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Emerging technologies of single-cell multi-omics

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Abstract

The heterogeneity of the hematopoietic system was largely veiled by traditional bulk sequencing methods, which measure the averaged signals from mixed cellular populations. In contrast, single-cell sequencing has enabled the direct measurement of individual signals from each cell, significantly enhancing our ability to unveil such heterogeneity. Building on these advances, numerous single-cell multi-omics techniques have been developed into high-throughput, routinely accessible platforms, delineating the precise relationships among the different layers of the central dogma in molecular biology. These technologies have uncovered the intricate landscape of genetic clonality and transcriptional heterogeneity in both normal and malignant hematopoietic systems, highlighting their roles in differentiation, disease progression, and therapy resistance. This review aims to provide a brief overview of the principles of single-cell technologies, their historical development, and a subset of ever-expanding multi-omics tools, emphasizing the specific research questions that inspired their creation. Amidst the evolving landscape of single-cell multi-omics technologies, our main objective is to guide investigators in selecting the most suitable platforms for their research needs.

Introduction

The hematopoietic system is a complex ecosystem comprising diverse cellular populations, each defined by different lineages, functions, and activation states. While cost-effective, the conventional approach of analyzing bulk blood specimens obscures detailed examination of heterogeneous cellular states. As distinct cellular populations are sequenced together, bulk sequencing yields an average mutant allele frequency or gene expression profile combined for the heterogeneous populations, masking the precise lineage or clonal relationship between the cells. In contrast, single-cell sequencing (SCS) has enabled direct assessment of cell-to-cell variabilities, reconstruction of evolutionary relationships, and identification of rare populations.

Historically, the hematopoietic system has been at the forefront of technological advancements due to the accessibility of blood samples and the facility to analyze cells that naturally exist in dissociated states. Over the past decade, SCS has continued to refine our understanding of the hematopoietic system, challenging the models of hematopoiesis [1] and characterizing unconventional leukemic stem cells (LSCs)[2] conferring resistance against targeted therapies, just to iterate a few.

This review aims to summarize the principles of SCS while outlining recent advancements in SCS. As the field rapidly evolves and new technologies continue to emerge, investigators are left with an almost overwhelming abundance of choices. The primary focus of this review is to present a subset of the many available multi-omics platforms, particularly in the context of studying tumor heterogeneity and clonal

evolution, while highlighting the types of research questions they are designed to address.

Overview of single-cell technologies

Single-cell isolation and library preparation techniques

The first crucial step in SCS is the isolation of individual cells. Initially, single cells were manually picked under a microscope [3], which was laborious and inherently low in throughput. Shortly after, the procedure was scaled up and partially automated using fluorescence-activated cell sorting (FACS) [4, 5], which was used to place each cell onto each well of the 96-well plates before researchers carried out library preparation.

The advancement of SCS gained considerable momentum with the introduction of microfluidics, which allowed a large number of cells to be automatically isolated and prepared for libraries in parallel. The earlier plate-based microfluidics, such as Fluidigm's C1 system [6], enabled the processing of hundreds of cells per sample. Later, the advent of droplet-based microfluidics [7, 8] scaled up the throughput to thousands of cells per sample, enhancing the ability to detect rare populations in the sample. Perhaps the most well-known droplet-based platform, the Chromium from 10X Genomics [9], quickly gained popularity over existing droplet-based methods [7, 8] by significantly improving cell capture rates.

A significant drawback of microfluidics is their propensity to isolate multiple cells together, known as multiplets. To circumvent this, some single-cell studies adopted a high-throughput technique called combinatorial indexing [10], which reduces the likelihood

of any two cells receiving the same barcode without necessitating each cell's physical isolation. Others sought to capture single cells in scalable, size-adjusted nanowells [11], minimizing the probability of isolating more than one cell per well. Nevertheless, a comparative study [12] found no significant difference in multiple rates between these methods and microfluidics, suggesting that factors like target sensitivity or ease of use should be prioritized when choosing high-throughput techniques.

In SCS, each fragmented genetic molecule is labeled with a cell-specific barcode and a unique molecular identifier (UMI) [13]. This dual tagging enables the tracing of the molecule's origin back to its respective single cell and the quantifying of the pre-amplification molecule abundance. The advent of UMI was detrimental to the refinement of SCS, as it significantly mitigated amplification errors by allowing the discernment of amplification-derived artifacts. Mammalian cells only contain picograms of DNA and RNA, far short of the nanograms of input materials required for sequencing. As a result, SCS often requires aggressive amplification, which serves as a major source of error.

