REVIEW

Single-cell Transcriptome Study as Big Data

Pingjian Yu a, Wei Lin * ,b

Genomics and Bioinformatics Lab, Baylor Institute for Immunology Research, Dallas, TX 75204, USA

Received 17 November 2015; revised 9 January 2016; accepted 10 January 2016
Available online 11 February 2016

Handled by Hongxing Lei

Abstract The rapid growth of single-cell RNA-seq studies (scRNA-seq) demands efficient data storage, processing, and analysis. Big-data technology provides a framework that facilitates the comprehensive discovery of biological signals from inter-institutional scRNA-seq datasets. The strategies to solve the stochastic and heterogeneous single-cell transcriptome signal are discussed in this article. After extensively reviewing the available big-data applications of next-generation sequencing (NGS)-based studies, we propose a workflow that accounts for the unique characteristics of scRNA-seq data and primary objectives of single-cell studies.

Introduction

Multi-institutional collaborative omics studies on the next-generation sequencing (NGS) platform have generated petabytes of data that constitute ‘big data’ from the perspective of scale and complexity [1–6]. Particularly, transcriptomics studies using the RNA-seq technique have become revolutionary and powerful [7–9]. Scientists have now moved one step forward to single-cell RNA sequencing (scRNA-seq) by employing new protocols for single cell isolation, low-input RNA extraction, reverse transcription, and unbiased amplification [9–13]. Given the high anticipated value of single-cell transcriptomics, explosive growth of scRNA-seq data is expected in the next 5–10 years. Consequently, uncovering the hidden pattern, connectivity, and interactions of such huge and heterogeneous data will be a major challenge.

Without a doubt, the detailed and extremely-valuable information that single-cell technology provides is at a significant cost due to sophisticated data acquisition, large data-storage requirements, as well as challenging data processing and management. Big data incorporate a body of technologies including computational parallelization and distribution, data visualization, and data integration that are used to reveal the hidden associations within large datasets that are diverse, complex, and of a massive scale. Data-intensive scientific discovery has been proposed as the 4th paradigm of scientific research [14], following and interacting with the other three paradigms – theory, experimentation, and simulation modeling. In 2001, Doug Laney defined characteristics of big data in three dimensions, i.e., increasing volume (amount of data), velocity (speed of data I/O), and variety (range of data types and sources) [15]. While agreeing that volume, variety, and velocity are the quantitative characteristics of big data, Ivanov et al. [16] added that variability (the inconsistency the data can show over time) and veracity (the quality of captured data) are the qualitative characteristics of big data.
Big-data technology has many applications in biomedical research [17–20]. Particularly, high-throughput molecular and functional profiling of patients using NGS or single-cell technology is the key driving force of precision medicine [21–24]. By examining the annual growth of scRNA-seq datasets uploaded to the NCBI Gene Expression Omnibus (GEO) database [25] and the increasing number of new articles in PubMed over the past 7 years that involve scRNA-seq and big-data (Figure 1), we expect the extensive integration of big data and scRNA-seq technologies.

In the following sections, we will discuss the characteristics of single-cell transcriptomics, especially scRNA-seq, data as examples of big data. We will discuss how to adapt single-cell transcriptomics study to big-data infrastructure such as Hadoop and MapReduce.

**Transcriptional stochasticity and cellular heterogeneity**

scRNA-seq is always compared to bulk RNA-seq in terms of signal profile and noise level. In addition to the descriptive key-
word like high resolution, stochasticity and heterogeneity are also frequently used to feature the single-cell transcription [26–29]. Most of the scRNA-seq investigators have experience with zero-inflation transcriptional signals. Some of them tend to regard this phenomenon as technical dropout. We prefer to use the phrase ‘bimodality’ to delineate the signal distribution, since recent results have shown that the low transcriptional values are biologically meaningful signals rather than technical dropout. Shalek et al. have revealed the bimodality of single-cell expression and splicing using both scRNA-seq and RNA fluorescence in situ hybridization (RNA-FISH) [30]. The two modes in an expression profile can be attributed to the ‘on’ or ‘off’ transcriptional status. Figure 2 demonstrates two clusters of cells showing different expression level and the change of the ratio of on/off status of a marker gene MYH2 over time during human myoblast cell differentiation using both scRNA-seq and RNA-FISH [31]. The aforementioned studies indicate that even from a seemingly homogeneous population, many genes are expressed in a stochastically-bursting fashion and their abundance exhibits a bimodal distribution in the cell population examined. The traditional RNA-seq analysis method rarely takes such transcriptional bimodality into account. Further investigation on co-bursting networks have validated the biological significance of the ‘bimodality’ rather than just relegating it to technical dropout [31].

