APPLICATION NOTE

Spotlight on single-cell transcriptomics

Uncover deeper insights into complex cellular biology at single-cell resolution

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Introduction

The transcriptome reveals key information about the functional elements of the genome under various conditions. Next-generation sequencing (NGS) technology has revolutionized the field of transcriptomics by enabling thousands of genes to be profiled simultaneously on a genome-wide scale, offering broad insights into gene expression in health and disease. NGS-based RNA sequencing (RNA-Seq) can quantify the number of transcripts for each gene, providing an unbiased snapshot of actively expressed genes under dynamic conditions. The flexibility and accuracy of RNA-Seq allows researchers to uncover novel features, such as transcript isoforms, gene fusions, and single nucleotide variants, in a strand-specific manner without the limitations of prior knowledge.[1,2]

The conventional approach to transcriptomics involves bulk analysis of gene expression averaged across thousands of cells in a sample. Though bulk RNA-Seq has been successfully applied to understand differential gene expression at a population scale, it does not quantify the inherent heterogeneity of cells within tissues. Transcripts can be expressed at different levels within a cell population, either due to environmental signals or stochastic changes that occur over time. Gene expression also varies substantially based on tissue of origin. In addition, bulk RNA-Seq may potentially fail to capture transcripts from rare but biologically relevant subpopulations, such as stem cells or circulating tumor cells (CTCs). A low-expressing gene identified in bulk RNA-Seq may instead be robustly expressed in a rare cell type. Obtaining data from single cells overcomes these limitations, enabling researchers to gain a clearer understanding of cellular function at single-cell resolution (Figure 1).

Over the past decade, the field of single-cell transcriptomics, or single-cell RNA-Seq (scRNA-Seq), has undergone exponential growth, enabling unprecedented insights into cellular function and how subpopulations of cells interact with their microenvironments.[3] Early single cell gene expression approaches involved manual methods for cell isolation, such as micropipetting, to characterize individual cells. Today, several higher throughput methods are available to capture single cells. Regardless of the single-cell isolation method used, Illumina NGS technology maximizes the discovery power of single-cell gene expression studies, enabling researchers to assay millions

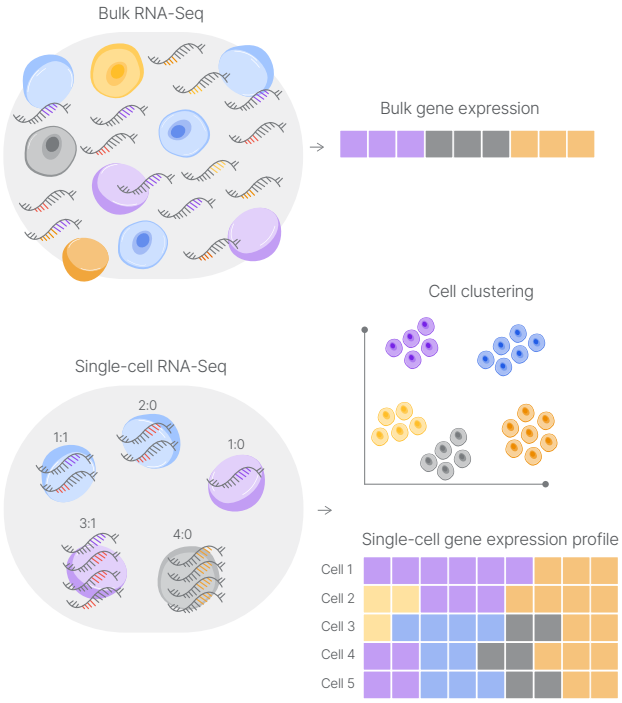

Figure 1: Comparison between bulk and single-cell RNA-Seq approaches—With bulk analysis (top), gene expression is averaged across all the cells included in the sample. However, with scRNA-Seq gene expression (bottom) data are generated for individual cells, enabling deeper insights into the nuanced distinctions between cells within the same sample.

of individual cells in a single assay with high accuracy and sensitivity. Illumina sequencing platforms, including the NextSeq™ 500, NextSeq 550, NextSeq 1000, NextSeq 2000, NovaSeq™ 6000, and NovaSeq X systems, deliver exceptional data quality and accuracy across a wide range of throughputs. Integration with the DRAGEN™ Bio-IT Platform single-cell analysis pipeline, and compatibility with third-party single-cell platforms, provides a streamlined solution to analyze the vast amounts of data generated by scRNA-Seq experiments. Flexible NGS readouts can be used to analyze genomes, transcriptomes, epigenomes, and proteomes, making it well suited for large-scale multiomics applications.

