

Deciphering the blueprint of life: integrated protocols for single-cell multi-omics and functional genomics

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The rapid evolution of single-cell technologies has fundamentally transformed our ability to interrogate cellular heterogeneity and biological complexity with unprecedented resolution. Over the past few years, the field has progressed beyond the creation of purely descriptive “cell atlases” toward a new frontier centered on functional validation and mechanistic dissection of regulatory landscapes. Among them, the launch and implementation of the Hematopoietic Ecosystem Multi-Omics Atlas of billions Blood Cells (HEMO ABC) Project is particularly significant. It aims to comprehensively and precisely characterize a vast number of blood cells, with the goal of constructing a massive database encompassing the characteristics and relationships of various cell types within the blood system. However, such an ambitious project faces numerous challenges. Data generated from different research platforms exhibit heterogeneity, making data integration a formidable task. Moreover, the absence of a unified and standardized technical system to ensure the quality and consistency of research. So this paradigm shift is particularly critical for the highly dynamic and complex hematopoietic ecosystem.

The hematopoietic system represents a finely regulated hierarchy with hematopoietic stem cells (HSCs) at its apex, in which homeostasis is maintained through intricate interactions among genetic perturbations,¹ epigenetic regulation, and phenotypic outputs. To achieve a comprehensive understanding of how this ecosystem preserves equilibrium or, alternatively, drives disease states such as leukemia and myelofibrosis, this special issue focuses on “single-cell multi-omics and functional genomics technologies” and systematically introduces four cutting-edge solutions. It aims to provide researchers with a comprehensive “toolbox” to establish a standardized and unified technical system, thereby offering solid technical

support for a thorough and in-depth understanding of the blood system.

1. SYSTEM-LEVEL INTERPRETATION VIA CRISPR SCREENS

While Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) screens have emerged as a transformative force in functional genomics,² traditional pooled approaches are often constrained by their reliance on cell fitness as the primary metric.³ This reliance distills complex biological impacts into a simplified measure of survival, failing to capture the high-dimensional shifts in cellular identity and regulatory pathways.⁴ The protocol “Dissecting cellular ecosystem with single-cell CRISPR screens” discusses how combining pooled screens with single-cell omics (such as Perturb-seq and CROP-seq) enables comprehensive profiling following individual genetic manipulations. This workflow is poised to bridge the gap between genetic perturbation and system-level biological interpretation.

2. MULTIMODAL RESOLUTION OF TRANSCRIPTOMES AND EPITOPES

Recognizing that transcriptomic data alone often fail to fully capture the complexity of cellular function, largely due to the decoupling between mRNA transcription and protein expression, as well as the inability to account for post-transcriptional and post-translational modifications that critically regulate cell phenotype and activity. To address these limitations, the CITE-seq protocol has emerged as a robust, standardized multimodal single-cell technology for the simultaneous profiling of full-length RNA transcriptomes and quantitative analysis of cell surface protein epitopes.⁵ Unlike traditional approaches, CITE-seq leverages a key innovation: antibody-oligonucleotide (Ab-oligo) conjugation, in which each monoclonal antibody targeting a specific cell surface protein is covalently linked to a unique, barcode-containing oligonucleotide tag. This design enables the concurrent capture of both polyadenylated mRNA molecules and Ab-oligo tags from individual cells, eliminating the need for cell sorting or sample splitting and preserving the native cellular context. The technical refinement significantly improves the resolution of cell type classification, allowing researchers to distinguish phenotypically similar but functionally distinct cell subsets that would be indistinguishable by transcriptomics alone. Most notably, in the fields of immunology and oncology—where rare cell populations often exert outsized effects on disease progression and therapeutic response—CITE-seq enables the precise, high-throughput characterization of rare cell subsets.^{6,7}

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3. DIRECT LINKAGE OF EXPRESSION AND ACCESSIBILITY

Independent analyses of scRNA-seq and scATAC-seq often rely on indirect integration to infer regulatory relationships, limiting mechanistic insight. The drop-based workflow for joint profiling of the transcriptome and chromatin accessibility presented in this Special Issue directly addresses this challenge by enabling simultaneous measurement of gene expression and chromatin accessibility within the same single cell. This scalable and reproducible droplet-based protocol allows parallel profiling of tens of thousands of cells, significantly improving the resolution of cellular states and the detection of rare or transitional populations.^{8–10} By directly coupling transcriptional output with its epigenetic context, this approach provides a powerful framework for dissecting regulatory programs underlying hematopoietic differentiation and disease, and represents a critical step toward advancing single-cell studies from descriptive atlases to functional and mechanistic understanding.

4. A STREAMLINED COMPUTATIONAL PIPELINE FOR scATAC-seq

As data complexity grows, robust analytical pipelines become paramount. One of the featured protocols addresses this challenge by presenting a sophisticated pipeline for scATAC-seq analysis, extending beyond basic cell clustering to deep mechanistic insights. This workflow integrates essential steps including robust peak calling via MACS2¹¹ and the inference of transcription factor (TF) activity using chromVAR.¹² Notably, the protocol emphasizes high-resolution regulatory mapping through TF footprinting with Tn5 bias subtraction and the reconstruction of transcriptional regulatory networks (TRNs) using SCENIC+.¹³ By linking enhancers to target genes (eRegulons), this integrative approach provides a powerful toolkit for decoding the logic of gene regulation and identifying the master switches that drive cellular identity in complex biological systems.

In summary, the experimental strategies presented in this Special Issue collectively define a robust and extensible framework for next-generation single-cell research. By transcending purely descriptive profiling and enabling systematic interrogation of gene function and regulatory circuitry, these approaches mark a decisive step toward a more mechanistic and actionable understanding of cellular systems.

Within the context of hematopoiesis and hematological diseases, the standardized and reproducible workflows featured here provide a critical foundation for bridging basic discovery and translational applications. As single-cell technologies continue to converge with artificial intelligence-guided experimental design and spatially resolved modalities, the methodologies highlighted in this collection are expected to play a central role in shaping future biological insights and accelerating the development of innovative therapeutic strategies.

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