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Review

Applications and techniques of single-cell RNA sequencing across diverse species

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Abstract

Single-cell ribonucleic acid sequencing (scRNA-seq) is an important tool in molecular biology, allowing transcriptomic profiling at the single-cell level. This transformative technology has provided unprecedented insights into cellular heterogeneity, lineage differentiation, and cell-type-specific gene expression patterns, significantly advancing our understanding of complex biological systems. scRNA-seq is broadly applied across various fields, including oncology, where it sheds light on intratumoral heterogeneity and precision medicine strategies, and developmental biology, where it uncovers cellular trajectories in both model and non-model organisms. Additionally, scRNA-seq has been instrumental in ecological genomics, which can help elucidate cellular responses to environmental perturbations and species interactions. Despite these advancements, several challenges remain, particularly technical and financial barriers, limiting its application to non-model organisms and tissues with complex cellular compositions. Addressing these issues will require continued innovation in single-cell isolation methods, cost-effective sequencing technologies, and sophisticated bioinformatics tools. As scRNA-seq advances, it can deepen our understanding of biological systems, with broad implications for personalized medicine, evolutionary biology, and ecological research.

Keywords: single-cell RNA sequencing; non-model organisms; cell differentiation; applications

Introduction

Single-cell ribonucleic acid sequencing (scRNA-seq) has revolutionized our understanding of complex biological systems by allowing the analysis of genetic information at a single-cell resolution [1]. This innovative technology extends the capability of RNA-seq, which has significantly advanced the study of gene expression by quantifying RNA molecules in large cell populations [2]. Developed alongside next-generation sequencing (NGS) technologies, RNA-seq has provided comprehensive insights into the transcriptome, facilitating the identification of new transcripts, splice variants, and differential gene expression under diverse conditions. However, while RNA-seq has substantially advanced transcriptomics, its reliance on bulk RNA samples averages gene expression across cell populations, obscuring cellular heterogeneity and masking unique phenotypes [3]. In contrast, scRNA-seq can be used for the analysis of gene expression at the single-cell level, allowing the identification of rare cell types, mapping of cellular differentiation pathways, and understanding of cell-specific responses. This technology has proven essential for medical research, developmental biology, and ecological studies, driving significant discoveries and new therapeutic approaches. In particular, scRNA-seq has identified rare cell types, mapped cellular differentiation pathways, and elucidated cell-specific responses to environmental stimuli. These discoveries have led to groundbreaking insights in various fields of biology.

Researchers utilize scRNA-seq to analyze the genetic information of individual cells, gaining a comprehensive understanding of complex biological processes for various analytical purposes. Several scRNA-seq studies have been conducted in the medical, developmental, and ecological fields [4–6]. In medical research, the investigation of pathological conditions and immune responses includes the heterogeneity of cancer cells, autoimmune diseases, inflammatory pathways, metabolic variability, and cellular responses to viral infections. Furthermore, scRNA-seq is critical for identifying diagnostic biomarkers, novel cellular subpopulations, and mechanisms of drug response and resistance, thereby enhancing the customization of diagnostics for tumor heterogeneity [7]. This detailed profiling significantly improves therapeutic selection and monitoring by exploring the diverse expression levels and interactions between receptors and ligands in both tumor and normal tissues. Additionally, scRNA-seq employs advanced techniques including trajectory inference and RNA velocity to track cell progression and differentiation in diseases such as Alzheimer's disease, revealing how stem cells and neurons evolve in response to the disease [8]. These methods provide a dynamic perspective on gene expression changes over time, improving our understanding of cell differentiation and maturation across various health and disease states. Moreover, scRNA-seq offers critical insights into epigenetic alterations, such as chromatin accessibility, deoxyribonucleic acid (DNA)/RNA methylation, histone modifications, and nucleosome positioning

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across diverse cell types within the tumor microenvironment, advancing our knowledge of the epigenetic mechanisms driving tumor heterogeneity [9]. This deepened understanding is essential for developing targeted therapeutic strategies and unraveling the complexities of tumor progression and treatment resistance.

In the field of ecological research, scRNA-seq provides transformative insights into complex interspecies interactions and ecosystem functions, particularly unraveling the symbiotic relationships within coral ecosystems. It has been instrumental in dissecting the metabolic interactions between coral cells and their symbiotic dinoflagellate algae, as observed in species such as *Stylophora pistillata* [10] and *Xenia* [11]. scRNA-seq identifies specific cell types involved in symbiosis, including the endosymbiotic cells in *Xenia* that can manage the uptake and maintenance of algae. This helps elucidate the molecular pathways critical for sustaining these ecological relationships, offering crucial insights into how corals adapt to and recover from environmental stressors, thereby maintaining reef health and stability. scRNA-seq is crucial for investigating biological adaptations to climate change, revealing the cellular mechanisms through which organisms respond and adapt to environmental challenges [6]. In a study of the estuarine oyster *Crassostrea hongkongensis*, scRNA-seq identified 1900 Cu-responsive genes in 12 hemocyte clusters, revealing the varied mechanisms of granulocytes for managing copper stress, such as metal transport and granule sequestration. This analysis demonstrates the ability of scRNA-seq in uncovering detailed cellular responses to environmental stressors, highlighting its utility in ecological research. Taken together, scRNA-seq is primarily utilized for three major purposes: enhancing medical diagnostics and therapies, advancing the understanding of developmental biology and plant science, and exploring ecological relationships. These areas continue to be the focus of active and productive research, underscoring the indispensable role of scRNA-seq in advancing the frontiers of biological science.

Advancement in NGS technologies has significantly enhanced genomic, RNA-seq, and ATAC-seq analyses, facilitating the open access and sharing of large genomic datasets. In particular, the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database provides extensive RNA expression data across various species (Fig. 1a) [12].

Bulk RNA-seq data, broadly categorized into animals, plants, fungi, and microorganisms, are mainly derived from animals, comprising ~83.2% of the data due to their relevance in disease research. In contrast, plants, fungi, and microorganisms account for smaller proportions. Based on the dataset, species such as *Homo sapiens* and *Mus musculus* are the most extensively studied, reflecting their critical role in medical and genetic research (Fig. 1b) [13]. This focus on animals is driven by their importance in understanding human diseases and developing therapeutic strategies. In contrast, plants, fungi, and microorganisms are underrepresented. Among plants, *Arabidopsis thaliana* [14] and *Oryza sativa* [15] are the primary subjects owing to their significance in agricultural research. Microorganisms such as *Klebsiella* [16] and *Pseudomonas aeruginosa* [17] play important roles in microbiology and infection biology, whereas fungi such as *Saccharomyces cerevisiae* [18] are vital in industrial and biotechnological applications. Despite ongoing research on model organisms, the use of scRNA-seq for non-model organisms remains relatively low (Fig. 1c). The complexity of isolating individual cells from plants [19], fungi, and microorganisms [20] limits scRNA-seq applications, underscoring the need for further technological advancements to facilitate single-cell studies across a broader range of organisms. Developing and refining methods for single-cell isolation and sequencing in these biological systems will gain more comprehensive and detailed biological insights. Despite the extensive use of scRNA-seq in animal research, its application to plants, fungi, and microorganisms is still in its nascent stages. This is particularly true for single-cell analyses, where the technical challenges of cell isolation and the complexity of these organisms present substantial barriers. As technology advances, it will be crucial to adapt and apply these methods to study non-model organisms, thereby broadening the scope of single-cell research and enhancing our understanding of various life forms.

Optimizing single-cell ribonucleic acid sequencing for species characterization

In scRNA-seq analysis across various species, each step from cell sorting to computational analysis must be carefully tailored to accommodate unique biological characteristics, such as cell size and rigidity. Techniques such as fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS) are chosen based on specific cell types [21, 22]. Sequencing methods can be droplet-based or plate-based, which are selected for their throughput and resolution. After sequencing, data filtering removes low-quality reads, and normalization adjusts for sequencing depth and cell-specific biases [23]. Dropout imputation addresses missing data caused by technical variability, and integration algorithms minimize batch effects from combining different datasets [24, 25]. Scaling standardizes features across the dataset, and dimensional reduction techniques such as PCA, t-SNE, or UMAP help visualize complex data structures. Cell clustering identifies groups with similar gene expression, and post-clustering annotation further refines these groups based on known markers [23]. Given the heterogeneity in cell properties among different species, selecting appropriate techniques is crucial for accurate analysis. This paper systematically categorizes techniques suited to different species, which may help improve the accuracy of scRNA-seq analyses and contribute to the understanding of biological phenomena at the single-cell level.

Technical workflow optimization for single-cell ribonucleic acid sequencing

Step 1: species and tissue assessment

Optimizing the scRNA-seq workflow is essential to account for the diverse biological characteristics of different species and tissue types (Fig. 2).