This application note highlights the latest advancements and emerging trends in the rapidly evolving field of single-cell transcriptomics. Also presented is a brief overview of the expanding portfolio of scRNA-Seq methods and technology solutions from Illumina available to support single-cell research goals.

Applications of scRNA-Seq

scRNA-Seq has demonstrated utility in various applications, ranging from basic to translational and clinical research. This powerful approach has been applied to several disciplines, including developmental biology, neurobiology, immunology, and cancer biology, enabling researchers to make significant strides in understanding complex biological systems (Figure 2). scRNA-Seq methods can also be used to investigate differential gene expression and cellular heterogeneity in time-dependent processes, such as differentiation, proliferation, and tumorigenesis.

Cell atlasing

A complete molecular cell atlas of human and model organism tissues is an essential first step for assessing cellular changes due to disease states, aging, or response to treatments. However, mammalian tissues are highly complex structures, composed of diverse cell types with varying abundances, making it challenging to capture the full scope of cellular heterogeneity using bulk RNA-Seq alone. Single-cell approaches have been instrumental in enabling researchers to build detailed cellular maps of tissues, transforming our understanding of biology and disease.[3,4] For instance, single-cell transcriptomics analysis with antibody-based protein profiling was recently used to create a single-cell type transcriptomics map of 13 human tissues, which included data for all protein-coding genes.[4] Building on this progress, the Human Cell Atlas (HCA) Project is using high-throughput scRNA-Seq data to create a comprehensive map of all cell types across complex tissues to reveal vital information about cellular functions and regulation, from individual cells up to tissues and organ systems, building a solid foundation for future biological research.[5]

Immunology

The immune system is highly complex, consisting of multiple cell types with the potential to alter their gene expression profiles based on environmental cues. Flow cytometry and fluorescence-assisted cell sorting (FACS) have been the mainstays of immunophenotyping studies. However, these single-approaches are low throughput and limited by the need for suitable antibodies for isolating cell populations of interest.

Figure 2: Applications of scRNA-Seq—Single-cell approaches have a broad range of applications across diverse research areas.

Advances in scRNA-Seq methodologies are powering large, unbiased investigations into immune cell phenotypes. As a result, researchers can now access new insights into immune cell development, activation-induced responses, rare immune cell subpopulations, and responses to vaccination or infections (Figure 3).

Immune repertoire profiling

A defining feature of the adaptive immune response is the ability of immune receptors to generate a rapid immune response against a wide range of pathogens. T- and B-cell receptors (TCR and BCR, respectively) undergo random recombination of variable (V), diversity (D), and joining (J) gene segments to produce a highly diverse antibody repertoire. BCRs also undergo somatic hypermutations to further refine antigen-binding affinity. scRNA-Seq provides a unique opportunity to investigate this extreme immunoreceptor diversity with high accuracy and resolution. Correctly identifying T-cell clonality, for example, may reveal T-cell subset-specific TCR diversity that may be associated with pathogenicity.[6,7] One of the challenges of immune repertoire sequencing using heterogenous bulk samples is determining which α and β chains combine to form the functional TCR or BCR unit. Unlike bulk RNA-Seq, scRNA-Seq enables pairing of both α and β chains of TCR and BCRs, revealing a more holistic view of their antigen specificity.

