, including comparing data sets across experimental conditions or organisms and integrating data from different genomics.

In short, single-cell analysis, in particular, single cell transcriptomic analysis has revolutionized our understanding of gene regulation networks, metastasis and the complexity of cell-to-cell heterogeneity, and this technology is expected to eventually benefit human in a way that has never been available at the bulk level. Researchers are still figuring out the way to deal with the data sets and the algorithms that are the most useful for analyzing a single cell. Further improvement of library preparation methods, single cell sequencing and bioinformatics will provide a deeper understanding of how gene regulation operates in a particular cell type.

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