Key factors such as cell size, viability, tissue dissociation feasibility, and the presence of rigid cell walls must be carefully considered during sample preparation and cell isolation. For species with small, viable, wall-free cells, standard protocols for generating single-cell suspensions are applicable. In contrast, plant, fungal, and microbial cells often require specialized dissociation methods or alternative approaches such as single-nucleus RNA sequencing [26, 27]. When standard single-cell suspension protocols cannot be applied, alternative strategies must be employed depending on tissue characteristics. For tissues that are difficult to dissociate, optimized combinations of mechanical and enzymatic dissociation are required to improve cell yield and viability [28]. In cases where viable single-cell isolation is not feasible, single-nucleus RNA sequencing (snRNA-seq) or fixed-cell scRNA-seq protocols can be adopted to enable transcriptomic profiling [29]. Thus, optimizing the scRNA-seq workflow requires careful consideration

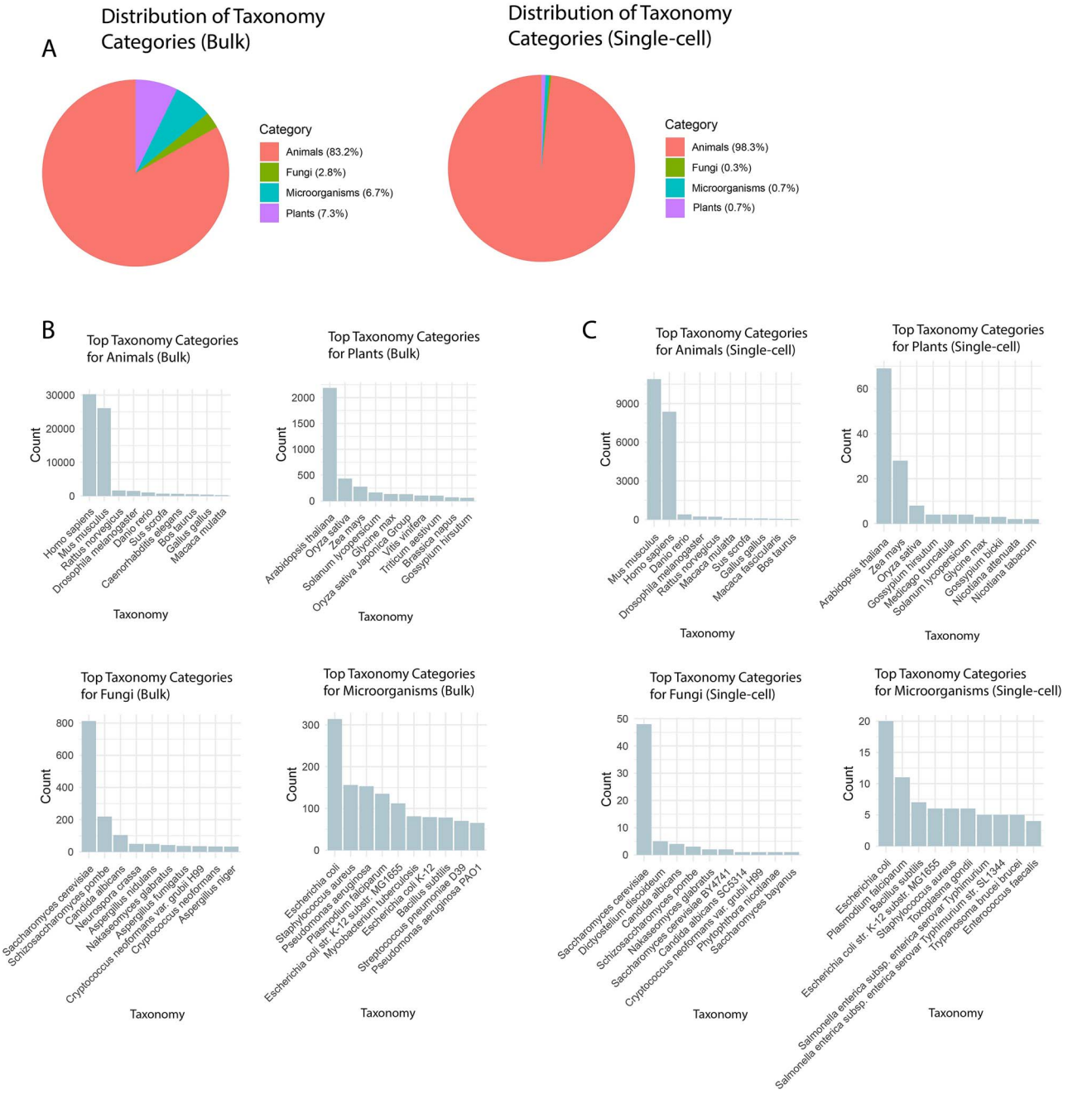

Figure 1. Distribution of bulk RNA-seq and scRNA-seq data according to taxonomy. (a) The pie charts show the distribution of RNA-seq data for bulk (left) and single-cell (right) analyses. Bulk RNA-seq data are dominated by animals (83.2%), with smaller proportions for plants (7.3%), microorganisms (6.7%), and fungi (2.8%). Single-cell RNA-seq data focus almost entirely on animals (98.3%), with minimal representation of other categories. (b) Bar plots show top taxa in bulk RNA-seq, with *H. sapiens* and *M. musculus* dominating animals, *A. thaliana* in plants, and *S. cerevisiae* and *Escherichia coli* in fungi and microorganisms. (c) Bar plots highlight single-cell RNA-seq taxa, mainly focusing on *H. sapiens* and *M. musculus*, with rare representation of *A. thaliana*, *S. cerevisiae*, and others.

of species- and tissue-specific factors, and no single protocol is universally optimal across all biological systems.

Step 2: library preparation and sequencing

After generating high-quality single-cell suspensions, appropriate library preparation and sequencing strategies must be selected according to the sample characteristics (Fig. 2). For samples yielding viable single cells, droplet-based platforms such as 10x Genomics are widely used due to their scalability and efficiency. For fixed cells, small samples, or rare cell populations, alternative approaches such as SPLiT-seq and SMART-seq2 enable high-resolution transcriptomic profiling even when standard droplet-based methods are not feasible.

Step 3: mapping

Mapping and quantification strategies depend on the availability of a suitable reference genome. For well-annotated species with available genomic resources, reference-based pipelines such as Cell Ranger are employed. This workflow begins by constructing a reference package using the mkref function. Raw sequencing

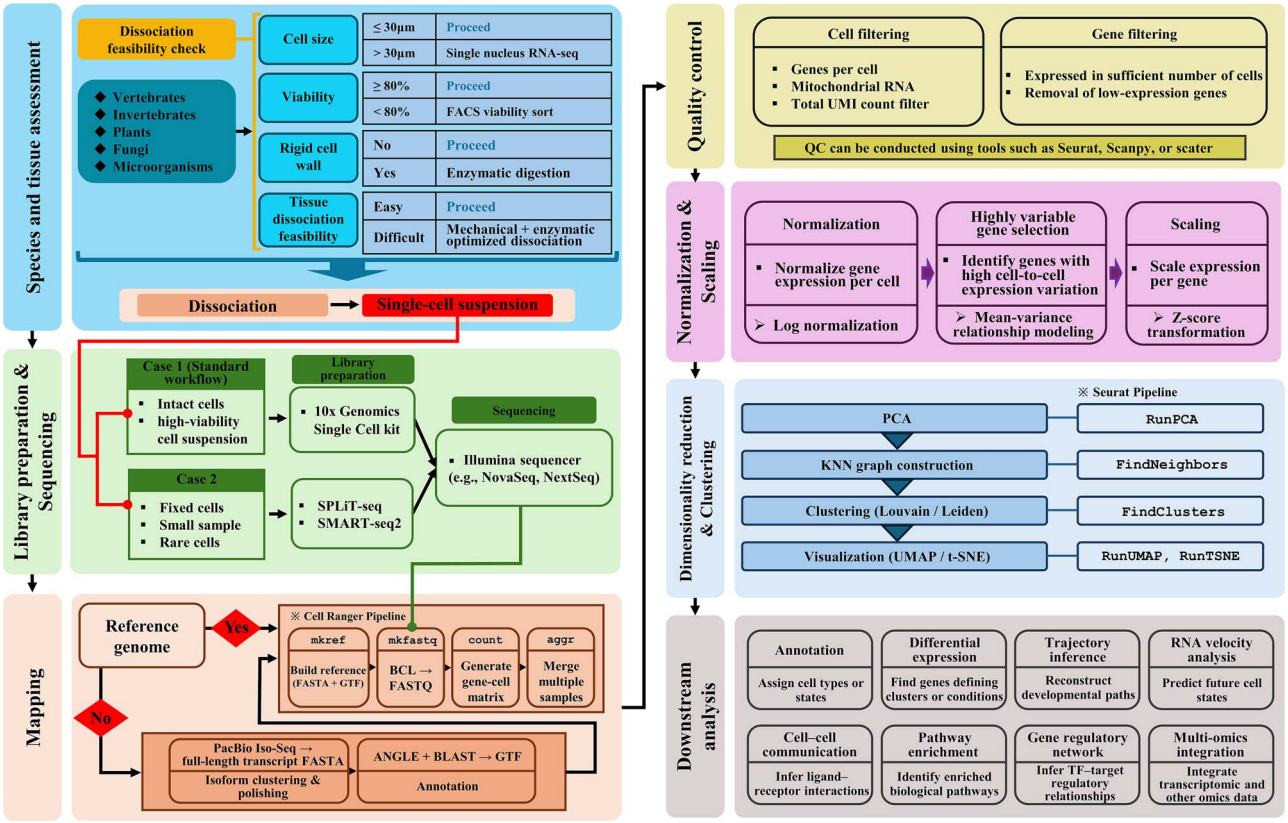

Figure 2. Workflow for single-cell RNA-seq from sample preparation to downstream analysis across diverse species. BCL, base call file. GTF, gene transfer format. PCA, principal component analysis. KNN, k-nearest neighbors. UMAP, uniform manifold approximation and projection. t-SNE, t-distributed stochastic neighbor embedding. TF, transcription factor.

outputs (BCL format) are converted into FASTQ files with mkfastq, followed by alignment and UMI-based quantification using count, which generates a gene-cell expression matrix. When necessary, multiple samples or batches can be integrated through the aggr function. For species lacking a high-quality reference genome, a pseudo-reference can be constructed from full-length transcriptome sequencing (e.g. PacBio Iso-Seq) [30]. This approach involves isoform clustering and polishing to obtain high-confidence full-length transcript FASTA sequences. Transcript annotation is performed by predicting coding regions with ANGLE and conducting functional annotation with BLAST to generate a GTF annotation file. The resulting pseudo-reference can then be used to build a custom reference for the Cell Ranger pipeline, enabling standard downstream quantification.