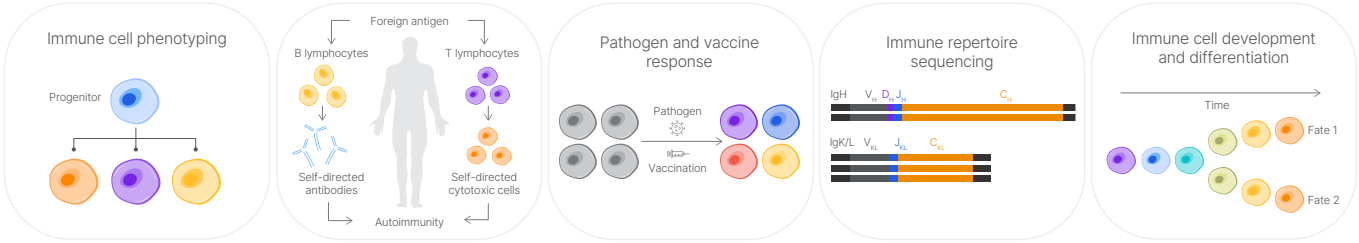

Figure 3: Applications of scRNA-Seq in immunology—The immune system consists of highly specialized cell types that work in concert to protect the host from invading pathogens, cancer cells, and self-directed antigens. Using scRNA-Seq and multiomics approaches, researchers can deconvolute the complex biology of the immune system at single-cell resolution.

TCR antigen-specificity profiling by scRNA-Seq provides key information about T-cell functionality, cellular phenotype, receptor sequence, and peptide-major histocompatibility complex (MHC).[8] This approach has also been applied to identify tumor-specific T-cells that can then be used for adoptive cell immunotherapy[9] or determine response to chimeric antigen receptor (CAR) T-cell therapy.[10]

Immune response to infection and vaccination

Delineating the changes in BCR repertoire in response to infection offers important information to aid in biomarker discovery and development of novel therapeutics. BCR-Seq and scRNA-Seq have been applied to characterize the TCR[11] and BCR[12] immune repertoire profiles in response to Pneumocystis infection, a life-threatening complication of immunosuppression. LIBRA-Seq (linking BCR to antigen specificity through sequencing) is a single-cell technique being increasingly used to assess BCR repertoire changes due to infection or vaccination.[13] This approach has been used to identify key changes in clonal proliferation and BCR repertoire dynamics in the lungs, draining lymph nodes, and the spleen after influenza infection.[14] Similarly, LIBRA-Seq has been used to create a comprehensive profile of the memory B cell transcriptomic signatures in response to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[15] In addition, LIBRA-Seq has identified multiple SARS-CoV-neutralizing antibodies that can be further investigated as potential therapeutics for coronavirus disease 2019 (COVID-19).[16]

For more information on immune receptor profiling, read the Multiomic interrogation of the immune system at single-cell resolution application note

Autoimmunity

Autoimmune disorders are challenging to study owing to the heterogeneity of disease phenotypes and variable responses to therapies. Molecular mechanisms underlying autoimmunity are also not well understood, further complicating diagnosis and treatment. A combination of scRNA-Seq and single-cell epigenomics was used to characterize diverse immune subsets in germinal centers (GCs).[17] This study revealed that the expression of key immune-regulatory genes was dysregulated in GCs, implicating aberrant GC-reactions in autoimmune disease pathogenesis.[17] Transcription profiles of key cell populations involved in ulcerative colitis have been characterized using scRNA-Seq to create a comprehensive single-cell atlas of the healthy and diseased colon.[18] Recently, multiplexed scRNA-Seq has been applied to characterize circulating immune cell populations and identify transcriptional signatures in systemic lupus erythematosus (SLE).[19]

Neurobiology

Single-cell approaches have the potential to address diverse areas of basic and translational neuroscience to understand brain physiology and pathology. However, isolation of single cells from samples, such as frozen human brain tissue, is challenging. Single-nucleus RNA-Seq (snRNA-Seq) is an alternative to scRNA-Seq that enables transcriptomic profiling at single-cell resolution.[20] Researchers at the Allen Institute of Brain Science are using snRNA-Seq data to develop a comprehensive brain atlas with transcriptional profiles for key brain regions, including the cortex, hippocampus, and thalamus.[21] Similar approaches have been used to create a single-

cell atlas of the developing human brain.[22] Single-nucleus chromatin accessibility and transcriptomic profiling has been applied to uncover disease-specific transcription factors and their regulatory targets in Alzheimer's disease.[23] Analysis of circulating cells in cerebrospinal fluid by scRNA-Seq has emerged as a promising approach to studying neurological diseases, including Alzheimer's disease,[24] multiple sclerosis,[24,25] and neurological sequelae of COVID-19.[27] Recently, a single-cell approach known as CITE-Seq (single-cell cellular indexing of transcriptomes and epitopes by sequencing) was used to characterize epileptic lesions.[28] Extensive microglial activation and proinflammatory immune cell infiltration in these lesions may inform future therapeutic development for drug-resistant epilepsy.[28]