To enhance efficiency and reduce costs, each molecule is now also tagged with a sample-specific barcode, allowing the pooling of multiple samples for simultaneous sequencing. Tools like cell hashing [14] directly assign sample-specific barcodes to molecules, while computational methods like demuxlet [15] utilize natural markers such as single-nucleotide variants (SNVs) to distinguish samples based on donor-specific mutational profiles. Despite these advances, challenges like cross-contamination persist, underscoring the need for further technical and bioinformatic improvements in sample multiplexing techniques.

Single-cell RNA sequencing

The human transcriptome, comprising approximately 20,000 genes, represents only 2% of the entire genome. This has uniquely positioned single-cell RNA sequencing (scRNA-seq) as a leading single-cell technology and a powerful, unbiased tool for capturing a cell's phenotypic state.

The inaugural single-cell study in 2009 [3] **(Figure 1)** conducted whole transcriptomic sequencing (WTS) on just a single mouse blastomere. Since then, a wide spectrum of scRNA-seq protocols have been developed to meet different needs. Given that the average mRNA transcripts are sized several kilobases, reading only 100-400 base pairs at either the 3' or 5' end of mRNA provides a cost-effective and high-throughput approach. However, this approach cannot profile RNA isoforms or most SNVs, which require full-length sequencing. Smart-seq2 [16], a pioneer of modern full-length sequencing, has been widely used in applications that require highly sensitive variant analysis but have lower demands on the throughput. More recently, it was updated as Smart-seq3 [17], incorporating 5' UMIs to enhance the accuracy of transcript quantification. Extending from these capabilities, recent advancements in scRNA-seq have made it compatible with long-read sequencing, such as Nanopore [18], which circumvents the error-prone processes of fragmentation and assembly. Although the technology has facilitated the study of larger structural variants in scRNA-seq, its current high error rates warrant careful application. The per-cell cost increases progressively from 3'/5' end-based sequencing to full-length sequencing and further to long-read sequencing. 5' end sequencing is slightly more expensive than 3' end sequencing but offers moderately improved mutation detection. In summary, cost

differences have created a tradeoff between cellular throughput and transcript coverage in scRNA-seq (**Figure 2a**), leading to the development of various platforms (**Table 1**).

One of the current limitations of scRNA-seq includes transcriptomic dropouts. Most mRNAs are expressed in only a few copies per cell, and the expression of mRNA at any given state varies significantly depending on the cell physiology and RNA stochasticity. This makes it difficult to discern whether the observed absence of a transcript is due to technical error or genuine biological variability. However, newer algorithms, as well as the construction of reference-level transcriptomic cell atlases, are gradually addressing these challenges.

Single-cell DNA sequencing

Single-cell DNA sequencing (scDNA-seq) technologies present a unique opportunity to analyze the clonality of individual cells and the order in which mutations arise, both of which have significant implications for clinical outcomes [19]. However, the development of scDNA-seq was relatively delayed compared to scRNA-seq (**Figure 1**), primarily due to the limited copy number of DNA in a cell (only two versus multiple copies of mRNA), as well as their larger size and complexity **(Figure 2).** These attributes of the genome present higher risks of misalignment, allele dropout (ADO), and artifact mutations, all of which can easily complicate scDNA-seq analysis.

Compared to the transcriptome, the human genome spans several gigabases. Subsequently, whole-genome amplification (WGA) remains a bottleneck in scDNA-seq, making single-cell whole-genome sequencing (WGS) costly, error-prone, and challenging. To reduce the WGA bottleneck, specific methods have emerged for

different intentions of analysis. In general, polymerase chain reaction (PCR)-based methods, such as Degenerate Oligonucleotide-Primed PCR (DOP-PCR) [20] or Multiple Annealing and Looping-Based Amplification Cycles (MALBAC) [21], are considered more suitable for analyzing larger changes in the chromosome, such as copy number alterations (CNA). Meanwhile, isothermal methods utilizing high-fidelity phi29 polymerases, such as Multiple Displacement Amplification (MDA) [22] or Primary Template-directed Amplification (PTA) [23], are deemed more suitable for precisely analyzing smaller changes such as SNVs. The range of options available for WGA stands in contrast to those commonly employed for whole-transcriptome amplification (WTA) in scRNA-seq, which are simply narrowed down to either *in vitro* transcription [24] or PCR.

The first scDNA-seq study in 2011 performed whole-genome sequencing (WGS) on a hundred single nuclei from human breast cancer [5] (**Figure 1**). Using DOP-PCR, the study focused on profiling CNAs to reconstruct the clonal history of breast cancer at the chromosomal level. Subsequent studies demonstrated the feasibility of single-cell whole-exome sequencing (WES) in human essential thrombocytopenia [25] and renal cell carcinoma [26]. Using MDA to profile SNVs, these studies aimed to characterize the clonal makeup of these diseases at the single nucleotide level.