Step 4: quality control

Rigorous quality control (QC) is essential to ensure that downstream analyses are based on high quality data. This step includes filtering out low-quality cells such as those with abnormal gene counts or high mitochondrial RNA content as well as removing lowly expressed genes. Such filtering helps eliminate technical artifacts and enhances the biological interpretability of the data.

Step 5: normalization and scaling

Normalization, highly variable gene selection, and scaling are applied to ensure that subsequent analyses accurately capture biological variation while minimizing technical noise. First, normalization adjusts gene expression levels to account for differences in sequencing depth and capture efficiency across cells. Next, highly variable genes are identified by modeling the mean–variance relationship, selecting genes with the greatest cell-to-cell expression variability, which are most informative for downstream analyses. Finally, scaling standardizes expression values to place all genes on a comparable scale, facilitating unbiased dimensionality reduction and clustering.

Step 6: dimensionality reduction and clustering

Dimensionality reduction techniques, such as PCA and UMAP, are used to summarize complex gene expression patterns. Combined with graph-based clustering methods, these approaches enable the identification of distinct cell populations and cellular states, providing a foundation for more detailed downstream analyses.

Step 7: downstream analysis

Finally, advanced downstream analyses including annotation of cell types or states, differential expression analysis, trajectory inference, and cell–cell communication modeling provide rich biological insights. Additional layers of analysis such as pathway enrichment, gene regulatory network inference, RNA velocity analysis, and multiomics integration further expand the interpretive power of scRNA-seq data across diverse species and experimental contexts.

Single-cell sorting methods

scRNA-seq begins with the critical step of isolating individual cells, a process that can significantly influence the accuracy and depth of subsequent analyses [3]. Various techniques have been developed to isolate single cells, each with unique advantages and limitations, and these can broadly be classified based on physical

Table 1. Single-cell isolation techniques.

Isolation techniques	Description	Advantage	Disadvantage	Ref.
Fluorescence-Activated Cell Sorting (FACS)	Utilizes fluorescent markers to sort cells by size, granularity, and fluorescence.	Enables high-speed, multiparametric analysis.	Requires a large quantity of cells and may cause cell damage due to high flow rates.	[21]
Magnetic-Activated Cell Sorting (MACS)	Employs magnetic beads conjugated with antibodies to isolate cells.	Provides a cost-effective and high-purity method.	Limited to surface markers and potential for non-specific binding.	[22]
Laser Capture Microdissection (LCM)	Utilizes laser to isolate specific cells from solid tissue samples on a microscope slide.	Offers high precision and preserves tissue morphology.	Requires high skill and poses a risk of technical issues.	[31]
Microfluidics-Based Cell Isolation	Utilizes microchannels to manipulate fluids and isolate cells based on various properties.	Consumes low sample volumes and integrates well with other techniques.	Demands high skill for operation.	[32]
Manual Cell Picking	Combines micromanipulators with an inverted microscope to manually isolate single cells using micro-pipettes.	Enables the isolation of live cells.	Characterized by low throughput and requires high skill.	[33]

or biological properties (Table 1). Among these methods, FACS is widely used for high-speed multiparametric analysis, allowing the sorting of cells based on size, granularity, and fluorescence; however, it requires large cell quantities and may cause cell damage due to high flow rates [21]. MACS offers a cost-effective and high-purity approach by using magnetic beads conjugated with antibodies; however, it is limited to surface markers and may suffer from non-specific binding [22]. Laser capture microdissection (LCM) provides high precision and preserves tissue morphology by utilizing a laser to isolate specific cells from solid tissue samples; however, it requires high skills and has technical challenges [31]. Microfluidics-based cell isolation, which leverages microchannels to manipulate fluids and isolate cells, is advantageous due to its low sample volume requirement and compatibility with other techniques; however, it requires skilled operation [32]. Lastly, manual cell picking, which uses micromanipulators and micropipettes to isolate live cells under an inverted microscope, is characterized by its low throughput and high skill requirement [33]. The choice of isolation method directly affects the purity and recovery rate of single cells, thereby influencing the overall quality of scRNA-seq data.

Single-cell ribonucleic acid sequencing methods

scRNA-seq technology uses various isolation and amplification strategies to analyze individual cell transcriptomes (Table 2). After converting RNA into first-strand cDNA, amplification is performed by either polymerase chain reaction (PCR) or *in vitro* transcription (IVT). PCR, a widely used non-linear amplification method, is employed by platforms such as VASA-seq [34], Quartz-seq2 [35], 10x Genomics [36], Seq-Well [37], MATQ-seq [38], Drop-seq [39], and STRT-seq [40] with unique molecular identifiers (UMIs) to barcode mRNA molecules, thereby reducing amplification biases. IVT, used in CEL-seq [41], MARS-seq [42], and inDrop-seq [43], provides linear amplification but can cause 3′ coverage biases. There are also differences in isolation strategies. Microfluidic droplets are used in VASA-seq [34], 10x Genomics [36], Drop-seq [39], and Seq-Well [37], allowing the high-throughput analysis of large cell populations, whereas FACS is used in Quartz-seq2 [35], MATQ-seq [38], MARS-seq [42], CEL-seq [41], and Smart-seq2 [27], allowing the precise sorting of individual cells. SPLiT-seq [44] uses combinatorial barcoding without the need for physical cell isolation, facilitating the high-throughput scRNA-seq of hundreds of thousands of fixed cells or nuclei at a relatively low cost. Technologies vary according to the scale of cells, from large-scale (VASA-seq, 10x Genomics) to small-scale (Quartz-seq2, MATQ-seq) methods. Gene coverage ranges from 3′-end sequencing (e.g. Quartz-seq2, Drop-seq, inDrop) to full-length transcript coverage (e.g. VASA-seq, MATQ-seq, Smart-seq2), affecting data depth and breadth. Throughput also varies, with some technologies offering very high throughput (10x Genomics) and or lower throughput (MATQ-seq).

Comprehensive tools and methods for single-cell ribonucleic acid sequencing

scRNA-seq provides insights into the diversity of cell types and states within tissues by allowing the analysis of gene expression at the individual cell level, facilitating discoveries in developmental biology, immunology, and cancer research. However, the complexity and scale of scRNA-seq data present significant challenges in data processing and analysis, demanding the use of specialized tools and methods (Table 3). Read mapping is the first step in scRNA-seq data analysis, where raw sequencing reads are aligned to a reference genome. Tools such as CellRanger and DropEst are commonly used for this purpose. CellRanger utilizes the STAR aligner to process 10x Genomics scRNA-seq data, providing a comprehensive pipeline that includes read alignment, UMI counting, and gene expression quantification [36]. In contrast to traditional alignment methods such as STAR, pseudo-alignment techniques, as employed in tools such as Kallisto and Alevin, take a different approach. QC is crucial for ensuring the reliability of scRNA-seq data by identifying and filtering low-quality cells and technical artifacts. Seurat, Scanpy, Scater, and DoubletFinder are prominent tools in this domain. Normalization is a vital step in scRNA-seq data processing as it corrects for technical variations such as differences in sequencing depth and capture efficiency, allowing an accurate comparison of gene expression levels across cells. Imputation methods are employed to address the pervasive issue of missing data in scRNA-seq datasets, which arises due to dropouts and other technical limitations. SAVER, MAGIC, scVI, and scGNN are leading tools for imputation. Batch effects, which stem from technical variations between different experimental batches, can obscure biological interpretations if not appropriately corrected. Tools such as Batchelor and Seurat offer methods for batch effect correction. Clustering is a critical analytical step

Table 2. Comparison of scRNA-seq technologies.