Several computational models have been proposed to analyze transcriptional stochasticity and cellular heterogeneity in scRNA-seq data in the context of zero-inflation or bimodality. Kim and Marioni [32] use a mixture of two Poisson distributions to model theoretical kinetics for ‘bursty’ gene expression. However, in the presence of massive variability, the model is compromised by excessive over-dispersion in read counts. Kharchenko et al. take the probability of “dropout” into consideration in their differential-expression algorithm [33]. Person and Yau proposed using zero-inflated factor analysis to perform dimensionality reduction [29]. Gu et al. use a mixture of two negative binomial distributions to model over-dispersed read counts generated from a gene’s two distinct biological states: an ‘on’ component and an ‘off’ component [31]. All of these four studies acknowledge the fact that single-cell transcription signals cannot be solved by unimodal statistics. Gu et al. first introduced the statistics term “bimodal proportion” to measure the ratio of two signal modes in a single-cell population. The functional enrichment of co-bursting transcription supports the biological significance of transcriptional bursting over technical dropout. The value of “bimodal proportion” ranges from 0 to 1 and notably, it can be compared across different datasets without additional normalization.

**The opportunities and challenges of scRNA-seq**

Single-cell transcriptomics provides us unprecedented opportunity to understand the transcriptional stochasticity and cellular heterogeneity in great detail, which are crucial for maintaining cell functions and for facilitating disease progression or treatment response [34–38]. Such stochasticity and heterogeneity are always masked in bulk-cell studies [27]. Recent single-cell applications have utilized a broad range of tissues [28,39–42], stem cell lines [43,44] and cell populations with clinical backgrounds [45]. The cell types that have been interrogated using scRNA-seq in the GEO database are briefly summarized in Table 1.

scRNA-seq is one of the most promising technologies for single-cell transcriptomics [46,47]. Nevertheless, it also poses big challenges, largely stemming from the aforementioned big-data characteristics with regard to the data management, query, and analysis. There are five ‘V’s to consider for scRNA-seq data. (1) Volume. NGS data has become one of the largest big-data domains in terms of data acquisition,
Just like bulk-cell RNA-seq and other NGS-based studies, scRNA-seq generates a high volume of raw sequencing data and high-dimensional transformed expression data. Moreover, due to the heterogeneity of cell populations, a typical scRNA-seq study usually incorporates hundreds or even thousands of cells and thus adds a few more orders of magnitude to the data volume. (2) Velocity. As aforementioned, the data volume of scRNA-seq is higher than that of bulk-cell RNA-seq. Consequently, high data-transfer bandwidth, parallel algorithms, and high-performance computers are required to generate and process data. (3) Variety. An scRNA-seq study may combine data from different single-cell isolation chips, protocols, and research environments. How to normalize the datasets and make them comparable becomes a major issue. (4) Variability. The transcriptional activity of a living cell is dynamic rather than static. Thus, scRNA-seq captures a snapshot of single cells in seemingly homogeneous populations that as a matter of fact, vary significantly from one to another. Substantial variability of the scRNA-seq signal comes from a variety of biological aspects, including transcriptional stochasticity and cellular heterogeneity, which cannot be investigated in bulk-cell studies. Therefore, scRNA-seq data exhibit significantly larger variance than bulk-cell RNA-seq data [33]. Solving the biological variability is the main goal of single-cell transcriptomics research. (5) Veracity. scRNA-seq is composed of sequential steps of target cell isolation, RNA extraction, fragmentation, reverse transcription, cDNA amplification, sequencing, alignment, and read counting. Every step introduces biases and artifacts that may significantly affect the coverage, accuracy, and timeliness of transcript expression and thus interfere with both the proper characterization and quantification of transcripts. It is therefore critical to control the data quality prior to including the datasets in a meaningful global study.