Stem cell and developmental biology

Rapid developments in the area of scRNA-Seq have facilitated deeper insights into embryogenesis and stem cell biology.[29] Induced pluripotent cells (iPSCs) have a relatively low rate of successful transformation[30] and understanding the underlying factors that drive this heterogeneity is critical to achieving the promise of regenerative medicine. scRNA-Seq has been used to assess endoderm differentiation across multiple human iPSCs.[31] This study characterized common genetic variants and their influence on defined time points during early differentiation, providing critical information about the causal role for genetic variation in developmental disorders. scRNA-Seq has also been applied to characterize transcriptional heterogeneity in mesenchymal stem cells[32] and limbal stem cells.[33] Using scRNA-Seq, researchers have uncovered a rare subpopulation of adult neural stem cells that undergo activation in response to brain injury and may play a role in tissue repair.[34] Cancer stem cell (CSC) profiling is another area of active investigation using scRNA-Seq. CSCs are rare subpopulations within tumors that are frequently resistant to conventional treatments and play a key role in promoting metastasis and recurrence. scRNA-Seq has been applied to characterize CSCs in several recurrent cancers,[35-38] revealing potential targets for therapy.

Cancer research

Single-cell methods have transformed the landscape of cancer research by uncovering crucial information about intratumoral heterogeneity and the tumor microenvironment (Figure 4). Tumors are known to be highly heterogenous, comprised of multiple clones of cancer cells interacting with cells in their microenvironment. Though bulk RNA-Seq approaches have been used extensively to identify gene expression signatures for multiple tumor types,[39-41] these findings are not uniformly reproducible due to the high degree of complexity that exists within and between tumors. Solid tumors also contain various infiltrating immune and stromal cells, each with their own transcriptional programs that can affect tumor progression, metastasis, and treatment response. scRNA-Seq can also be leveraged to discover novel therapeutic targets and identify drug candidates that can be advanced to clinical trials.[42]

Tumor heterogeneity

Single-cell transcriptomics has emerged as a powerful method for investigating transcriptome heterogeneity, identifying rare biologically important clones, characterizing which molecular pathways they utilize, and predicting therapy response in cancer research (Figure 5). scRNA-Seq was applied to dissect divergent tumor

Figure 4: Applications of scRNA-Seq in cancer research—scRNA-Seq has powered breakthrough discoveries in understanding tumor evolution and heterogeneity, monitoring disease progression and metastasis, predicting response to immunotherapies, and overcoming chemoresistance.

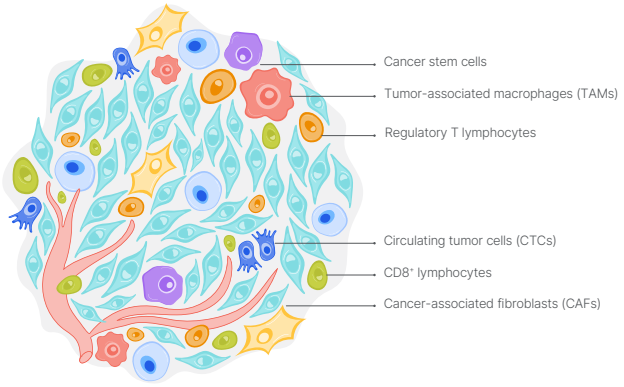

Figure 5: Tumor heterogeneity uncovered by scRNA-Seq—Single-cell transcriptomics studies can be leveraged to characterize rare cancer cell subpopulations, circulating tumor cells, and tumor infiltrating immune cells at high resolution.

composition and regulators of stemness in primary glioblastoma[43] and oligodendroma.[44] A similar approach was used to investigate intratumor diversification in colorectal cancer (CRC), revealing that CRC clones contain substantial levels of somatic mutations that can influence their response to anticancer agents.[45] Bulk RNA-Seq data were further refined using scRNA-Seq to re-define lung cancer[46] and head and neck cancer[47] subtypes, and predict their tendency to metastasize.

