Single cell biology and single cell RNA sequencing

Modeling of Complex Biological Systems

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Introduction

Instant movement within the event of next generation sequencing provided many valuable informations about complex biological systems. The facility to estimate the transcriptomes of single cells is becoming a very popular test. Single-cell analysis allow researchers to uncover modern and potentially progressive biological discoveries relative to profiling methods that assess bulk populations (sequenced RNA isolated from group of cells). Single-cell RNA sequencing (scRNA-seq) modify differentiating cells into populations in the examined tissue. Single cell RNA sequencing has emerged as technology to characterize complex tissues and to answer questions that may not be addressed using bulk RNA-seq. While many sorts of analyzes and questions is answered by sequencing single-cell RNA, the foremost goal is to be able to examine the range of cell types in an exceedingly very sample. Cataloging different types of cells requires an oversized number of cells. Technological advances and protocol refinements have contributed to a gradual, exponential increase within the amount of cells tested in RNA-seq single cell analyzes (see Figure 1 below).

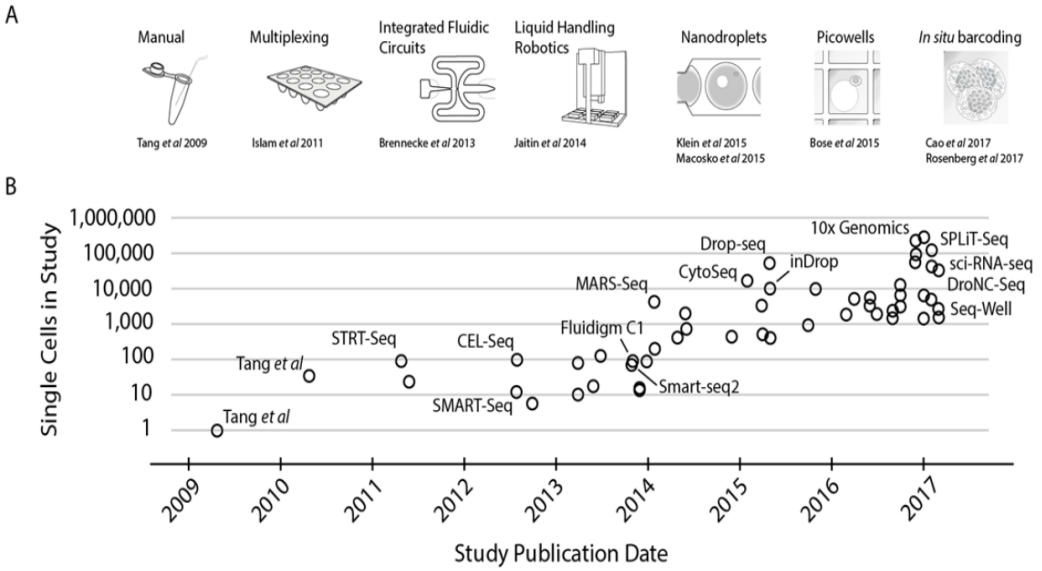

Figure 1: (A) Key technologies enabling progress in an experimental scale. (B) Cell numbers are provided in representative publications [1].

Methodology

There are different scRNA-seq methods, but all of them follow the identical basic principles. Transcriptome profiling of individual cells will be attenuated into this major components: RNA capture, RT (reverse transcription) and transcriptome amplification, library preparation for sequencing, and finally sequencing. However, creating appropriate experimental designs is difficult as each experiment requires the user to create informed decisions about sample preparation and RNA sequencing so as to realize interpretable results. When selecting methods for a given experiment, high accuracy, efficiency and value should be taken under consideration.

1. Sample preparation that include RNA capture, reverse transcription and transcriptome amplification.

   Preparation of high quality samples is essential to successful testing. High quality material is fundamental for an efficient cell capture process and optimal conduct of scRNA-seq protocols. During sample preparation (in most methods) cells are physically separated by mechanical dissection or by enzymatic degradation into an answer containing individual cells. Although high-throughput experiments use microfluidics or fluorescence activated cell sorting that's FACS. Then, certain cell types can then be optionally excluded from this solution. After the cells are trapped within the wells or drops, individual cells are lysed. the utilization of scRNA-seq normally isn't limited to specific tissues as long as poly(A)-tailed RNA is present. Poly(A) tail (after polyadenylation) could be a stretch of mRNA sequence that has only adenine bases.