Table 1 Summary of cell types in GEO datasets

| Cell type     | No. of datasets |
|---------------|----------------|
| Neuron        | 11             |
| Embryonic     | 80             |
| Blood         | 18             |
| Lung          | 17             |
| Renal         | 4              |
| Brain         | 17             |
| Skin          | 26             |
| Heart         | 9              |
| Bone marrow   | 17             |
| Stem cell     | 43             |
| Tumor         | 23             |
| Cell line     | 71             |
| Total No. of unique datasets | 195 |

Figure 2 MYH2 gene is the marker of mature myotubes
The increased bulk expression of MYH2 is primarily driven by the growing proportion of “on-” component cells (upper cluster) over time (0, 24, 48, and 72 h after myoblast differentiation is induced). Figures were derived from the dataset in Trapnell et al [41]. A. The growth of MYH2 expression in bulk cell replicate samples (n = 3 over time). B. Beeswarm plots of the growing bimodal proportion of MYH2 from scRNA-seq over time. C–F. RNA-FISH signals at 0, 24, 48, and 72 h, respectively. MYH2 and nucleus are shown in red and blue (DAPI staining), respectively. Scare bar: 25 nm. G. MYH2 RNA molecule counts per cell over time, based on RNA-FISH analyses. RNA-FISH, RNA-fluorescence in situ hybridization.
Due to the much lower starting amount of RNA in a single cell, it takes more cycles of amplification using a template-switching strategy, compared to the bulk-cell sequencing [49,50] and thus introduces much larger technical variations to the scRNA-seq data. Technical variations in scRNA-seq include but not limit to the ones introduced by RNA extraction, transcript fragmentation, reverse transcription, PCR amplification, sequencing sampling, sequencing error, short-read mapping error, and miscount. Because the technical variation introduced during earlier steps will be carried over to the later steps and even be amplified further, it is critical to control the technical variations in the earlier steps. Artificial RNA molecules such as the External RNA Controls Consortium spike-in molecules (ERCC) can be doped into the assayed RNA samples at the same level. Since there is no expected biological variation for the ERCC transcriptions in the samples, the variation in the ERCC quantification measurements in the scRNA-seq will be due to technical variability. This is a reliable way to quantify technical variation in scRNA-seq [51]. Technical variations may confound with biological variations, and we can only observe total variation in gene expression. Efforts have been made to distinguish the technical variation from biological variation in scRNA-seq by computational methods with or without ERCC control [52,53].

The best efforts at mitigating the technical variations have been made by protocol modification. Saliba et al. [10] and Kołodziejczyk et al. [54] have reviewed a variety of single-cell RNA-seq techniques. Besides including external molecule controls, improved single-cell chemistry and physics [8,9,55], as well as incorporation of molecular barcoding system [56], have significantly reduced the noise level within each study.

### Big data—the norm of NGS technology

A typical RNA-seq study on the most popular NGS platform such as the Illumina HiSeq 2500 usually generates hundreds of gigabytes (GB) of raw read data. It usually takes hours to align these raw reads to the human or other mammalian reference genomes. The NGS throughput and computer processors are in a race and the growth of NGS data always seems to win. Moreover, a robust data storage, management and analysis framework is in need.

The National Center for Biotechnology Information (NCBI) hosts RNA-seq data using two data storage/sharing platforms, *i.e.,* Gene Expression Omnibus database (GEO) [25,58] and Sequence Read Archive database (SRA) [59]. Both of these databases provide comprehensive metadata structure, including information about the data producer, study design, sample description, technical details, keywords, *etc.* The metadata that they collect has been considered as data-sharing standards and the overall bioinformatics infrastructure in a big-data system.

Apache Hadoop is an open-source software framework for distributed storage and distributed processing of very large datasets on computer clusters. The key modules in the Apache Hadoop framework are the Hadoop Distributed File System (HDFS) and Hadoop MapReduce. Apache Hadoop uses its HDFS to store data on commodity machines, providing very high aggregate bandwidth across the cluster. In addition, Apache Hadoop implements MapReduce technology [60] to decompose a large-scale problem into small independent sub-problems and schedule the sub-problems to computer clusters. MapReduce allows the development of approaches that can handle larger volumes of data using a larger number of processors simultaneously. By utilizing parallel-based approaches, Apache Hadoop improves the flexibility and scalability of computer clusters.

scRNA-seq utilizes the most common short-read mapping, as well as data storage and query procedures of common NGS applications. The Hadoop-based bioinformatics applications [61–91] are reviewed in Table 2. To our best knowledge, there is no Hadoop application specially designed for scRNA-seq so far. Given the unique bimodal signal profile of scRNA-seq data, the long-used unimodal statistics in bulk RNA-seq cannot satisfy the need to determine differential expression in scRNA-seq. It has also been validated that change of bimodal proportion/burst frequency as well as the coordination of transcriptional bursts are biologically meaningful [31]. Thus the new analytic components should be included when mining the scRNA-seq data in the big-data domain.