Immunotherapy response

Immune cell infiltration is commonly observed in multiple tumors. The biological characteristics of these immune cells can have a significant impact on tumor progression and can predict the success of immunotherapy. Characterization of the divergent transcriptional profiles of these immune cell populations is now possible as a result of scRNA-Seq.[48] For instance, scRNA-Seq has been applied to categorize tumor-infiltrating myeloid cells into over 25 different states based on their gene expression signatures.[49] Using scRNA-Seq researchers have identified that a high proportion of infiltrating CD8⁺ T lymphocytes is associated with better outcomes in nonsmall cell lung cancer.[50] Similarly, a rare subpopulation of CD8⁺ T lymphocytes has been linked to a favorable response to adoptive cell transfer immunotherapy in melanoma.[51] Conversely, scRNA-Seq has identified that higher regulatory T-cell infiltration is associated with poorer prognosis in liver cancer.[51] Single-cell approaches have also been used to determine checkpoint inhibitor immunotherapy response in sarcoma[53] and metastatic melanoma.[54-56]

Treatment selection

Data from scRNA-Seq can detect rare treatment-resistant cell populations within heterogenous tumors, which can aid in selection of appropriate therapeutic approaches. For example, small populations of metastatic melanoma cells expressing high levels of AXL have been identified by scRNA-Seq.[57] This treatment-resistant subpopulation of cells can be predicted to undergo clonal selection in response to conventional treatment with RAF or MEK inhibitors. Instead, tumors with the AXLhi transcriptional signature would be more likely respond to treatment with AXL inhibitors.[58] Similarly, scRNA-Seq data has been used to create prediction models to determine drug responses in breast cancer cell lines.[59]

Tumor microenvironment

Most solid tumors contain cancer-associated fibroblasts (CAFs) that can participate in tumorigenesis and metastasis. Though these cells are known to play a key role in establishing and maintaining the tumor microenvironment,[60] the mechanisms by which they do so is under active investigation. The lack of reliable biomarkers for CAFs makes it challenging to study these cells using conventional methods. Researchers have successfully leveraged scRNA-Seq to identify multiple populations of CAFs, including their cellular origins, in breast[61,62] and colorectal[63] tumors. This critical information has paved the way for future research into CAFs as potential therapeutic targets.[42]

Circulating tumor cells (CTCs)

An emerging application of scRNA-Seq is the characterization of CTCs for noninvasive liquid biopsy to detect early or recurrent disease. Tumor-derived CTCs are highly heterogeneous. Single-cell transcriptomics is a promising approach to characterize CTCs accurately, enabling researchers to predict cancer progression and response to treatment. scRNA-Seq has been applied for CTC analysis in multiple cancer types, including breast,[64-67] liver,[68] prostate,[69,70] and gastric[71] cancers.

scRNA-Seq workflow

The scRNA-workflow consists of four key steps, tissue preparation, single cell isolation and library preparation, sequencing, and data analysis (Figure 6). Illumina offers a growing portfolio of sequencing and data analysis solutions that integrate with commercially available single-cell platforms to support various scRNA-Seq research needs.

For detailed methods, see the Illumina single-cell methods guide

Tissue preparation

A successful scRNA-Seq experiment begins with a high-quality monodispersed suspension of live cells. Tissues of interest are dissociated with mechanical or enzymatic means, or a combination of these methods, to break down the extracellular matrix and obtain a suspension of viable cells for further processing. This step can be followed by protocols to enrich or eliminate specific cell populations, including gradient centrifugation, flow cytometry, or magnetic bead-based enrichment methods. Optimized enrichment and quality control (QC) steps are essential to ensure a high yield of viable cells for scRNA-Seq.

Single-cell isolation

The single-cell isolation method used in transcriptomics studies has a significant impact on the overall scRNA-Seq results. Several high- and low-throughput methods are available to isolate single cells from cell suspensions for library preparation and sequencing.