Technology	Isolation strategy	Amplification method	Cell scale	UMI use	Gene coverage	Throughput	Year	Ref.
VASA-seq	Microfluidic (droplet)	PCR	Large	Yes	Full length	High (1000–10 000)	2022	[34]
SPLiT-seq	Split-pool	PCR	Small	Yes	3′	Very high (>10 000)	2018	[44]
Quartz-seq2	FACS	PCR	Small	Yes	3′	High (1000–10 000)	2018	[35]
10x Genomics	Microfluidic (droplet)	PCR	Large	Yes	3′ or 5′	Very high (>10 000)	2017	[36]
Seq-Well	Microfluidic	PCR	Large	Yes	3’	High (1000–10 000)	2017	[37]
MATQ-seq	FACS	PCR	Small	Yes	Full length	Low (100–200)	2017	[38]
Drop-seq	Microfluidic (droplet)	PCR	Large	Yes	3′	High (1000–10 000)	2015	[39]
inDrop	Microfluidic (droplet)	IVT	Large	Yes	3′	High (1000–10 000)	2015	[43]
MARS-seq	FACS	IVT	Small	Yes	3′	High (1000–10 000)	2014	[42]
CEL-seq	FACS	IVT	Small	Yes	3′	Low (1–200)	2012	[41]
Smart-seq2	FACS	PCR	Small	No	Full length	Median (100–1000)	2012	[27]
Fluidigm C1	Microfluidic	PCR	Small	No	Full length	Low (1–200)	2012	[45]
STRT-seq	FACS	PCR	Small	Yes	5′	Median (100–1000)	2011	[40]

in scRNA-seq data processing, which allows the identification of distinct cell types and subpopulations. Tools such as SC3, Seurat, and Scanpy provide robust clustering methods tailored for single-cell data. The tools and methods introduced provide a comprehensive framework for scRNA-seq data analysis from raw data processing to clustering. By carefully selecting and applying these tools, researchers can navigate the complexities of scRNA-seq data, ensuring that their analyses derive accurate and biologically meaningful insights. As scRNA-seq technology evolves, the tools and methods for data analysis will similarly advance, further enhancing our understanding of cellular heterogeneity and the mechanisms underlying complex biological systems.

Single-cell transcriptomics in model research

Homo sapiens

Advances in scRNA-seq have revolutionized our understanding of the cellular landscape in human diseases, offering deep insights into cell heterogeneity, interactions, and molecular mechanisms. The application of scRNA-seq has been vital for studying tumors, rare diseases, inflammatory responses, and viral infections, providing new avenues for therapeutic strategies. In tumor biology, scRNA-seq has shed light on the diversity of immune and non-immune cells within the tumor microenvironment, helping to unravel the mechanisms of drug resistance and potential therapeutic targets. For example, in an acute myeloid leukemia (AML) study, immune cell heterogeneity in peripheral blood mononuclear cells (PBMCs) was identified after chemotherapy, highlighting shifts in NK cells, monocytes, and dendritic cells [58]. Similarly, research on high-risk neuroblastoma identified the NECTIN2-TIGIT axis as a critical immune checkpoint suppressing T and NK cell activity [59]. Targeting this axis, in combination with chemotherapy, showed promising results in overcoming drug resistance. Studies on ovarian cancer and tamoxifen-resistant breast cancer further emphasize the power of scRNA-seq in identifying key cellular subpopulations and gene expression changes, which could be leveraged for precision therapies [60, 61]. Rare diseases have also benefitted from single-cell transcriptomics, with scRNA-seq allowing the detailed characterization of immune dysfunction and disease progression. In a study of autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), scRNA-seq mapped the changes in blood transcriptome profiles during pregnancy, revealing distinct immune signatures [62]. In particular, SLE patients exhibited sustained interferon signaling postpartum, indicating long-term immune dysregulation. In another study of atherosclerosis, suppression of IL-1β promoted the beneficial accumulation of fibroblast-like cells, stabilizing plaques and offering potential therapeutic approaches for patients with clonal hematopoiesis [63]. Additionally, biomarkers (e.g. serglycin) were identified in a late-stage intervertebral disc degeneration (IVDD) study, suggesting new targets for treatment [64]. In a previous study, IL-1β + macrophages were identified as key drivers of pathogenic inflammation in pancreatic cancer, which could interact with pancreatic ductal adenocarcinoma cells to form a feedback loop that promotes tumor progression [65]. Blocking this loop through IL-1β-targeted therapies holds promise for suppressing tumor-associated inflammation. Moreover, scRNA-seq was used to clarify the metabolic adaptation of T cells in the intestinal environment, revealing how prostaglandin E2 (PGE2) control mitochondrial function and glutathione production to regulate CD8+ T cell responses [66]. These findings provide new therapeutic opportunities for diseases characterized by chronic inflammation, such as inflammatory bowel disease (IBD) and certain cancers. Viral infections have also been a focus of single-cell studies, particularly in attempts to understand the effects of SARS-CoV-2 on human cells. scRNA-seq revealed that SARS-CoV-2 can infect dopaminergic neurons, inducing senescence and neuroinflammation [67]. These findings raise concerns about potential long-term neurological sequelae, such as Parkinson’s disease, in COVID-19 survivors. Several FDA-approved drugs, including riluzole and metformin, have shown efficacy in suppressing the virus-induced senescence response, providing potential therapeutic interventions to mitigate this risk. scRNA-seq is transforming our understanding of disease biology by providing detailed information on cellular interactions and gene expression in diverse contexts. This technology is especially useful in disease research, which can reveal key pathways and cellular targets that are critical for developing precision therapies across a wide range of conditions, from cancer to rare diseases and viral infections.

Table 3. Summary of tools and methods for scRNA-seq data processing and analysis.

Task	Function or method	Tool	Language	Description	Year	URL	Ref.
Read mapping	STAR	Cell-Ranger	Python	Comprehensive pipeline for processing 10x Genomics scRNA-seq data.	2017	https://support.10xgenomics.com	[36]
	STAR	DropEst	C++	Tool for processing droplet-based scRNA-seq data with improved UMI handling and error correction.	2018	https://github.com/kharchenkolab/dropEst	[46]
	Pseudo-alignment	Kallisto-BUStools	Python, R	Fast pseudo-aligner with UMI support, particularly efficient for droplet-based scRNA-seq.	2021	https://pachterlab.github.io/kallistobustools/	[47]
	Pseudo-alignment	Alevin	C++	Efficient tool for scRNA-seq data processing with integrated UMI handling.	2017	https://github.com/COMBINE-lab/salmon	[48]
QC	CreateSeuratObject, PercentageFeatureSet	Seurat	R	Functions in Seurat for creating a Seurat object, calculating the percentage of mitochondrial genes, and subsetting cells based on quality metrics.	2015	https://github.com/satijalab/seurat	[23]
	scanpy.pp.filter_cells, scanpy.pp.calculate_qc_metrics scanpy.pp.highly_variable_genes	Scanpy	Python	Key preprocessing functions in Scanpy for filtering cells, calculating QC metrics, and identifying highly variable genes.	2018	https://github.com/scverse/scanpy	[49]
	calculateQCMetrics isOutlier	Scater	R	Functions for calculating QC metrics, filtering low-quality cells, and identifying outliers based on QC metrics.	2017	https://github.com/jimhester/scater	[50]
	paramSweep	DoubletFinder	R	Functions used in DoubletFinder for parameter sweeping and identifying doublets in single-cell RNA-seq data.	2019	https://github.com/ddiez/DoubletFinder	[51]
Normal-ization	NormalizeData, ScaleData, SCTransform	Seurat	R	Functions in Seurat for normalizing scRNA-seq data by scaling and transforming data to correct for technical variation.	2015	https://github.com/satijalab/seurat	[23]
	scanpy.pp.normalize_total, scanpy.pp.log1p,	Scanpy	Python	Functions in Scanpy for total count normalization and logarithmic transformation.	2018	https://github.com/scverse/scanpy	[49]
	SCnorm	SCnorm	R	An scRNA-seq normalization method based on quantile regression, which effectively normalizes data while preserving biological variation.	2017	https://github.com/rhondabacher/SCnorm	[52]
	Dino	Dino	R	A normalization method based on a flexible negative binomial mixed model, which is robust to shallow sequencing and sample heterogeneity.	2021	https://github.com/JBrownBiostat/Dino	[53]
Imputa-tion	Bayesian Model	SAVER	R	A Bayesian-based method for recovering true gene expression levels in UMI-based scRNA-seq data.	2018	https://github.com/mohuangx/SAVER	[24]
	Markov Affinity	MAGIC	Python	Constructs Markov affinity-based graphs to impute gene expression values, which can smooth the data while preserving important structures.	2018	https://github.com/KrishnaswamyLab/magic	[25]
	Variational Inference, Autoencoder	scVI	Python	A comprehensive tool using hierarchical Bayesian models with variational inference for imputation and analysis of scRNA-seq data.	2018	https://github.com/scverse/scvi-tools	[54]
	Graph Neural Network, Autoencoder	scGNN	Python	A method based on graph neural networks with an autoencoder that performs well for noisy large-scale datasets, which is useful for imputation and clustering.	2021	https://github.com/juexinwang/scGNN	[55]
Batch effect correction	Mutual Nearest Neighbors	Batche-lor	R	Batchelor uses MNN to identify and correct batch effects by aligning similar cells across batches, adjusting for systematic differences.	2018	https://github.com/LTLA/batchelor	[56]
	IntegrateData, FindIntegrationAnchors	Seurat	R	Seurat integrates datasets from different batches by identifying shared cell populations across batches using MNN-based methods.	2015	https://github.com/satijalab/seurat	[23]
Clustering	SC3, consensus clustering	SC3	R	SC3 is an unsupervised approach combining multiple clustering methods, offering high accuracy and robustness in single-cell clustering.	2017	https://github.com/hemberg-lab/SC3	[57]
	SNN, FindClusters, RunPCA	Seurat	R	Seurat utilizes a Shared Nearest Neighbor (SNN) algorithm for identifying cell clusters. Clustering is performed after dimension reduction by PCA.	2015	https://github.com/satijalab/seurat	[23]
	Density-based clustering, graph-based clustering	Scanpy	Python	Scanpy provides various methods, including density-based and graph-based clustering, suitable for clustering large-scale single-cell datasets.	2018	https://github.com/scverse/scanpy	[49]