A robust normalization underlies the success of analyses across datasets. The goal of using a big-data approach for scRNA-seq studies is not just to take full advantage of the computational resources on the cloud but also to integrate the sample power of multiple single-cell datasets to uncover the global associations and the molecular mechanisms that maintain the cellular function of biological systems. Reference like ERCC for signal normalization, as discussed above, can be added on the bench side. On the computational side, signal-rescaling algorithms (Table 3) based on the putative abundance of internal references (e.g., ERCC and housekeeping genes) can be implemented. Reads per million mapped reads (RPM), reads per kilobase per million mapped reads (RPKM) [92], median, and upper-quantile normalizations [93] rescale the raw counts based on mean, mean with gene length considered, median, and upper-quantile of read counts, respectively, in a sample. Full-quantile normalization [94] aligns all quantiles of the count distributions among samples. Other than direct comparison of the rescaled RNA abundance signal across samples, dataset normalization also involves a variety of analyses, including statistical modeling and hypothesis testing that are used to delineate and compare the read-count-based profiles of samples and datasets (Table 3). GC-content [95], DESeq [96], trimmed mean of M values (TMM) [97], remove unwanted variation (RUV) [98], Poisson beta [32], and Sphinx [31] utilize statistical modeling to infer normalized read counts. Owing to the distribution assumed, DESeq, TMM, RUV, Poisson beta, and Sphinx allow over-dispersion on read counts. In particular, Poisson beta and Sphinx can identify transcriptional status through bimodality, which characterizes the single-cell RNA-seq signal profiles. Because of the regression method used, GC-content, DESeq, TMM, and RUV can model batch effect and other known factors such as cycles of PCR amplification and length distribution of fragments.

We hereby propose a workflow for inter-institutional scRNA-seq data integration and analysis (Figure 3). The workflow consists of four layers: Hadoop, normalization, analysis, and verification. (1) Hadoop layer. Inter-institutional scRNA-seq data is stored and managed in this layer using HDFS. Parallel algorithms, such as short-read alignment and read count per transcript algorithms, can be implemented under Hadoop framework provided in this layer. (2)
| Function                        | Name            | Weblink                                      | Description                                                                 | Ref. |
|--------------------------------|-----------------|----------------------------------------------|-----------------------------------------------------------------------------|------|
| Sequence file management       | LFQC            | [link](http://engr.uconn.edu/rajasek/lfqc-v1.1.zip) | A lossless compression algorithm for FASTQ files                            | 61   |
| Quake                          | [link](http://www.cbcb.umd.edu/software/quake) | Quality-guided error detection and correction of short reads                  | 62   |
| SeqPig                         | [link](http://sourceforge.net/projects/seqpig) | Simple and scalable scripting for large sequencing datasets                    | 63   |
| Hadoop-BAM                     | [link](http://sourceforge.net/projects/hadoop-bam/) | Library for scalable manipulation of aligned NGS data                        | 64   |
| smallWig                       | [link](http://publish.illinois.edu/milenkovic/) | Parallel compression of RNA-seq WIG files                                    | 65   |
| Search engine                  | SeqWare         | [link](http://seqware.sourceforge.net)       | Pipeline and query engine for storing and searching sequence                | 66   |
| Hydra                          | [link](http://code.google.com/p/hydra-proteomics/) | A protein sequence database search engine                                    | 67   |
| SparkSeq                       | [link](https://bitbucket.org/mwiewiorka/sparkseq/) | Interactive data querying of genomic data analysis                           | 68   |
| GMQL                           | [link](http://www.bioinformatics.deib.polimi.it/GMQL/) | Large-scale genomic data query and management                                | 69   |
| Genomic sequence mapping       | CloudAligner    | [link](http://mine.cs.wayne.edu:8080/CloudAligner/) | A MapReduce-based application for short read alignment                      | 70   |
| CouldBurst                     | [link](http://cloudburst-bio.sourceforge.net/) | A parallel short read mapper                                                  | 71   |
| BigBWA                         | [link](https://github.com/citiususc/BigBWA) | Hadoop implementation of BWA                                                  | 72   |
| SEAL                           | [link](http://biodoop-seal.sourceforge.net/) | Alignment, manipulation, and analysis of short reads                         | 73   |
| DistMap                        | [link](http://code.google.com/p/distmap/) | A toolkit for distributed short read mapping                                  | 74   |
| SOAP3                          | [link](http://www.cs.hku.hk/2bwt-tools/soap3) | Short sequence read alignment with GPU acceleration                          | 75   |
| GPU-BLAST                      | [link](http://archimedes.cheme.cmu.edu/biosoftware.html) | NCBi-BLAST with GPU acceleration                                              | 76   |
| Expression analysis            | Myrna           | [link](http://bowtie-bio.sf.net/myrna)       | RNA sequencing differential expression analysis                            | 77   |
| Eoulsan                        | [link](http://transcriptome.ens.fr/eoulsan/) | Pipeline for calculating differential gene expression                        | 78   |
| YunBe                          | [link](http://tinyurl.com/yunbedownload) | A gene set analysis algorithm for biomarker identification                    | 79   |
| FX                             | [link](http://fx.gmi.ac.kr) | Gene expression estimation and genomic variant calling                        | 80   |
| Phylogenetic analysis          | FVGWAS          | [link](http://www.nitrc.org/projects/fvgwas) | Fast voxel-wise genome-wide association analysis                            | 81   |
| GATK                           | [link](http://www.broadinstitute.org/gsa/wiki/index.php/The_Genome_Analysis_Toolkit) | Variant calling                                                              | 82   |
| Crossbow                       | [link](http://bowtie.bio.sourceforge.net/crossbow/) | Alignment and SNP genotyping with Bowtie and SoapSNP                        | 83   |
| MrsRF                          | [link](http://mrsrf.googlecode.com) | Calculate Robinson–Foulds distance between trees                             | 84   |
| BlueSNP                        | [link](http://github.com/ibm-bioinformatics/bluesnp) | Genome-wide association studies using Hadoop clusters                        | 85   |
| GeneCOST                       | [link](www.igbam.bilgem.tubitak.gov.tr/en/softwares/genecost-en/index.html) | Scoring-based prioritization to identify disease-causing genes               | 86   |
| Nephele                        | [link](http://code.google.com/p/nephele/) | Genotyping via complete composition vector                                   | 87   |
| Miscellaneous                  | PeakRanger      | [link](http://www.modencode.org/software/ranger/) | A cloud-enabled peak caller for ChIP-seq data                              | 88   |
| SeqHBase                       | [link](http://seqhbase.omicspace.org) | A big-data toolset for family-based sequencing data analysis                 | 89   |
| ProKinO                        | [link](http://vulcan.cs.uga.edu/prokino) | A unified resource for mining the cancer kinome                              | 90   |
| BioPig                         | [link](https://sites.google.com/a/ibl.gov/biopig/) | An analytic toolkit for large-scale sequence data                            | 91   |
Normalization layer. To make the single-cell expression profiles comparable across different studies or even across different chips/runs for the same study, normalization is not just the most important task but also the biggest challenge. Normalization covers the analyses that are performed to control the cross-assay technical variation. Nonetheless, different normalization strategies should be extensively tested and compared.