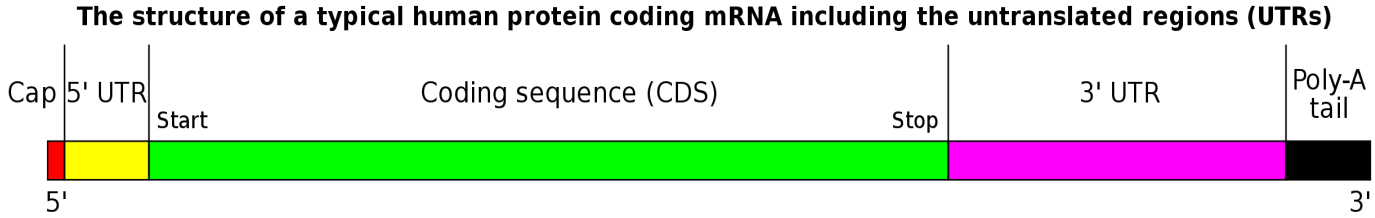

Figure 2: Structure of eukaryotic mRNA [sourced from en.wikipedia].

2. Library construction and scRNA sequencing.

   Converting RNA into sequencing-ready libraries requires captured them by poly (T) oligonucleotides, which can contain UMI sequences (Unique Molecular Identifier) and single cell specific barcodes. To enable for amplification of the RNA by polymerase chain reaction (PCR) or in vitro transcription (IVT), adaptors or T7 polymerase promoter sequences, respectively, are included within the oligonucleotides. After RT into cDNA transcriptome may be amplified (RT is a fundamental step, different protocols are optimized in numerous ways using efficient enzymes and various performance enhancement additives). So as to be transformed into sequencing libraries, the amplicons are fragmented enzymatically or mechanically. Adapters are included during the ultimate stage of amplification. Full-length sequencing are often performed, or the 5 'or 3' ends of the transcription may be selected for sequencing. The ultimate stage is paired-ed or single-end sequencing.

For better visualisation and understanding each step of scRNA-seq, Figure 3 below has been attached.

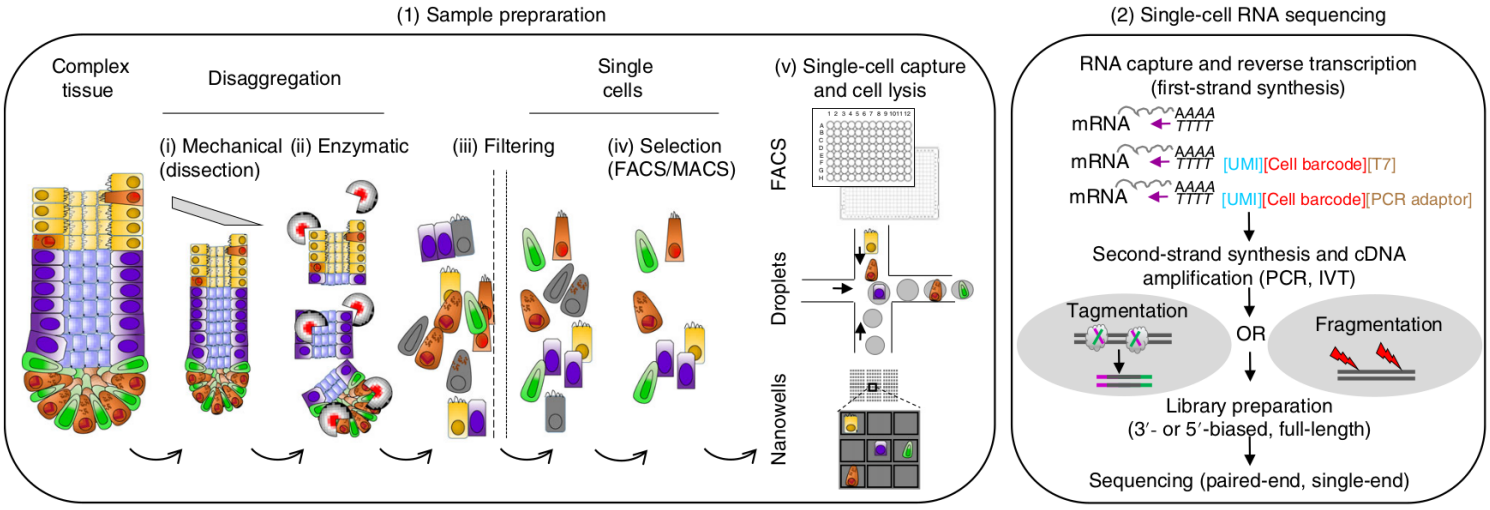

Figure 3: Single cell RNA sequencing process [2].

References

1. Svensson, V., Vento-Tormo, R. Teichmann, S. Exponential scaling of single-cell RNA-seq in the past decade. Nat Protoc 13, 599–604 (2018). https://doi.org/10.1038/nprot.2017.149

2. Lafzi, A., Moutinho, C., Picelli, S. et al. Tutorial: guidelines for the experimental design of single-cell RNA sequencing studies. Nat Protoc 13, 2742–2757 (2018). https://doi.org/10.1038/s41596-018-0073-y