To minimize technical artifacts from *in vitro* WGA, some researchers sought to utilize amplification methods naturally employed by cells. One group [27] performed *ex vivo* WGA via a method known as single-cell cloning, deriving colonies from individual hematopoietic stem and progenitor cells (HSPC) that are capable of forming colonies. This approach has found numerous applications in studies investigating the clonal

architecture of HSPCs, such as in healthy individuals [28, 29] and patients with myeloproliferative neoplasms [30]. Another group captured nuclei undergoing the G2/M phase of the cell cycle, leveraging their duplicated genomic material [31].

The large size of the genome presents a distinct tradeoff between genome coverage and throughput in scDNA-seq **(Figure 2b)**. Targeted scDNA-seq [32, 33] only requires a small region of DNA amplification but provides an incomplete picture of the genome, trading coverage for cost and efficiency. To give a practical example within commercial programs, Mission Bio's Tapestri platform uses targeted scDNA-seq to [32] profile thousands of cells at the expense of sequencing only tens or hundreds of genes. In contrast, Bioskryb's ResolveDNA platform [34], which relies on the PTA technique for WGA, provides whole-genome or whole-exome analyses for sequencing just a few hundred cells. At the single-cell level, WES is done by enriching the exome region after performing WGA **(Figure 2b)**, facing the same bottleneck as WGS.

One of the core applications of scDNA-seq is to trace the evolutionary trajectory through examination of the mutation co-occurrence. In this sense, targeted scDNA-seq offers an efficient means for clonal analysis by focusing on common driver mutations. However, due to the limited number of mutations profiled for each cell, the resolution of clonality is inherently limited. Albeit costly, whole-genome analysis is necessary to obtain a complete and unbiased view of the genome.

Single-cell mitochondrial sequencing

To address the limitations of high cost, error rates, and lack of scalability in single-cell WGS, recent studies have also shown the potential of analyzing clonality

through profiling the mitochondrial DNA (mtDNA). A landmark study [35] has reported that mtDNA has proven to be a reliable source for lineage tracing due to its smaller size (16.6 kilobases) and higher copy number per cell. Most importantly, as the mitochondrial genotype is already, and often inadvertently, captured during routine scRNA-seq or single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq)[36] experiments, this approach is spearheading advancements in single-cell lineage tracing techniques. However, it is currently unknown whether the clonalities inferred from mtDNA match those inferred from genomic DNA.

Bioinformatics

With the increasing standardization of experimental workflows, data analysis now constitutes the majority of the effort in single-cell analysis. Repurposing many tools used for processing bulk sequencing data, the upstream preprocessing, such as trimming, alignment, and variant calling, has become relatively well-defined and automated. For instance, many commercial SCS platforms provide cloud-based interfaces that allow users to simply upload FASTQ files and run all the steps of preprocessing. Interestingly, a study [37] has even demonstrated that the choice of scRNA-seq preprocessing pipelines had minimal impact on downstream clustering results once effective normalization and clustering methods are applied.

In contrast, downstream analysis requires advanced programming skills and domain knowledge, presenting a hurdle that often necessitates hiring a trained bioinformatician to manage the analysis. The analytic workflow of scRNA-seq has been well-established, supported by publications such as '*Best Practices of Single-Cell*

Analysis' [38] and renowned tools such as Seurat [39] or SCANPY [40]. Conversely, scDNA-seq has yet to achieve a comparable level of establishment, although new tools like Mission Bio's Mosaic or Bioskryb's BaseJumper are beginning to make the analysis more simple and accessible.

Multimodal single-cell technologies

The maturation of unimodal single-cell technologies soon motivated researchers to overlay different modalities to understand relationships among various components of the cell, such as the genome, epigenome, transcriptome, or proteome (**Table 1** and **Figure 3**). Some of these technologies are *bona fide* multi-omics, directly measuring multiple elements from the same cell, while others attempt to infer unmeasured modalities based on a measured modality.

Integrating genomics with transcriptomics

Although scRNA-seq effectively resolves granular cell types within a tumor sample, it poses challenges in precisely demarcating leukemic cells from normal cells in the sample. In this sense, concurrent examination of a cell's mutational status enables the assessment of malignancy in a sample of transcriptionally heterogeneous cells. This approach offers a unique advantage in discovering the underlying transcriptional pathways that may drive the predominance of certain clonal populations and elucidating the impact of mutations in the alteration of transcriptional activities.