Mus musculus

Murine models, particularly *M. musculus*, have significantly advanced our understanding of disease mechanisms across a wide range of biological processes. In tumor research, *M. musculus* models have facilitated the study of immune evasion, resistance mechanisms, and therapeutic responses. For example, research on the co-activating myeloid receptors CD40 and Dectin-1 showed that dual activation could enhance anti-tumor immunity in pancreatic cancer, overcoming resistance to immune checkpoint blockade (ICB) [68]. Furthermore, investigations of the SOX9-B7x axis in breast cancer demonstrated how dedifferentiated tumor cells escape immune surveillance by inhibiting T cell infiltration, with implications for targeted immunotherapies [69]. In colon cancer, cannabidiol (CBD) was shown to reprogram the tumor microenvironment, shifting macrophages toward a more anti-tumor phenotype and synergizing with anti-PD-1 therapy [70]. These findings underscore the potential of targeting myeloid and tumor-infiltrating immune cells to enhance cancer treatment outcomes. In a study of inflammatory responses, *M. musculus* models have been essential for understanding the role of regulatory B cells (Bregs) in autoimmune diseases such as SLE [71]. scRNA-seq revealed the heterogeneity of IL-10-producing Bregs during disease progression, showing a decrease in marginal zone Bregs (MZ Bregs) and an increase in plasma cell-like Bregs (PB-PC Bregs) in active disease stages. This shift, accompanied by the increased expression of inflammation-related genes, suggests that Bregs lose their immune-regulatory functions as lupus progresses, contributing to the disease. Another study on inflammatory responses in chronic limb-threatening ischemia (CLTI) revealed that pro-inflammatory macrophages could hinder skeletal muscle regeneration by driving the premature differentiation of satellite cells (MuSCs), reducing their regenerative capacity [72]. The study identified disruptions in the IGF1 signaling pathway and activation of SPP1-CD44 signaling, which exacerbated inflammation and impaired tissue repair. These findings suggest potential therapeutic strategies that may target macrophages and MuSCs to improve muscle regeneration. Viral infections, particularly SARS-CoV-2, have been investigated using *M. musculus* models to understand both the cell-intrinsic and cell-extrinsic effects of viral RNA [73]. Single-cell meta-analysis of various animal models revealed that infection in K18-hACE2 transgenic mice could most closely mimic severe human COVID-19, providing valuable insights into inflammatory responses, including IL1B and CXCL10 expression, even in the absence of viral RNA. This study underscores the complexity of inflammatory signaling during viral infection, suggesting that paracrine and autocrine signaling mechanisms drive most of the immune responses to SARS-CoV-2. In development, *M. musculus* has proven critical for understanding neurogenesis and the genetic regulation of cellular differentiation. For example, the Tsc2 gene was found to regulate neuroprogenitor differentiation, with the loss of Tsc2 leading to impaired translation control and defective neuronal development, resembling pathologies observed in human tuberous sclerosis [74]. Additionally, recent advancements in single-nucleus multiomic mapping technologies (e.g. sn-m6A-CT) have allowed the simultaneous analysis of m6A methylomes and transcriptomes at the single-cell level [75]. This approach has provided unprecedented insights into how m6A modifications regulate gene expression during cellular differentiation and development in mouse models. These studies demonstrate that murine models remain indispensable in biomedical research, particularly in exploring the cellular and molecular mechanisms underlying diseases. The application of cutting-edge single-cell and multiomic technologies to *M. musculus* can not only enhance the understanding of complex biological processes but also facilitate the development of novel therapeutic strategies across a range of diseases, from cancer to viral infections and developmental disorders.

Danio rerio

The zebrafish (*D. rerio*) has been extensively studied as a model organism to understand various biological processes, including inflammatory responses, immune system dynamics, regenerative capabilities, and disease modeling. Recent studies have highlighted the versatility of zebrafish in elucidating mechanisms relevant to human diseases across multiple domains. For instance, in the context of autism spectrum disorders (ASDs), mutations in CHD8 were shown to disrupt neural crest cell development, resulting in impaired gut serotonergic function and disruption of epithelial homeostasis, which may explain the gastrointestinal symptoms observed in ASD patients [76]. Transcriptomic analyses further revealed altered expression in serotonergic and cholinergic signaling pathways, leading to epithelial thinning, immune dysregulation, and subsequent inflammation, underscoring the multifaceted impact of CHD8 mutations. In a study on aging, single-cell analysis of the zebrafish brain revealed significant alterations in immune cell heterogeneity, particularly an increase in T lymphocytes and distinct microglial subpopulations in aged fish, which could be linked to chronic neuroinflammation and neurodegeneration [77]. These findings provide insights into the cellular and molecular interactions that may underlie age-related neurological decline. Additionally, a study has investigated the role of macrophages in tissue injury and regeneration, which demonstrated that macrophage activation follows a sequential anti-inflammatory pathway involving glucocorticoid receptor (GR), IL-10, and IL-4/polyamine signaling, each contributing uniquely to tissue regeneration and inflammation resolution [78]. This sequential activation mechanism suggests a conserved strategy in immune modulation and tissue repair, potentially applicable across species. In cardiac regeneration, zebrafish epicardial regeneration was found to depend on the activation of adult epicardial progenitor cells (aEPCs), which undergo epithelial-to-mesenchymal transition (EMT) to generate mesenchymal and vascular support cells essential for effective cardiac repair [79]. Genetic ablation of aEPCs led to impaired cardiac regeneration, highlighting their critical role and identifying potential targets for therapeutic intervention. Furthermore, single-cell ATAC-seq analysis of *gata2b*-deficient zebrafish uncovered distinct chromatin accessibility changes in myeloid and lymphoid progenitors, mirroring key features of *GATA2* deficiency syndrome in humans [80]. Zebrafish *gata2b* deficiency resulted in impaired myeloid differentiation, reduced accessibility to monocyte-specific regulatory elements, and increased but incomplete lymphoid differentiation, particularly affecting B cell lineage commitment. These findings emphasize the importance of *GATA2* in regulating early hematopoietic differentiation and lineage fidelity. Collectively, these studies demonstrate how zebrafish serve as a powerful model for investigating disease mechanisms, elucidating inflammatory and immune pathways, and advancing regenerative medicine and therapeutic strategies.

Drosophila melanogaster

The fruit fly (*D. melanogaster*) has emerged as a powerful model organism for dissecting complex biological processes,

including tumor development, cell differentiation, and aging-related changes. In tumor biology, scRNA-seq of tumor-associated macrophages (TAMs) in Drosophila models has revealed the distinct transcriptional profiles of macrophages and hemocytes during early tumorigenesis induced by *RasV12* [81]. Five distinct hemocyte clusters were identified, with macrophages in the *RasV12* model activating genes related to cell division. In development, the single-cell expression profile of Drosophila ovarian follicle stem cells (FSCs) has provided insights into spatial differentiation within the germarium [82]. FSCs were shown to be regulated by pathways such as Wnt and JAK–STAT, which guide cell division and differentiation. Additionally, the temporal gene network of medulla neuroblasts in Drosophila has been characterized, revealing a series of temporal transcription factors (TTFs) that control the sequential generation of neuronal subtypes [83]. The study identified new TTFs involved in medulla neuroblast development and demonstrated their roles in regulating neuroblast division and glial cell differentiation, offering a comprehensive view of temporal patterning in neural development. In a study on aging, Drosophila intestinal stem cells (ISCs) were found to exhibit age-related changes in lineage fidelity, driven by the altered regulation of Polycomb (Pc) genes [84]. Aging led to changes in chromatin accessibility, resulting in the increased conversion of ISCs to enteroendocrine (EE) cells, ultimately contributing to dysplasia and impaired gut function. Pc genes play a key role in maintaining normal stem cell function and inhibiting excessive differentiation; however, their reduced function during aging can lead to disrupted homeostasis and increased disease risk. Collectively, these studies demonstrate the utility of *D. melanogaster* as a model for research on tumor biology, stem cell differentiation, and aging processes, providing valuable insights into the underlying genetic and cellular mechanisms.

Arabidopsis thaliana

The model plant *A. thaliana* has been used extensively to study various aspects of cell development and adaptation to environmental changes, particularly through single-cell analyses that can reveal the intricate details of cellular heterogeneity and regulatory mechanisms. In a recent study, the role of brassinosteroids, a class of plant hormones, in regulating root cell proliferation and elongation was investigated using time-resolved scRNA-seq [85]. Brassinosteroids were found to increase cell wall-related gene expression in the cortex, facilitating the transition from proliferation to elongation. The study also revealed how hormonal signaling networks act spatially and temporally to optimize plant growth under varying environmental conditions, contributing to a better understanding of plant development and environmental adaptation. Additionally, single-cell transcriptional profiling of leaf palisade mesophyll cells sheds light on their unique role in UV light protection [86]. Despite minimal morphological differences from other photosynthetic cells, palisade cells were found to exhibit a higher expression of genes involved in the phenylpropanoid pathway, particularly those linked to the synthesis of the UV-B protective compound sinapoyl malate. Together, these studies demonstrate the power of single-cell approaches in elucidating plant responses at a cellular resolution, providing valuable insights into the regulatory networks that underpin plant development, immunity, and adaptation to environmental stresses.