As discussed, single-cell RNA-seq exhibits unique bimodal transcriptional profiles that can be resolved into “on” and “off” components. This unique transcriptional pattern distinguishes the single-cell RNA-seq analyses from traditional bulk-cell RNA-seq and provides a naturally normalized signal profile for comparison. (3) Analysis layer. In this layer, the normalized single-cell gene expression profiles are loaded as input. The output of the analysis is the target gene sets that drive the divergence of the cellular phenotypes or experimentally-controlled cellular groups. Determination of differential expression and co-expression, as well as biclustering will be implemented in this layer to identify the pattern in gene expression profiles. The target gene sets or the classification of the cell populations will be further interpreted in the verification layer. (4) Verification layer. In this layer, the biological significance of the input gene set will be analyzed, interpreted, and verified using tools such as the gene set enrichment analysis (GSEA), and gene ontology (GO)-term enrichment analysis, as well as the database for annotation, visualization and integrated discovery (DAVID) functional analysis, etc.

Outlook

Big data and scRNAseq are two rapid-growing technologies. Big data not only can provide the framework to host, process, transform, and visualize the data from different sources, but also can increase the sample power by including comprehensive sample descriptions and ruling out cross-study batch effects. Notably, the big-data framework offers the opportunity to identify significant correlation in new dimensionalities, with the sample power that cannot be reached by individual studies on these dimensionalities. One possible application of big data for scRNA-seq is in mammalian single-cell studies, which are often associated with the origin of cells from different body parts. This means the assayed single cells can be mapped spatially. The atlas of cell phenotypes or interactive behaviors can be further explored in this way. This spatial data infrastructure has been widely used in geoinformatics and has now become a popular methodology of big data. For instance, the Human Protein Atlas project is one of the research efforts that is taking the idea to the protein level [99]. As the vehicles of the DNA, RNA, and protein molecules, single cells carry the molecular signature of the phenotypic and functional elements. They should also be able to be systematically assayed and organized in the big-data domain.