Several platforms have enabled direct measurement of DNA and RNA from the same cell. For instance, ResolveOME [34], an extension of the WGS-based ResolveDNA

platform, combines WGS with full-length WTS to allow for unbiased screening of a cell's genome and transcriptome. To focus on only a few genes of interest instead, TARGET-seq [41] could be used, as demonstrated in a recent study [42] that investigated two representative clonal hematopoiesis mutations in *DNMT3A* and *TET2*. Using TARGET-seq, the study demonstrated that HSCs harboring these mutations gain a fitness advantage over wild-type HSCs by attenuating inflammation-associated transcriptional programs, which enhances the survival of mutant HSCs in the inflammatory tumor environment.

However, direct measurement of DNA remains expensive and technically challenging, prompting the development of various alternatives. Inferring CNAs from scRNA-seq data is relatively straightforward and can be achieved using simple estimation [43] or advanced computational algorithms [44]. On the contrary, detecting oncogenic SNVs and indels proves to be more challenging. Truncating or non-sense mutations are less likely to be transcribed, and 3' or 5' biased scRNA-seq may not efficiently cover the mutated loci. Nonetheless, several groups have successfully inferred specific driver mutations from scRNA-seq reads by experimentally modifying the scRNA-seq protocols, such as spiking in mutant-specific primers [45, 46], circularizing cDNA [46], or utilizing long-read sequencing [46, 47]. A notable multi-omic platform adopting this approach is the Genotyping of Transcriptomes (GoT) [46], which analyzed patients with essential thrombocytopenia and provided *in situ* evidence that the transcriptional outcome of CALR mutations may confer a clonal advantage specifically in the compartment of megakaryocyte progenitors.

Integrating genomics with epigenomics

Following the development of scRNA-seq and scDNA-seq, newer technologies were rapidly developed to study epigenomic processes at the single-cell level (**Figure 1**). For instance, scATAC-seq profiles open chromatin regions of the DNA, and single-cell reduced-representation bisulfite sequencing (scRRBS) [48] analyzes DNA methylation patterns in the CpG-rich regions. These technologies were quickly integrated with genomic analysis to understand how alterations in gene regulation are affected by mutations at the DNA level.

Two multi-omic protocols have enabled high-throughput analysis of a few target genes with chromatin accessibility by adding specific DNA primers onto scATAC-seq [49, 50] protocols. For example, the Genotyping of Targeted loci with single-cell Chromatin Accessibility (GoT-ChA) [50], an extension of a droplet-based scATAC-seq protocol, was applied to study patients with myeloproliferative neoplasm. Focusing on *JAK2* V617F mutations, the study revealed epigenetic rewiring specifically in HSCs and megakaryocyte progenitors, marked by increased chromatin accessibility in inflammation-related genes. A notable aspect of GoT-ChA is that, beyond the two directly sequenced modalities, it sought computational integration with other multi-omic tools, such as DOGMA-seq [51], through the common grounds of ATAC-seq modality. Hence, GoT-ChA was able to infer modalities such as mtDNA, immunophenotype, and gene expression, which were not directly sequenced by the platform itself. Such an imputation approach has become increasingly popular to complement missing information and cross-validate the findings that have been made based on measured

modalities, overcoming current limitations in multi-omic capabilities that can directly sequence only two to three modalities.

Currently, multi-omics technologies that integrate DNA mutations and methylation status are rooted in joint methylome and transcriptomic analysis. Earlier methods like the single-cell triple omics sequencing technique (scTrio-seq)[52] enabled genome, methylome, and transcriptome analysis by combining scRRBS with scRNA-seq and inferring CNAs from their reads. More recent methods attempted to integrate scRRBS with Smart-seq2 (Smart-RRBS) [53] and, further, with genotyping [54] to profile DNA methylome, transcriptome, and SNVs simultaneously. This technique was applied in chronic lymphocytic leukemia (CLL) study [54] that aimed to reconstruct the evolutionary history of tumor cells based on “epimutations”, the heritable changes in DNA methylation. The study initially constructed the CLL lineage tree based on the scRRBS data and subsequently integrated genotyping data to validate and refine the lineage tree.

Integrating genomics with proteomics

Prior to the development of scRNA-seq, cell types were traditionally identified based on surface proteins or immunophenotypes. Integrating single-cell immunophenotypes offers several advantages over single-cell transcriptomics for characterizing a cell's phenotypic state. Not only is immunophenotype the clinically established gold standard for cell type identification, but it also exhibits greater stability compared to RNA species, holding greater potential for applications like in vitro diagnostic tools. Moreover, certain immunophenotypes serve as canonical markers for

leukemic cells, enabling their use as surrogates of malignancy to demarcate leukemic cells within a sample.