Saccharomyces cerevisiae

The yeast *S. cerevisiae* has also been extensively studied using scRNA-seq to investigate various aspects of cell and gene development. In a recent study used scRNA-seq to observe early transcriptional heterogeneity during the aging process of *S. cerevisiae*, demonstrating that transcriptional variability increases with age [87]. This study identified FIT3, a gene involved in iron transport, as an early marker of aging-related heterogeneity and found that its deletion extended replicative lifespan. Additionally, the study highlighted the roles of the transcription factors YAP1 and RPN4 in regulating oxidative stress responses and proteasome activity, respectively, both of which contribute to lifespan extension. These findings provide crucial insights into the early molecular events associated with cellular aging. Furthermore, a new technology called mDrop-seq was introduced for the massively parallel scRNA-seq of *S. cerevisiae* and *Candida albicans*, overcoming the challenges posed by microbial cell walls and low RNA yield [88]. Using mDrop-seq, researchers analyzed 35 109 *S. cerevisiae* cells and 39 705 *C. albicans* cells, revealing the diversity of stress response gene expression under heat shock conditions in *S. cerevisiae* and changes in stress response, drug target pathways, cell cycle patterns, and histone activity in *C. albicans* exposed to fluconazole. This technology has significant potential for studying pathogenic gene changes, drug resistance, and infection outcomes and developing new therapies. Collectively, these studies highlight the utility of *S. cerevisiae* as a model for understanding gene regulation, cellular aging, and responses to environmental stress at the single-cell level, providing a deeper understanding of gene development and regulation.

Escherichia coli

E. coli has been the focus of recent studies using scRNA-seq to investigate antibiotic response mechanisms, resistance, and cell development. One such study introduced BacDrop, a droplet-based scRNA-seq technology designed to address challenges in bacterial transcriptomics, particularly single-cell analysis [89]. BacDrop can analyze both Gram-negative and Gram-positive bacteria with support for thousands to millions of cells, allowing high-throughput analysis using ribosomal RNA removal and combinatorial barcoding. When applied to *Klebsiella pneumoniae*, BacDrop revealed heterogeneous responses to antibiotic stress, with mobile genetic element expression driving heterogeneity under antibiotic-free conditions and diverse resistance-associated subpopulations emerging upon exposure. Another study introduced PETRI-seq, an *in situ* combinatorial indexing method, for low-cost, high-throughput bacterial scRNA-seq [90]. PETRI-seq uses a split-pool approach to analyze transcriptomes from thousands of bacterial cells, even those with thick cell walls, without relying on droplets or microfluidics. This method has been successfully applied to *E. coli* and *Staphylococcus aureus*, which detected prophage induction in a rare *S. aureus* subpopulation. Together, these single-cell technologies highlight the potential of scRNA-seq in improving the understanding of bacterial resistance, transcriptional heterogeneity, and individual cellular roles within microbial communities. The relevant information on the model organisms and their studies is summarized in Table 4, which provides an organized overview of the key research findings obtained using single-cell transcriptomics across various species.

Single-cell transcriptomics in non-model research

Vertebrates

Recent studies using non-model vertebrates with single-cell technologies have provided valuable insights into the diversity of immune, developmental, and regenerative processes across

Table 4. Model organism research utilizing single-cell transcriptomics.

Main category	Species	Title	Sequencing method	Year	Data source	Ref.
Tumor	*H. sapiens*	Single-cell transcriptomic profiling reveals immune cell heterogeneity in AML peripheral blood mononuclear cells after chemotherapy	10x Genomics	2024	GSE235857	[58]
Tumor	*H. sapiens*	Integrative analysis of neuroblastoma by single-cell RNA sequencing identifies the NECTIN2-TIGIT axis as a target for immunotherapy	CEL-Seq2	2024	GSE218003	[59]
Tumor	*H. sapiens*	Exploring the tumor micro-environment in primary and metastatic tumors of different ovarian cancer histotypes	Drop-seq	2023	GSE235931	[60]
Tumor	*H. sapiens*	Tamoxifen response at single-cell resolution in estrogen receptor–positive primary human breast tumors	10x Genomics	2023	GSE245601	[61]
Tumor	*H. sapiens*	IL-1β + macrophages fuel pathogenic inflammation in pancreatic cancer	10x Genomics	2023	GSE217845	[65]
Rare disease	*H. sapiens*	Single-cell resolution of longitudinal blood transcriptome profiles in RA, SLE, and healthy control pregnancies	10x Genomics	2024	GSE235508	[62]
Rare disease	*H. sapiens*	Suppression of IL-1β promotes beneficial accumulation of fibroblast-like cells in atherosclerotic plaques in clonal hematopoiesis	10x Genomics	2024	GSE248394	[63]
Rare disease	*H. sapiens*	Serglycin secreted by late-stage nucleus pulposus cells is a biomarker of IVDD	10x Genomics	2024	GSE244889	[64]
Rare disease	*H. sapiens*	Identification of unstable regulatory and autoreactive effector T cells that are expanded in patients with FOXP3 mutations	10x Genomics	2023	GSE247274	[91]
Inflammatory response	*H. sapiens*	PGE2 controls the metabolic adaptation of T cells to the intestinal microenvironment	10x Genomics	2024	GSE200567	[66]
Inflammatory response	*H. sapiens*	PIM kinases regulate early human Th17 cell differentiation	10x Genomics	2023	GSE231651	[92]
Viral infection	*H. sapiens*	SARS-CoV-2 infection causes dopaminergic neuron senescence	10x Genomics	2024	GSE248989	[67]
Tumor	*M. musculus*	Cancer immunotherapy via synergistic co-activation of myeloid receptors CD40 and Dectin-1	10x Genomics	2023	GSE233171	[68]
Tumor	*M. musculus*	A SOX9-B7x axis safeguards dedifferentiated tumor cells from immune surveillance to drive breast cancer progression	10x Genomics	2023	GSE219110	[69]
Tumor	*M. musculus*	*In vivo* macrophage engineering reshapes the tumor microenvironment leading to eradication of liver metastases	10x Genomics	2023	GSE221357	[93]
Tumor	*M. musculus*	Single-cell analyses reveal CBD rewires tumor microenvironment via inhibiting alternative activation of macrophage and synergizes with anti-PD-1 in colon cancer	10x Genomics	2023	GSE220635	[70]
Tumor	*M. musculus*	Lung tumor–infiltrating Treg have divergent transcriptional profiles and function linked to checkpoint blockade response	10x Genomics	2023	GSE235602	[94]
Tumor	*M. musculus*	Formate supplementation enhances anti-tumor CD8+ T cell fitness and efficacy of PD-1 blockade	10x Genomics	2023	GSE240231	[95]
Tumor	*M. musculus*	Broad-spectrum kinome profiling identifies CDK6 upregulation as a driver of lenvatinib resistance in hepatocellular carcinoma	10x Genomics	2023	GSE218010	[96]
Inflammatory response	*M. musculus*	Single-cell RNA sequencing analysis reveals the heterogeneity of IL-10 producing regulatory B cells in lupus-prone mice	10x Genomics	2023	GSE242200	[71]

(continued)

Table 4. Continued.