Many diseases, especially cancer, are heterogeneous when considered from two different perspectives. On the one hand, cancer tissues are heterogeneous and thus require the high-resolution information that can be obtained from single-cell technology. On the other hand, certain cancer categories are actually defined from a heterogeneous patient population that requires personalized solutions. Big-data technology has been recognized by Doudican et al. for its ability to inform personalized therapeutics [100]. Irish and Doxie have recently

---

**Table 3: Read count normalization methods**

| Name    | Normalization method | Assumed distribution | Parameter estimation | Over-dispersion capability | Gene status identification capability | Correction factor | Sequecing depth | Gene length | GC content | Batch |
|---------|----------------------|----------------------|----------------------|-----------------------------|---------------------------------------|------------------|----------------|-------------|------------|--------|
| RPM     | Rescale              | N/A                  | GLM                  | No                          | No                                    | No               | No             | No          | No         | No     |
| RPKM    | Rescale              | N/A                  | GLM                  | No                          | No                                    | Yes              | Yes            | Yes         | Yes        | Yes    |
| Median  | Rescale              | N/A                  | GLM                  | No                          | No                                    | No               | Yes            | Yes         | Yes        | Yes    |
| Full-quantile Rank average | Non-parametric | GLM                  | Local regression | Yes            | Yes                                    | No               | No             | No          | No         | No     |
| GC-content | Statistical model | Non-parametric | GLM                  | Yes                         | Yes                                    | No               | No             | No          | No         | No     |
| DESeq   | Statistical model    | Negative binomial    | GLM                  | No                          | No                                    | No               | No             | No          | No         | No     |
| TMM     | Statistical model    | Negative binomial    | GLM                  | No                          | No                                    | No               | No             | No          | No         | No     |
| RUV     | Statistical model    | Negative binomial    | Bayesian             | Yes                         | Yes                                    | Yes              | No             | No          | No         | No     |
| Sphinx  | Statistical model    | Mixed negative binomial | Bayesian             | Yes                         | Yes                                    | Yes              | No             | No          | No         | No     |

Note: RPM, reads per million mapped reads; RPKM, reads per kilobase per million mapped reads; TMM, trimmed mean of M values; RUV, remove unwanted variation; GLM, generalized linear model.
reviewed the progress of applying single-cell technology to cancer biology [101], and the advancements are significant. The big-data infrastructure of the ever-increasing number of single-cell RNA-seq datasets will eventually facilitate the decisions that are based on the comparison of clinical sample characteristics at a higher resolution, as well as interrogation of previous treatment responses within larger datasets.

Competing interests

The authors have declared no competing interests.

Acknowledgments

This work was supported by Baylor Research Institute start-up funding, USA to WL. We thank Dr. Carson Harrod for editing the manuscript.

References

[1] 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, et al. An integrated map of genetic variation from 1,092 human genomes. Nature 2012;491:56–65.

[2] Genome 10K Community of Scientists. Genome 10K: a proposal to obtain whole-genome sequence for 10,000 vertebrate species. J Hered 2009;100:659–74.

[3] Harrow J, Frankish A, Gonzalez JM, Tapanari E, Diekhans M, Kokocinski F, et al. GENCODE: the reference human genome annotation for The ENCODE Project. Genome Res 2012;22:1760–74.

[4] Gerstein MB, Lu ZJ, Van Nostrand EL, Cheng C, Arshinoff BI, Liu T, et al. Integrative analysis of the Caenorhabditis elegans genome by the modENCODE project. Science 2010;330:1775–87.

[5] Mouse ENCODE Consortium, Stamatoyannopoulos JA, Snyder M, Hardison R, Ren B, Gingeras T, et al. An encyclopedia of mouse DNA elements (Mouse ENCODE). Genome Biol 2012;13:418.

[6] Cancer Genome Atlas Research Network, Weinstein JN, Collisson EA, Mills GB, Shaw KRM, Ozenberger BA, et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet 2013;45:1113–20.

[7] Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 2009;10:57–63.

[8] Ossolak F, Milos PM. RNA sequencing: advances, challenges and opportunities. Nat Rev Genet 2011;12:87–98.

[9] Hashimshony T, Wagner F, Sher N, Yanai I. CEL-Seq: single-cell RNA-Seq by multiplexed linear amplification. Cell Rep 2012;2:666–73.