To remain compatible with high-throughput droplet-based microfluidic platforms, standard flow cytometry has been modified by conjugating protein-binding antibodies with oligonucleotides instead of fluorophores [55]. This strategy allows protein abundance to be sequenced and quantified just like DNA. Another benefit of using oligonucleotides is their ability to generate nearly infinite combinations of barcodes. In practice, this allows the unique identification of hundreds of surface proteins, far exceeding the capabilities of conventional mass or flow cytometry, which can typically trace only a few dozen. However, antibody-oligonucleotide tags are not fully optimized yet, with efforts to refine antibody titration [56] only recently underway.

Building on targeted scDNA-seq platform like Tapestri, DNA-Antibody sequencing (DAb-seq) [32] integrates the analysis of surface proteins and target genes to elucidate the relationship between cellular phenotypes and driver mutations. In acute myeloid leukemia (AML) research, DAb-seq has been employed to map the mutational landscape across diverse AML cell types [57, 58], outline genotype-phenotype evolution in response to targeted therapies [59], and distinguish non-malignant clonal hematopoiesis mutations from common driver mutations by identifying the absence of leukemia-associated immunophenotypes within the same cell [60].

Integrating transcriptomics with proteomics

Several multi-omics platforms, including the widely-used Cellular Indexing of Transcriptomes and Epitopes sequencing (CITE-seq) [61], have been developed to

measure mRNA expression and surface protein abundance from the same cell. Integrating scRNA-seq with immunophenotype data improves cell type identification by providing a more comprehensive view of a cell's phenotypic state. For instance, a recent study [56] using CITE-seq produced a high-resolution atlas of human hematopoietic progenitors, identifying novel surface markers to isolate transitional cell populations. These isolated progenitors exhibited restricted clonal outputs in experimental data, supporting a discrete stem and progenitor cell state model [1] rather than the continuous hematopoiesis model proposed with the advent of scRNA-seq.

Until recently, multi-omic techniques linking transcriptomics with proteomics were primarily limited to surface protein analysis. However, emerging techniques like Single-cell Protein And RNA Co-profiling (SPARC) [62] are now enabling the analysis of intracellular proteins as well.

Integrating mitochondrial genomics with transcriptomics or epigenomics

Clonal relationships can be inferred by enriching mitochondrial RNAs that are already captured in low amounts by standard scRNA-seq platforms. Techniques such as Mitochondrial Alteration Enrichment from Single-cell Transcriptomes to Establish Relatedness (MAESTER) [63] have demonstrated the feasibility of this approach. Similarly, integrating scATAC-seq with mitochondrial genotyping has proven effective for inferring clonal structures. For instance, the single-cell Regulatory Multimaps with Deep Mitochondrial Mutation Profiling (ReDeeM) [64] technique uses deep sequencing to profile mtDNA while simultaneously performing scRNA-seq and scATAC-seq, easily integrating the clonal analysis into the investigation of transcriptomic and regulatory landscapes.

Summary

The past decade has witnessed a significant expansion in the capabilities of single-cell analytics, greatly enhancing our understanding of the biology underlying hematopoiesis and hematologic malignancies. Advances in single-cell multi-omics technologies provided unprecedented insights into cellular heterogeneity, lineage differentiation, and molecular mechanisms driving the disease progression. Such rapid technological progress, coupled with the decreasing cost of sequencing, is poised to further improve cellular throughput and accessibility of these technologies.

Despite the current predominance of single-cell tools in research settings, there is a growing potential for their application in clinical practice. For instance, platforms like scDNA-seq and its multi-omic adaptations could be used to identify minimal residual disease, monitor clonal evolution during therapy, and tailor personalized treatment strategies in leukemia patients [19, 65]. However, translating these tools to clinical use will require rigorous clinical validation and evidence that they enhance clinical decision-making.

The remarkable advancements observed over the past decade suggest that integrating single-cell technologies into clinical workflows is not far off. Continued investment and research in single-cell analytics are essential to making these tools accessible and routine in both research and clinical settings.

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Table 1. Summary of single-cell sequencing technologies introduced in this review.