Main category	Species	Title	Sequencing method	Year	Data source	Ref.
Inflammatory response	*M. musculus*	Skeletal muscle regeneration failure in ischemic-damaged limbs is associated with pro-inflammatory macrophages and premature differentiation of satellite cells	10x Genomics	2023	GSE227076	[72]
Viral infection	*M. musculus*	Cell-intrinsic and -extrinsic effects of SARS-CoV-2 RNA on pathogenesis: single-cell meta-analysis	10x Genomics	2023	GSE239835	[73]
Development	*M. musculus*	Tsc2 coordinates neuroprogenitor differentiation	10x Genomics	2023	GSE229549	[74]
Development	*M. musculus*	Single-nucleus multiomic mapping of m6A methylomes and transcriptomes in native populations of cells with sn-m6A-CT	10x Genomics	2023	GSE217255	[75]
Development	*M. musculus*	KDM6A/UTX promotes spermatogenic gene expression across generations and is not required for male fertility	10x Genomics	2023	GSE215112	[97]
Inflammatory response	*Danio rerio*	Loss of autism-candidate CHD8 perturbs neural crest development and intestinal homeostatic balance	Smart-seq	2023	GSE184363	[76]
Inflammatory response	*D. rerio*	Alterations in immune cell heterogeneities in the brain of aged zebrafish using single-cell resolution	10x Genomics	2023	GSE197673	[77]
Inflammatory response	*D. rerio*	An anti-inflammatory activation sequence governs macrophage transcriptional dynamics during tissue injury in zebrafish	10x Genomics	2022	GSE209884	[78]
Inflammatory response	*D. rerio*	Activation of a transient progenitor state in the epicardium is required for zebrafish heart regeneration	10x Genomics	2022	GSE202836	[79]
Inflammatory response	*D. rerio*	Single-cell ATAC-seq reveals GATA2-dependent priming defect in myeloid and a maturation bottleneck in lymphoid lineages	inDrops	2021	GSE151231	[80]
Tumor	*Drosophila melanogaster*	Single-cell sequencing of TAMs in a Drosophila model	Smart-seq2	2023	GSE242774	[81]
Development	*D. melanogaster*	Single-cell expression profile of Drosophila ovarian follicle stem cells illuminates spatial differentiation in the germarium	10x Genomics	2023	GSE208674	[82]
Development	*D. melanogaster*	A comprehensive temporal patterning gene network in Drosophila medulla neuroblasts revealed by single-cell RNA sequencing	10x Genomics	2022	GSE168553	[83]
Development	*D. melanogaster*	Age-related changes in Pc gene regulation disrupt lineage fidelity in ISCs	10x Genomics	2021	GSE157775	[84]
Development	*A. thaliana*	Brassinosteroid gene regulatory networks at cellular resolution in the Arabidopsis root	10x Genomics	2023	GSE212230	[85]
Development	*A. thaliana*	Leaf cell-specific and single-cell transcriptional profiling reveals a role for the palisade layer in UV light protection	10x Genomics	2022	GSE184511	[86]
Development	*S. cerevisiae*	Single-cell RNA-seq reveals early heterogeneity during aging in yeast	Smart-seq2	2022	GSE210032	[87]
Development	*S. cerevisiae*	mDrop-Seq: Massively parallel single-cell RNA-seq of *S. cerevisiae* and *Candida albicans*	mDrop-seq	2022	GSE154515	[88]
Development	*Escherichia coli*	Bacterial droplet-based single-cell RNA-seq reveals antibiotic-associated heterogeneous cellular states	BacDrop	2023	GSE180237	[89]
Development	*E. coli*	Prokaryotic single-cell RNA sequencing by *in situ* combinatorial indexing	PETRI-seq	2020	GSE141018	[90]

various species. For example, using *Camelus bactrianus*, single-cell 5' RNA sequencing was employed to investigate B cells, which revealed the transcriptional landscape of heavy-chain and typical antibody-producing B cells and uncovered a unique immune adaptation in camels that involves VHH and VH hybrid B-cell receptors [98]. Similarly, a comprehensive single-cell atlas of the respiratory system of *Protopterus annectens* shed light on the cellular mechanisms that support extreme terrestrial adaptation, highlighting evolutionary parallels with the lung structures of fully terrestrial vertebrates [99]. In a study using *Ambystoma mexicanum*, combinatorial hybridization sequencing (CH-seq) of various tissues mapped cellular differentiation states across neotenic and metamorphic conditions, which elucidated the regenerative abilities and tissue remodeling mechanisms of axolotls [100]. Single-cell multiomics of the skin fibroblasts of *Rangifer tarandus* differentiated the pro-regenerative state of velvet skin and the fibrotic response of flank skin, providing important clues into the cellular and molecular basis for scar-free wound healing [101]. Furthermore, transcriptomic analyses of *Taeniopygia guttata* vocal circuits revealed evolutionary similarities between the songbird DVR and mammalian neocortex, demonstrating a convergence in sensory processing and complex behavior regulation [102]. Studies on immune responses in *Anas platyrhynchos* identified the key role of myeloid cells in antiviral defense, highlighting gender-specific differences in immune modulation [103]. Investigations of *Astyanax mexicanus* demonstrated the influence of parasite scarcity on immune investment, showing shifts toward adaptive immunity in cave-dwelling morphotypes, which had reduced pro-inflammatory responses compared with those of surface-dwelling forms [104]. scRNA-seq of the mesenchymal stromal cells (MSCs) of *Equus caballus* from multiple tissue sources revealed notable heterogeneity in immune modulation and cell motility even among donor-matched MSCs [105]. These studies collectively underscore the potential biological insights that single-cell sequencing can offer into non-model organisms, expanding the current understanding of evolutionary adaptations, immune strategies, and developmental processes in vertebrates beyond traditional model species.

Invertebrates

With non-model invertebrates, single-cell technologies have similarly yielded significant discoveries. In a study with *Dugesia japonica*, a high-resolution single-cell transcriptomic atlas was constructed to investigate the diverse cell types in planarians with a focus on neoblasts, the pluripotent stem cells responsible for their remarkable regenerative abilities [106]. The data provide insights into the cellular and molecular mechanisms underpinning planarian regeneration, which could be beneficial for regenerative medicine and stem cell biology. In a study of *Lytechinus variegatus*, scRNA-seq of sea urchin embryos provided insights into the developmental trajectories of multiple cell lineages, demonstrating the synchronous and asynchronous differentiation pathways of various germ layers [107]. Such detailed lineage tracking reveals the complexity of early embryonic development and offers a model for studying the regulatory networks that drive differentiation. In another study, single-cell profiling of the developing brain tissues of *Octopus vulgaris* identified distinct neural and glial cell types, providing a deeper understanding of the cellular basis for the sophisticated behaviors observed in cephalopods [108]. The identification of diverse neural cell types, along with their spatial organization, could reveal the evolutionary innovations that underpin the complex cognitive abilities of octopuses, such as problem-solving and tool use. The data generated from this study may pave the way for comparative analyses of neural development across different animal phyla, offering valuable insights into the evolution of nervous system complexity. These studies collectively underscore the biological insights provided by single-cell sequencing into non-model organisms, expanding our understanding of evolutionary adaptations, immune strategies, and developmental processes in both vertebrates and invertebrates beyond traditional model species. The application of single-cell technologies to these non-model organisms allows the exploration of unique evolutionary trajectories and discovery of novel biological mechanisms, providing a more comprehensive view of life's diversity and complexity.

Plants

Recent advancements in single-cell transcriptomics have significantly enhanced our understanding of plant developmental biology, particularly in non-model organisms, across various domains such as organ development, evolutionary biology, and genetic regulation. A study of *Eucalyptus grandis* employed scRNA-seq alongside LCM to elucidate the developmental pathways and evolutionary relationships of xylem cells with a focus on vessel elements, libriform fibers, and tracheids [109]. This research examined xylem tissue development and formation with the aim of identifying evolutionary differences and commonalities in xylem cells among diverse tree species, highlighting both conserved genetic mechanisms and species-specific trajectories. Similarly, experiments with *Gossypium bickii* utilized scRNA-seq to dissect the developmental trajectory and transcriptional regulatory networks of pigment glands containing gossypol [110]. By analyzing differences in gene expression related to pigment gland development, the study aimed to breed cotton varieties that retain pigment glands in the plant but not in the seeds, thus addressing agricultural limitations. In the context of symbiotic organogenesis, research on *Medicago truncatula* used single-cell transcriptome analysis to examine the transcriptional reprogramming of root cells during nodule development, which revealed key hormonal interactions and identified novel regulatory genes such as STYLISH 4 [111]. This study covers cell development, genetic regulation, and organ formation, offering insights into how plants adapt to environmental changes. Furthermore, a study of *Nicotiana attenuata* mapped the biosynthetic pathway of floral scent compounds such as benzylacetone at the single-cell level, uncovering critical enzymes and regulatory genes such as NaPAL4, NaPKS2, and NaAER1 present predominantly in epidermal cells [112]. This research highlights metabolic heterogeneity and gene expression differences in specific cell types, contributing to our understanding of genetic development. A study on root development in *Phyllostachys edulis* constructed a single-cell transcriptome atlas of basal roots, revealing unique spatiotemporal developmental trajectories and identifying regulatory factors such as PheWOX13a and PheWOX13b [113]. The findings emphasize the importance of organ development studies with non-model species. Lastly, research on *Solanum lycopersicum* demonstrated that a suberized exodermis, regulated by the MYB92 transcription factor and ASFT enzyme, is essential for drought tolerance [114]. This observation is contrary to the endodermal suberization observed in *A. thaliana*, highlighting species-specific adaptations and the role of genetic development in environmental responses. Collectively, these studies illustrate how single-cell technologies are being applied to non-model plants to unravel complex developmental processes, including organ formation, evolutionary differences, and genetic regulation,

thereby enriching our understanding of plant biology in various species.