Frequently used single-cell isolation methods include:

- Flow sorting: Microdroplets containing single cells are isolated using an electric charge. This method selects cell types accurately based on size, morphology, internal complexity, and protein expression by antibody labeling.
- Droplet fluidics platform: This approach uses compartmentalization of individual cells in droplets using a microfluidics device followed by lysis and capture of target DNA and RNA.[72-74] The ability to use unique molecular identifiers (UMIs) and cell barcodes enables cell and gene-specific identification.
- Microwells: Microwells containing fabricated arrays are used to capture individual cells.[75,76] This method is ideal for adherent cells and is also compatible with UMIs and cell barcoding.
- Combinatorial indexing or plate-based methods: Intact cells or nuclei are tagged via multiple rounds of splitting, pooling, and ligation to generate different barcode combinations, enabling thousands of cells to be profiled simultaneously.[77,78]

Library preparation

Single-cell isolation is followed by library preparation. A range of library preparation methods are available to generate barcoded single-cell gene expression libraries ready for sequencing on Illumina systems (Table 1). Prepared libraries are assessed for quality and quantity before proceeding to sequencing. Researchers can use lower throughput Illumina sequencing systems, including the iSeq™ 100 System, for library QC and rebalancing of pooled samples.

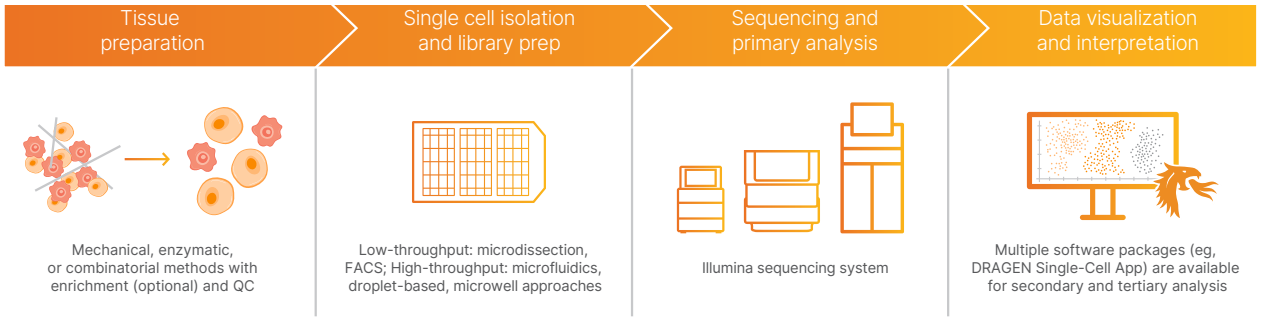

Figure 6: Single-cell sequencing workflow—The scRNA-Seq workflow begins with initial tissue dissociation, followed by isolation of single cells, library preparation, sequencing, and finally, data analysis and visualization. FACS, fluorescence-assisted cell sorting.

Table 1: Library preparation methods for scRNA-Seq applications

Method	Description	Sequencing depth
Full-length RNA-Seq	Switching mechanism at 5′ end of template (SMART) technology enables amplification of full-length cDNA	10–20K read pairs/cell
mRNA end-tag amplification (3′ WTA or 5′ WTA)	Capture of mRNA by 3′ polyadenylated (poly-A) tails enables sequencing of the coding transcriptome with strand-specific information	15–50K read pairs/cell
Targeted panels	Pre-designed single-cell targeted RNA sequencing panels enable T-cell, breast cancer profiling, and more	200 reads/amplicon/cell
Immune repertoire sequencing (IR-Seq)	Targeted sequencing method used to quantify the composition of BCR or TCR repertoires	5K reads/cell

WTA, whole-transcriptome amplification; BCR, B-cell receptor; TCR, T-cell receptor.

Sequencing

Prepared single-cell libraries are sequenced on Illumina sequencing systems to generate highly accurate and reliable sequencing data across a range of throughputs. Illumina NextSeq 550, NextSeq 1000, and NextSeq 2000 systems are suitable for mid- to high-throughput single-cell studies. NovaSeq 6000 and NovaSeq X systems are powerful, scalable Illumina platforms, ideal for high-throughput applications. All Illumina sequencing systems provide a single NGS readout that can be applied across a range of single-cell applications, including scRNA-Seq, spatial RNA-Seq, assay for transposase-accessible chromatin using sequencing (ATAC-Seq), immunoreceptor sequencing (IR-Seq), CITE-Seq, and a host of emerging applications.