Modality	Name of Technology	Base study	Scope of targets in the study
Transcriptome	Chromium (10X)	Zheng et al. (2017)	Whole-transcriptome (3' or 5' end)
	Smart-seq2	Picelli et al. (2013)	Whole-transcriptome (full-length)
	Smart-seq3	Hagemann-Jensen et al. (2020)	Whole-transcriptome (full-length)
	Nanopore	Byrne et al. (2017)	Whole-transcriptome (long-read)
Genome	Tapestri (Mission Bio)	Pellegrino et al. (2018)	63 genomic loci (23 genes)
	ResolveDNA (Bioskryb)	Marks et al. (2023)	Whole-genome
Genome+ Transcriptome	TARGET-seq	Rodriguez-Meira (2019)	Whole-transcriptome, 12 genomic loci
	GoT	Nam et al. (2019)	Whole-transcriptome, 45 genes
	ResolveOME	Marks et al. (2023)	Whole-transcriptome, whole-genome
Genome+ Chromatin Accessibility	Got-ChA	Izzo et al. (2024)	Open chromatin regions, 5 genomic loci
Genome+ Methylome	scTrio-seq	Hou et al. (2016)	Whole-transcriptome, CpG-rich DNA methylome, CNAs[1]
	Smart-RRBS + genotyping	Gaiti et al. (2019)	Whole-transcriptome, CpG-rich DNA methylome, CNAs, SNVs[2]
Genome+ Proteome	DAb-seq	Demaree et al. (2021)	49 genomic loci (19 genes), 23 surface proteins
Transcriptome+ Proteome	CITE-Seq	Stockeius et al. (2017)	Whole-transcriptome, 17 surface proteins
	SPARC	Reimegård et al. (2021)	Whole-transcriptome, 89 intracellular proteins
Mitochondrial Genome+ Transcriptome	MAESTER	Miller et al. (2022)	Whole-transcriptome, mtRNA
	ReDeeM	Weng et al. (2024)	Whole-transcriptome, open chromatin regions, mtDNA

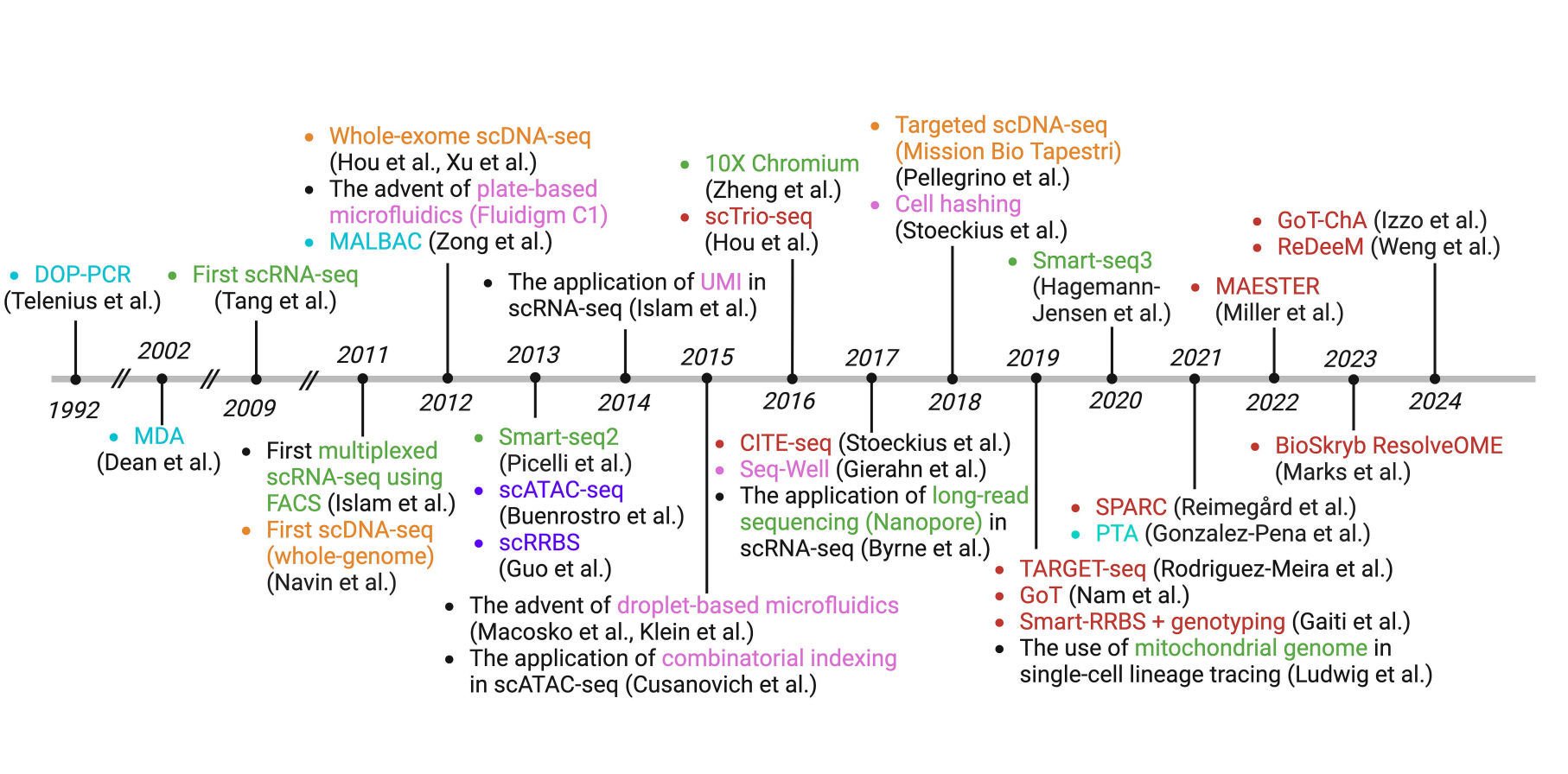
[1] Copy number alterations
[2] Single nucleotide variants