Microorganisms

Recent advances in scRNA-seq have significantly expanded our understanding of microbial development and interactions, particularly in non-model microorganisms. The development of the TRANSITome approach allowed the high-resolution analysis of *Burkholderia pseudomallei* during its intracellular transit within host cells [115]. Single-cell transcriptomics uncovered dynamic changes in gene expression during the three key intracellular stages: the pathogen's entry into the vacuole, its escape into the cytoplasm and subsequent replication, and its spread between cells. These findings led to the identification of novel virulence factors essential for the pathogen's development and ability to cause disease. Experiments with the unicellular alga *Chlamydomonas reinhardtii* using scRNA-seq showed heterogeneity in diurnal cycle phases among individual cells, even under constant light conditions [116]. The study highlights how cells individually respond to nutritional stresses such as iron or nitrogen deficiency without changes in circadian rhythms and unique transcriptional states, thus contributing to our understanding of cell development and environmental adaptation. Research on *Dictyostelium discoideum* demonstrated that collective cAMP signaling could drive rapid transitions between cell states during development, with single-cell analyses revealing how cells synchronize gene expression through amplified signaling while maintaining the ability to revert to original states upon nutrient resupply [117]. Single-cell transcriptomics of the marine microalga *Emiliania huxleyi* shed light on the sequential transcriptional programs and heterogeneous infection states during interactions with a specific giant virus [118]. The persistence of chloroplast and mitochondrial transcripts during infection stages suggests strategic manipulation by the virus to optimize host resources, offering insights into complex interspecies interactions and evolutionary development. Lastly, a study of *Serratia marcescens* showed that hosts such as *Drosophila* larvae can manipulate the lifestyle switch and pathogenicity heterogeneity of opportunistic pathogens at a single-cell resolution [119]. The host-induced suppression of bacterial virulence genes and promotion of commensal states through antimicrobial peptides underscore the sophisticated mechanisms of gene regulation and adaptation. Collectively, these studies demonstrate how single-cell technologies are being leveraged to dissect the complexities of cell development, gene expression, and interspecies interactions in non-model microorganisms, thereby contributing to a better understanding of microbial physiology, ecology, and evolution. The relevant information on the non-model organisms and their studies is summarized in Table 5, which provides an organized overview of the key research findings obtained using single-cell transcriptomics across various non-model species.

Future prospects and conclusion

scRNA-seq has transformed biological research, offering a high-resolution view of gene expression at the individual cell level. With this technology, researchers can observe cellular heterogeneity, trace developmental processes, and identify cell-specific responses in ways that were previously unattainable [3, 4]. scRNA-seq has applications in various fields, including medicine, developmental biology, and ecology, allowing scientists to investigate complex biological processes or architectures such as tumor microenvironments, immune responses, and ecosystem dynamics. The rapid advancements in scRNA-seq technology have contributed significantly to our understanding of both health and disease, as well as broader ecological interactions. A notable trend in scRNA-seq research is the heavy focus on human and mouse models. In comparison with bulk RNA sequencing, which aggregates signals from large populations of cells, scRNA-seq provides a more detailed and precise view of individual cells. However, most scRNA-seq data are derived from human and mouse samples, with the primary focus on human diseases and therapeutic applications. This focus limits the diversity of biological insights that could be gained from applying scRNA-seq to a broader range of species, particularly in fields outside of human disease research. Despite its potential, scRNA-seq has significant limitations, particularly when applied to non-model organisms or tissues with highly diverse cell types [115]. Isolating individual cells from such tissues can be technically challenging, and achieving accurate single-cell resolution across species remains a key hurdle [31, 32]. Furthermore, the high costs and technical demands of spatial transcriptomics, which aims to map gene expression to the exact location of cells within tissues, add complexity to the application of scRNA-seq across various organisms [33]. To fully realize the potential of scRNA-seq, it is essential to expand its application beyond traditional model organisms such as humans and mice. Non-model organisms should also be studied using scRNA-seq to address questions related to developmental biology, disease mechanisms, and ecological interactions. Technological improvements in single-cell sorting, sequencing methods, and data analysis tools are critical to overcome current limitations. Additionally, reducing the costs of these techniques will make scRNA-seq more accessible, allowing broader applications across a wider variety of species, ultimately increasing both the accuracy and efficiency of single-cell studies.

In conclusion, this review highlights the current advancements and applications of scRNA-seq across a diverse range of species. It highlights how scRNA-seq is being increasingly utilized not only in human and mouse models but also in non-model organisms to investigate developmental processes, disease mechanisms, and ecological interactions. As the technology continues to evolve, it is poised to further expand its reach, offering deeper insights into the complexities of cellular biology across different biological systems.

Key Points

- Presented a detailed framework addressing species-specific requirements for single-cell ribonucleic acid sequencing (scRNA-seq) workflows in both cell isolation and computational analysis.
- Explored the connections between scRNA-seq research in model organisms and its applications to non-model species, highlighting strategies to overcome technical limitations.
- Discussed the potential of scRNA-seq to identify immune adaptations, developmental pathways, and evolutionary processes across diverse biological systems.
- Provided practical recommendations for applying scRNA-seq tools and methods to research in non-model species.

Table 5. Non-model organism research utilizing single-cell transcriptomics.

Main category	Species	Title	Sequencing method	Year	Data source	Ref.
Vertebrates	*Camelus bactrianus*	Single-cell 5′ RNA sequencing of camelid peripheral B cells provides insights into cellular basis of heavy-chain antibody production	10x Genomics	2024	GSE238082	[98]
Vertebrates	*Protopterus annectens*	A single-cell atlas of West African lungfish respiratory system reveals evolutionary adaptations to terrestrialization	10x Genomics	2023	GSE240094	[99]
Vertebrates	*Ambystoma mexicanum*	Construction of the axolotl cell landscape using combinatorial hybridization sequencing at single-cell resolution	CH-seq	2022	GSE201446	[100]
Vertebrates	*Rangifer tarandus*	Fibroblast inflammatory priming determines regenerative versus fibrotic skin repair in reindeer	10x Genomics	2022	GSE180653	[101]
Vertebrates	*Taeniopygia guttata*	Cellular transcriptomics reveals evolutionary identities of songbird vocal circuits	10x Genomics	2021	GSE153665	[102]
Vertebrates	*Anas platyrhynchos*	Single-cell analysis of the *in vivo* dynamics of host circulating immune cells highlights the importance of myeloid cells in avian flaviviral infection	10x Genomics	2021	GSE153825	[103]
Vertebrates	*Astyanax mexicanus*	Adaptation to low parasite abundance affects immune investment and immunopathological responses of cavefish	10x Genomics	2020	GSE128306	[104]
Vertebrates	*Equus caballus*	Single-cell RNA sequencing of equine MSCs from primary donor-matched tissue sources reveals functional heterogeneity in immune modulation and cell motility	10x Genomics	2020	GSE156467	[105]
Invertebrates	*Dugesia japonica*	A resource of single-cell gene expression profiles in a planarian *D. japonica*	BD Rhapsody	2023	GSE223929	[106]
Invertebrates	*Lytechinus variegatus*	Developmental single-cell transcriptomics in the *L. variegatus* sea urchin embryo	10x Genomics	2021	GSE184538	[107]
Invertebrates	*Crassostrea gigas*	Single-cell atlases of two lophotrochozoan larvae highlight their complex evolutionary histories	10x Genomics	2023	GSE231713	[120]
Invertebrates	*Octopus vulgaris*	Cell type diversity in a developing octopus brain	10x Genomics	2022	GSE193622	[108]
Invertebrates	*Parasteatoda tepidariorum*	Reconstruction of the global polarity of an early spider embryo by single-cell and single-nucleus transcriptome analysis	BD Rhapsody	2022	GSE201705	[121]
Plants	*Medicago truncatula*	The single-cell transcriptome program of nodule development cellular lineages in *M. truncatula*	10x Genomics	2024	GSE224539	[111]
Plants	*Solanum lycopersicum*	A suberized exodermis is required for tomato drought tolerance	10x Genomics	2024	GSE212403	[114]
Plants	*Eucalyptus grandis*	Single-cell transcriptomics unveils xylem cell development and evolution	10x Genomics	2023	GSE180121	[109]
Plants	*Phyllostachys edulis*	Single-cell transcriptome atlas reveals spatiotemporal developmental trajectories in the basal roots of moso bamboo (*P. edulis*)	10x Genomics	2023	GSE229126	[113]
Plants	*Gossypium bickii*	Single-cell transcriptomic analysis reveals the developmental trajectory and transcriptional regulatory networks of pigment glands in Gossypium bickii	10x Genomics	2023	GSE224635	[110]
Plants	*Nicotiana attenuata*	Single-cell RNA-sequencing of *N. attenuata* corolla cells reveals the biosynthetic pathway of a floral scent	10x Genomics	2022	GSE193464	[112]
Fungi	*Candida albicans*	An ultra-high–throughput, massively multiplexable, single-cell RNA-seq platform in yeasts	SPLiT-seq	2024	GSE251966	[26]
Microorganisms	*Serratia marcescens*	Hosts manipulate lifestyle switch and pathogenicity heterogeneity of opportunistic pathogens in the single-cell resolution	M20 Genomics	2024	GSE232120	[119]
Microorganisms	*Dictyostelium discoideum*	Collective signaling drives rapid jumping between cell states	10x Genomics	2023	GSE220242	[117]
Microorganisms	*Burkholderia mallei*	The *Burkholderia pseudomallei* intracellular ‘TRANSITome’	TRANSITome	2021	GSE156938	[115]
Microorganisms	*Chlamydomonas reinhardtii*	Single-cell RNA sequencing of batch Chlamydomonas cultures reveals heterogeneity in their diurnal cycle phase	10x Genomics	2021	GSE157580	[116]
Microorganisms	*Emiliania huxleyi*	A single-cell view on alga-virus interactions reveals sequential transcriptional programs and infection states	MARS-seq	2020	GSE135429	[118]

Author contributions

Hyunmin Woo (Investigation, Formal analysis, Data curation, Writing—original draft, Writing—review & editing, Visualization) and Seong-il Eyun (Conceptualization, Writing—original draft, Writing—review & editing, Supervision, Project administration).

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Data availability

Not applicable.

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