For a detailed scRNA-Seq protocol using 10x Genomics Chromium Single-Cell Gene Expression on Illumina platforms, read the Explore the transcriptome at single-cell resolution technical note

Data analysis

The analysis pipeline for single-cell experiments consists of three phases: primary analysis or base calling, secondary analysis, and tertiary analysis and data visualization.

- Base calling: Illumina sequencing systems generate raw data files in the binary base call (BCL) format. Illumina BCL Convert standalone app or CellRanger software from 10x Genomics can be used to convert BCL outputs to FASTQ format, which can then be used as input for a wide range of secondary data analysis tools.
- Secondary analysis: Following primary analysis, scRNA-Seq data can be transferred, stored, and analyzed securely in Illumina BaseSpace Sequence Hub, a cloud-based environment for data analysis. Software packages, such as the DRAGEN Single-Cell App, can be used for alignment, variant calling, and data QC. The 10x Genomics CellRanger software also contains multiple secondary analysis pipelines for scRNA-Seq data.
- Tertiary analysis and data visualization: This step involves data interpretation and visualization to gain novel insights into cellular function at single-cell resolution. Popular tertiary analysis tools for single-cell data include Seurat, Scanpy, AnnData, 10x Genomics Loupe Cell Browser, and MissionBio Tapestri Insights. SeqGeq is a desktop application cell developed by BD Biosciences, offering direct integration with BaseSpace Sequence Hub for simplified clustering analysis and visualization of single-cell data. Cell by gene expression matrix outputs generated by the DRAGEN Single-Cell App are compatible with all these tertiary analysis software tools.

For information on the DRAGEN Single-Cell App, read the DRAGEN v3.7: Single-cell RNA, PrecisionFDA accuracy gains, and more blog article

Sample scRNA-Seq data sets and test runs on Illumina sequencing systems are available on BaseSpace Sequence Hub data page

Emerging trends in single-cell transcriptomics research

Multiomics

Multiomics combines insights gleaned through multiple 'omics' approaches, such as genomics, transcriptomics, epigenomics, and proteomics, providing a high-dimensional view of cellular processes (Figure 7). CITE-Seq is a multiomics approach that uses oligonucleotide-labeled antibodies to convert protein detection into a quantitative assay by NGS.[79] By linking single-cell transcriptomics to cellular protein expression, CITE-Seq provides a novel approach to cellular phenotyping. A newer application of CITE-Seq, known as expanded CRISPR-compatible cellular indexing of transcriptomes and epitopes by sequencing (ECCITE-Seq or Perturb-Seq), is a powerful multiomics approach to interrogate single-cell transcriptomics and cell surface protein markers in CRISPR screens.[80] Perturb-Seq provides crucial insights into the phenotype and clonotype of immune cells, along with their gene expression signatures. Single-cell ATAC-Seq and Gene Expression (ATAC-Seq + GE)[81] is another multiomics tool enabling simultaneous detection of gene expression and chromatin accessibility from the same cell. Multiomic profiling of the transcriptome and epigenome at single-cell resolution enables deeper insights into gene regulatory networks and cellular heterogeneity in health and disease.[82]

For more information on multiomics, read the Illumina multiomics e-book

For details on CITE-Seq using BioLegend TotalSeq antibodies, read the Correlated expression of protein and RNA reveals a unique molecular signature in Th1 polarized cells application note

For details on single-cell ATAC-Seq + GE, read the Unify single-cell gene expression and chromatin accessibility technical note

Spatial analysis

The native tissue microenvironment has a significant impact on gene expression. While single-cell transcriptomics data provides valuable data about cellular phenotypes and functional characteristics, the isolation of individual cells results in the loss of critical context

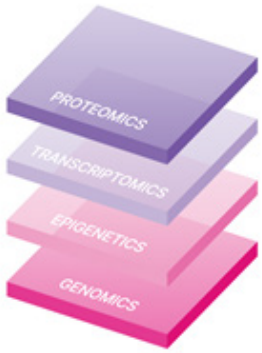

Figure 7: Multiomics in single-cell research—Multiple layers of information provide novel insights into complex biological systems that cannot be resolved by scRNA-Seq studies alone.

regarding cellular localization. Spatial transcriptomics enables researchers to uncover cellular networks within tissues, gain a deeper understanding of cell-to-cell communication.[83] Spatially resolved scRNA-Seq has been applied to characterize tissue architecture and cellular dynamics in the bone marrow[84] as well as breast,[85] colorectal,[86] and pancreatic[42,87] cancers.