Figure Legends

Figure 1. The milestones of single-cell sequencing platforms in the past decade. Color coding is as follows: green for single-cell RNA sequencing, orange for single-cell DNA sequencing, purple for single-cell epigenomics sequencing, red for single-cell multi-omics sequencing, pink for single-cell isolation techniques, and turquoise for whole-genome amplification methods.

Figure 2. The tradeoff between coverage and throughput due to the high sequencing cost of single-cell sequencing. (a) The tradeoff between cellular throughput and transcript coverage in scRNAseq requires a choice between high-throughput sequencing with partial transcript coverage on thousands of cells and low-throughput sequencing with full transcript coverage on tens or hundreds of cells. (b) The tradeoff between cellular throughput and genome coverage in scDNA-seq requires a choice between narrow, targeted sequencing on thousands of cells and broad, genome-wide sequencing on tens or hundreds of cells.

Figure 3. Summary of capabilities across multi-modal single-cell platforms.


1992
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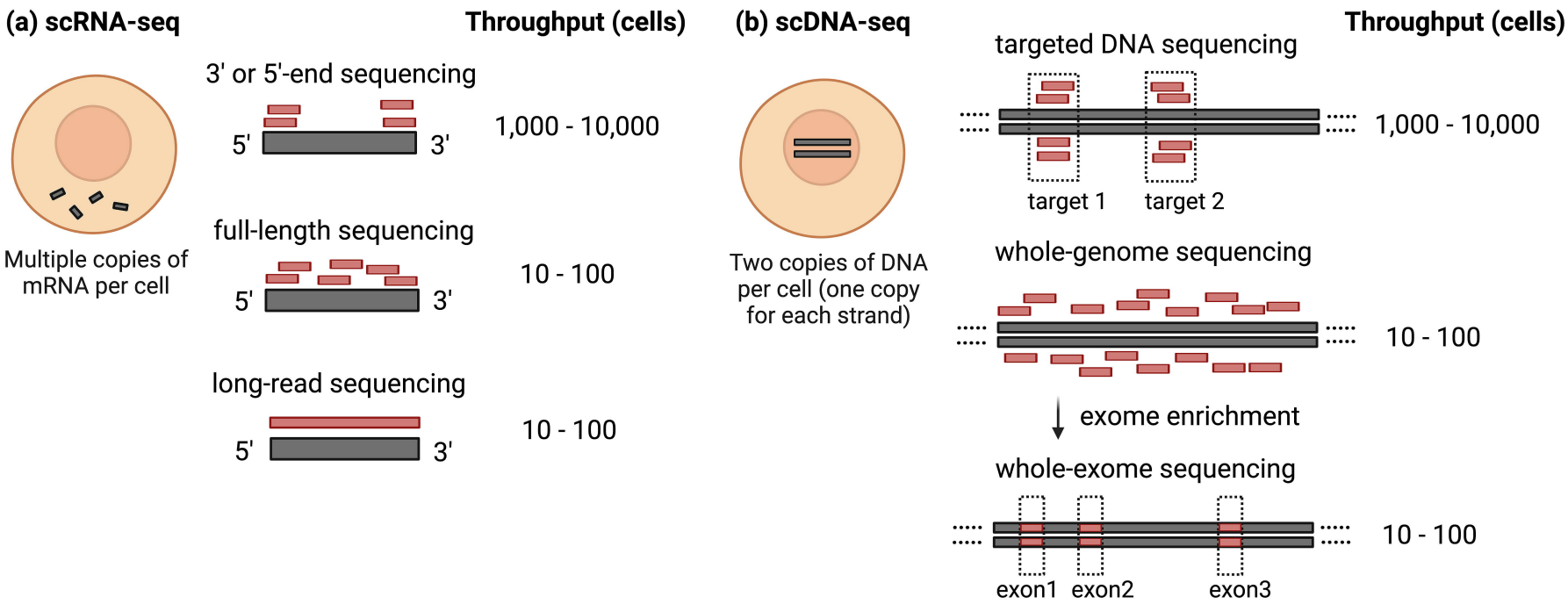
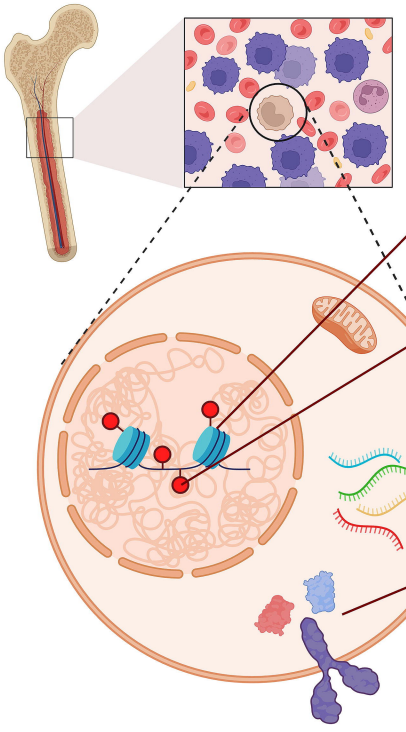
2024
DOP-PCR (Telenius et al.)
MDA (Dean et al.)
First scRNA-seq (Tang et al.)
First multiplexed scRNA-seq using FACS (Islam et al.)
First scDNA-seq (whole-genome) (Navin et al.)
Whole-exome scDNA-seq (Hou et al., Xu et al.)
The advent of plate-based microfluidics (Fluidigm C1)
MALBAC (Zong et al.)
The application of UMI in scRNA-seq (Islam et al.)
Smart-seq2 (Picelli et al.)
scATAC-seq (Buenrostro et al.)
scRRBS (Guo et al.)