[10] Saliba A-E, Westermann AJ, Gorski SA, Vogel J. Single-cell RNA-seq: advances and future challenges. Nucleic Acids Res 2014;42:8845–60.
[11] Shintaku H, Nishikii H, Marshall LA, Kotera H, Santiago JG. On-chip separation and analysis of RNA and DNA from single cells. Anal Chem 2014;86:1953–7.

[12] Nawy T. Single-cell sequencing. Nat Methods 2014;11:18.

[13] Lasken RS. Single-cell genomic sequencing using Multiple Displacement Amplification. Curr Opin Microbiol 2007;10:510–9.

[14] Tolle KM, Tansley DSW, Hey AJG. The fourth paradigm: data-intensive scientific discovery [Point of view]. Proc IEEE 2011;99:1334–7.

[15] Laney D. 3D data management: controlling data volume, velocity and variety. META Group Res Note 6 2001:70.

[16] Ivanov T, Korfatis N, Zicari R. On the inequality of the 3V’s of Big Data Architectural Paradigms: a case for heterogeneity. ArXiv Prepr 2013, arXiv:1311.0805.

[17] Costa FF. Big data in biomedicine. Drug Discov Today 2014;19:433–40.

[18] O’Driscoll A, Daugelaite J, Slator RD. “Big data”, Hadoop and cloud computing in genomics. J Biomed Inform 2013;46:774–81.

[19] Zou Q, Li XB, Jiang WR, Lin ZY, Li GL, Chen K. Survey of MapReduce frame operation in bioinformatics. Brief Bioinform 2014;15:637–49.

[20] Taylor RC. An overview of the Hadoop/MapReduce/HBase framework and its current applications in bioinformatics. BMC Bioinformatics 2010;11:S1.

[21] Yadav SS, Li J, Lavery HJ, Yadav KK, Tewari AK. Next-generation sequencing technology in prostate cancer diagnosis, prognosis, and personalized treatment. Urol Oncol 2015;33:el–13.

[22] Vicini P, Fields O, Lai E, Litwack E, Martin A-M, Morgan T, et al. Precision medicine in the age of big data: the present and future role of large-scale unbiased sequencing in drug discovery and development. Clin Pharmacol Ther 2016;99:198–207.

[23] Zhang X, Zhang C, Li Z, Zhong J, Weiner LP, Zhong JF. Investigating evolutionary perspective of carcinogenesis with single-cell transcriptome analysis. Chin J Cancer 2013;32:636–9.

[24] Campton DE, Ramirez AB, Nordberg JJ, Drovetto N, Clein AC, Varshavskaya P, et al. High-recovery visual identification and single-cell retrieval of circulating tumor cells for genomic analysis using a dual-technology platform integrated with automated immunofluorescence staining. BMC Cancer 2015;15:360.

[25] Edgar R. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002;30:207–10.

[26] Buettner T, Natarajan KN, Casale FP, Proserpio V, Scialdone A, Theis FJ, et al. Computational analysis of cell-to-cell heterogeneity in single-cell RNA-sequencing data reveals hidden subpopulations of cells. Nat Biotechnol 2015;33:155–60.

[27] Marinov GK, Williams BA, McCue K, Schroth GP, Gertz J, Myers RM, et al. From single-cell to cell-pool transcriptomes: stochasticity in gene expression and RNA splicing. Genome Res 2014;24:496–510.

[28] Pollen AA, Nowakowski TJ, Shuga J, Wang X, Leyrat AA, Lui JH, et al. Low-coverage single-cell mRNA sequencing reveals cellular heterogeneity and activated signaling pathways in developing cerebral cortex. Nat Biotechnol 2014;32:1053–8.

[29] Pierson E, Yau C. ZIFA: dimensionality reduction for zero-inflated single-cell gene expression analysis. Genome Biol 2015;16:241.

[30] Shalek AK, Satija R, Adiconis X, Gertner RS, Gabdoullame JT, Raychowdhury R, et al. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature 2015;498:637–42.

[31] Gu J, Du Q, Wang X, Yu P, Lin W. Sphinx: modeling transcriptional heterogeneity in single-cell RNA-Seq. bioRxiv 2015. http://dx.doi.org/10.1101/027870.

[32] Kim JK, Marioni JC. Inferring the kinetics of stochastic gene expression from single-cell RNA-sequencing data. Genome Biol 2013;14:R7.

[33] Shalek AK, Satija R, Shuga J, Trombetta JJ, Gennert D, Lu D, et al. Single-cell RNA-seq reveals dynamic paracrine control of cellular variation. Nature 2014;510:363–9.