For an overview of spatially-resolved transcriptomics using the NanoString GeoMx® Digital Spatial Profiler with Illumina NGS readout, read the High-resolution, high-throughput spatial transcriptomics of complex tissues application note

For more information on spatial proteogenomics, read the High-plex spatial proteogenomics of FFPE tissue sections application note

For a detailed protocol to map the spatially-resolved transcriptome from tissue sections with 10x Genomics Visium Spatial Gene Expression, read the Resolve the whole transcriptome within tissue architecture technical note

Temporal analysis

Current scRNA-Seq methods capture static gene expression to characterize cellular phenotype and function. However, the process of gene expression in tissues is inherently dynamic. Innovations in library preparation methods enabling fixation of single cells combined with increased affordability of scRNA-Seq have paved the way for time-course sampling in single-cell analyses. Single-cell RNA velocity (scVelo) is a useful

indicator of transcriptional dynamics in cell populations.[88] RNA velocity measures the ratio of unspliced to spliced reads, which can be used to infer temporal changes in gene expression, even from a sample taken at a single time point. This is an area of active investigation, with computational and modeling methods being developed to resolve the temporal characterization of gene expression in single cells.[89]

Sample multiplexing

As single-cell transcriptomics research continues to progress, there is a growing need for cost-effective approaches to run more samples per experiment. One method of sample multiplexing, known as MULTI-Seq, uses lipid-tagged indexes to barcode samples.[90] Another approach uses sample-specific genetic polymorphisms as fingerprints to identify individual cells in a pooled sample.[91] Cell hashing is a multiplexing approach that builds on the CITE-Seq method to use barcoded antibody signals as a fingerprint for demultiplexing, allowing for robust sample multiplexing and 'multiplet' determination, thereby increasing overall scRNA-Seq data quality.[92] For ultra-high–throughput applications, combinatorial preindexing of entire transcriptomes inside permeabilized cells enables cost-effective scRNA-Seq for millions of individual cells.[78,93] Sample multiplexing, regardless of the method used, can be used to reduce the cost per sample of library preparation, detect doublets and other technical artifacts, and enable sample pooling to mitigate batch effects. Ultimately, highly multiplexed experiments can combine many treatments, analogous to high-content screening approaches.[78]

VASA-Seq

Conventional scRNA-Seq methods amplify polyadenylated termini of transcripts, failing to capture long noncoding, short noncoding, and nonpolyadenylated protein coding transcripts that may be present in the cellular transcriptome. Vast transcriptome analysis of single cells by dA-tailing, or VASA-Seq, is a scRNA-Seq method that captures the total transcriptome in single cells.[94] Data obtained using VASA-Seq also provides improved alternative splicing detection and RNA velocity analysis.

Summary

Single-cell transcriptomics is a powerful discovery tool, enabling deeper insight into cellular heterogeneity in complex biological systems. scRNA-Seq can be applied across a breadth of research areas, with the potential to transform our understanding of cellular function in health and disease. Illumina is committed to developing technology solutions to support the evolving landscape of single-cell research. Researchers can leverage Illumina sequencing systems to access highly accurate and sensitive scRNA-Seq data. Flexible NGS readouts are well suited to be used with high-throughput multiomics workflows. Integration with the DRAGEN Single-Cell App simplifies data analysis and generates outputs that are compatible with commercially available single-cell data visualization pipelines.

Learn more

Single-cell RNA-Seq, illumina.com/techniques/sequencing/rna-sequencing/ultra-low-input-single-cell-rna-seq

Illumina sequencing systems, illumina.com/systems/sequencing-platforms

Illumina DRAGEN Bio-IT Platform, illumina.com/products/by-type/informatics-products/dragen-bio-it-platform

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