The advent of droplet-based microfluidics (Macosko et al., Klein et al.)
The application of combinatorial indexing in scATAC-seq (Cusanovich et al.)
10X Chromium (Zheng et al.)
scTrio-seq (Hou et al.)
CITE-seq (Stoeckius et al.)
Seq-Well (Gierahn et al.)
The application of long-read sequencing (Nanopore) in scRNA-seq (Byrne et al.)
Targeted scDNA-seq (Mission Bio Tapestri) (Pellegrino et al.)
Cell hashing (Stoeckius et al.)
Smart-seq3 (Hagemann-Jensen et al.)
TARGET-seq (Rodriguez-Meira et al.)
GoT (Nam et al.)
Smart-RRBS + genotyping (Gaiti et al.)
The use of mitochondrial genome in single-cell lineage tracing (Ludwig et al.)
MAESTER (Miller et al.)
SPARC (Reimegård et al.)
PTA (Gonzalez-Pena et al.)
GoT-ChA (Izzo et al.)
ReDeeM (Weng et al.)
BioSkryb ResolveOME (Marks et al.)


(a) scRNA-seq
Throughput (cells)
Multiple copies of mRNA per cell
3' or 5'-end sequencing
5'
3'
1,000 - 10,000
full-length sequencing
5'
3'
10 - 100
long-read sequencing
5'
3'
10 - 100
(b) scDNA-seq
Throughput (cells)
Two copies of DNA per cell (one copy for each strand)
targeted DNA sequencing
target 1
target 2
1,000 - 10,000
whole-genome sequencing
10 - 100
exome enrichment
whole-exome sequencing
exon1
exon2
exon3
10 - 100



	GoT	TARGET-seq	ResolveOME	GoT-ChA	scTrio-seq	Smart-RRBS + genotyping	DAb-seq	CITE-seq	SPARC	MAESTER	ReDeeM
Genome											
• Nuclear	☒	☒	☒	☒	☒	☒	☒	☐	☐	☐	☐
• Mitochondrial	☐	☐	☐	☐	☐	☐	☐	☐	☐	☒	☒
Epigenome											
• Chromatin accessibility	☐	☐	☐	☒	☐	☐	☐	☐	☐	☐	☒
• Methylome	☐	☐	☐	☐	☒	☒	☐	☐	☐	☐	☐
Transcriptome	☒	☒	☒	☐	☒	☒	☐	☒	☒	☒	☒
Proteome											
• Surface proteins	☐	☐	☐	☐	☐	☐	☒	☒	☐	☐	☐
• Intracellular proteins	☐	☐	☐	☐	☐	☐	☐	☐	☒	☐	☐