[34] Kowalczynk MS, Tirol H, Heckl D, Rao TN, Dixit A, Haas BJ, et al. Single-cell RNA-seq reveals changes in cell cycle and differentiation programs upon aging of hematopoietic stem cells. Genome Res 2015;25:1860–72.

[35] Peterson EA, Bauer MA, Chavan SS, Ashby C, Weinhold N, Heuck CJ, et al. Enhancing cancer clonality analysis with integrative genomics. BMC Bioinformatics 2015;16:S7.

[36] Freeman BT, Jung JP, Ogbe BM. Single-cell RNA-Seq of bone marrow-derived mesenchymal stem cells reveals unique profiles of lineage priming. PLoS One 2015;10:e0136199.

[37] Min JW, Kim WJ, Han JA, Jung YJ, Kim KT, Park WY, et al. Identification of distinct tumor subpopulations in lung adenocarcinoma via single-cell RNA-seq. PLoS One 2015;10:e0155817.

[38] Kim KT, Lee HW, Lee HO, Kim SC, Seo YJ, Chung W, et al. Single-cell mRNA sequencing identifies subclonal heterogeneity in anti-cancer drug responses of lung adenocarcinoma cells. Genome Biol 2015;16:127.

[39] Treutlein B, Brownfield DG, Wu AR, Neff NF, Mantalas GL, Espinoza FH, et al. Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. Nature 2014;509:371–5.

[40] Usoskin D, Furlan A, Islam S, Abdou H, Lönnberg P, Lou D, et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. Nat Neurosci 2015;18:145–53.

[41] Trapnell C, Cacchiarelli D, Grimsby J, Pokharel P, Li S, Morse M, et al. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. Nat Biotechnol 2014;32:381–6.

[42] Jaitin DA, Kenigsberg E, Keren-Shaul H, Elefant N, Paul F, Zaretzky I, et al. Massively parallel single-cell RNA-seq for marker-free composition of tissues into cell types. Science 2014;343:776–9.

[43] Liu N, Liu L, Pan X. Single-cell analysis of the transcriptome and its application in the characterization of stem cells and early embryos. Cell Mol Life Sci 2014;71:2707–15.

[44] Yan L, Yang M, Guo H, Yang L, Wu J, Li R, et al. Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat Struct Mol Biol 2013;20:1131–9.

[45] Henley BM, Williams BA, Srinivasan R, Cohen BN, Xiao C, Mackey EDW, et al. Transcriptional regulation by nicotine in dopamine neurons. Biochem Pharmacol 2013;86:1074–83.

[46] Hebenstreit D. Methods, challenges and potentials of single cell RNA-seq. Biology 2012;1:658–67.

[47] Tang F, Barbacioru C, Nordman E, Li B, Xu N, Bashkirov VI, et al. RNA-Seq analysis to capture the transcriptome landscape of a single cell. Nat Protoc 2010;5:516–35.

[48] Stephens ZD, Lee SY, Faghri F, Campbell RH, Zhai C, Efron MB, et al. Big data: astronomical or genomical? PLoS Biol 2015;13:e1002195.

[49] Zhu YY, Machleder EM, Chenchik A, Li R, Siebert PD. Reverse transcriptase template switching: a SMART approach for full-length cDNA library construction. Biotechniques 2001;30:892–7.

[50] Picelli S, Björklund ÅK, Faridani OR, Sagasser S, Winberg G, Sandberg R. Smart-seq2 for sensitive full-length transcriptome profiling in single cells. Nat Methods 2013;10:1096–9.

[51] Ding B, Zheng L, Zhu Y, Li N, Jia H, Ai R, et al. Normalization and noise reduction for single cell RNA-seq experiments. Bioinformatics 2015;31:2225–7.

[52] Kharchenko PV, Silberstein L, Scaddden DT. Bayesian approach to single-cell differential expression analysis. Nat Methods 2014;11:740–2.
[96] Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol 2010;11:R106.

[97] Robinson MD, McCarthy DJ, Smyth GK. EdgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010;26:139–40.

[98] Rio D, Ngai J, Speed TP, Dudoit S. Normalization of RNA-seq data using factor analysis of control genes or samples. Nat Biotechnol 2014;32:896–902.

[99] Uhlen M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Tissue-based map of the human proteome. Science 2015;347:1260419.

[100] Doudican NA, Kumar A, Singh NK, Nair PR, Lala DA, Basu K, et al. Personalization of cancer treatment using predictive simulation. J Transl Med 2015;13:43.

[101] Irish JM, Doxie DB. High-dimensional single-cell cancer biology. Curr Top Microbiol Immunol 2014;377:1–21.