DANISH SINGLE-CELL NETWORK NEWSLETTER

OCTOBER - 2024

THIS MONTH'S HIGHLIGHTS:

Multiplexed, image-based pooled screens in primary cells and tissues with PerturbView

Kudo, T., Meireles, A.M., Moncada, R. et al.

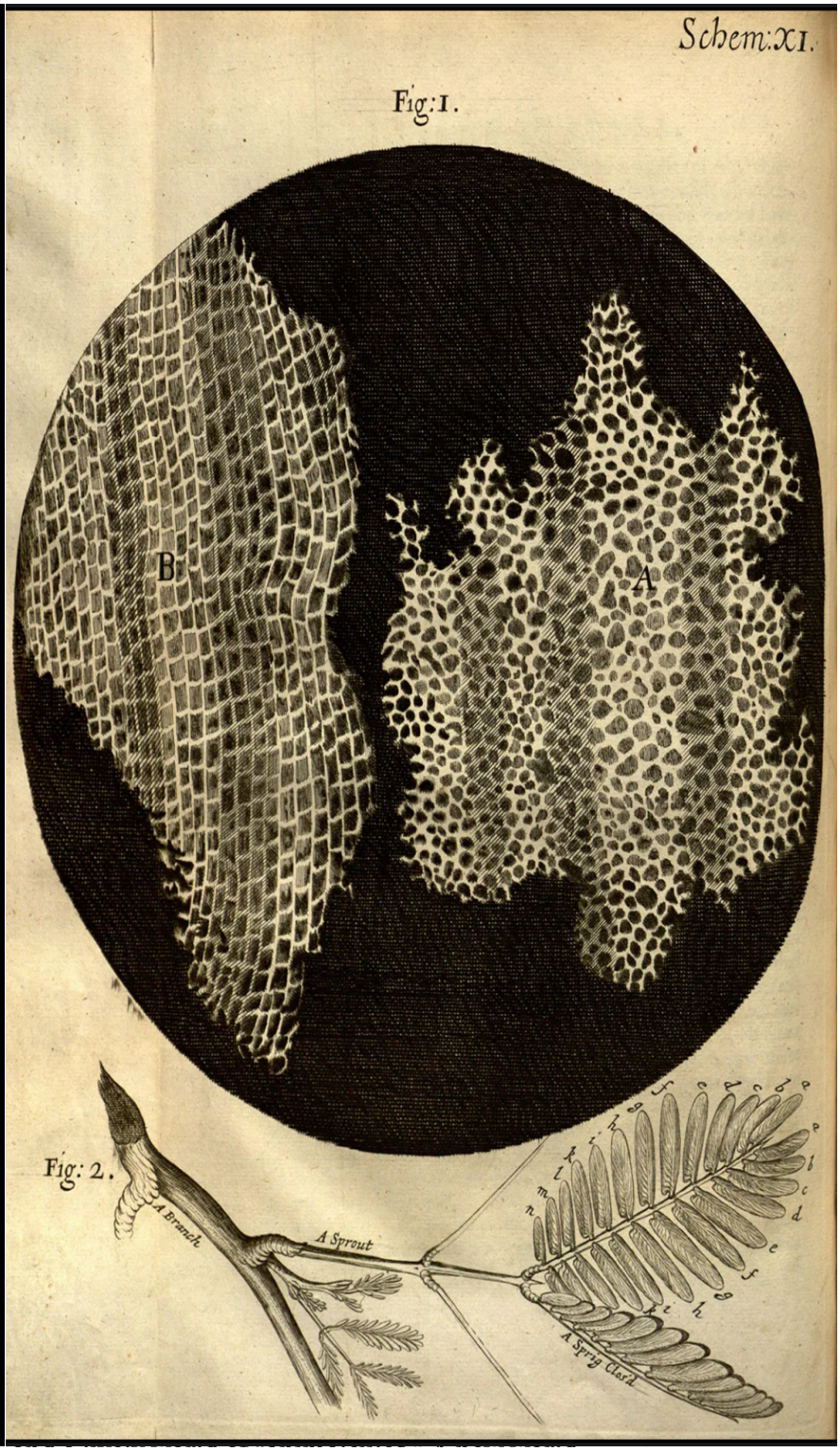
Real-time and programmable transcriptome sequencing with PROFIT-seq

Zhang, J., Hou, L., Ma, L. et al.

Multiplexed profiling of intracellular protein abundance, activity, interactions and druggability with LABEL-seq

Simon, J.J., Fowler, D.M. & Maly, D.J

COVER IMAGE
Robert Hooke's drawings of the cellular structure of cork and a sprig of sensitive plant from Micrographia (1665).



UNIVERSITY OF COPENHAGEN

Multiplexed, image-based pooled screens in primary cells and tissues with PerturbView

Kudo, T., Meireles, A.M., Moncada, R. et al.. *Nature Biotechnology* (2024),
https://doi.org/10.1038/s41587-024-02391-0

The latest advancements in image-based screening are embodied in PerturbView, a groundbreaking platform designed for high-throughput, **multiplexed genetic screens in primary cells and tissues**. With an emphasis on scalability and precision, PerturbView enables researchers to **assess a large number of genetic perturbations simultaneously** within complex, physiologically relevant environments. This tool addresses a significant gap in drug discovery and genomics research, where traditional cell lines often fail to replicate the intricate dynamics of primary tissues.

PerturbView's approach integrates state-of-the-art imaging technologies with computational algorithms to generate deep, quantitative phenotypic data. This combination enables scientists to **detect subtle morphological and functional changes at the cellular level**. One of the primary advantages of PerturbView is its **capacity to handle pooled screens**, where multiple perturbations are applied to the same sample. By tagging each perturbation uniquely, researchers can observe specific genetic and cellular responses in detail, providing insights into the functional roles of genes in diverse biological contexts.

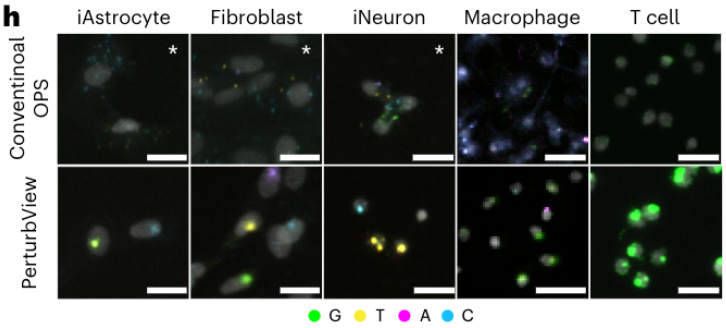


Figure 1. Images of sgRNA barcode detection by conventional in situ sequencing and PerturbView across cell types

This platform opens new possibilities for personalized medicine, as it allows for high-fidelity studies of genetic mutations in native tissues, yielding insights that could translate directly to human models. Applications range from drug response evaluations to investigating disease mechanisms and testing gene-editing outcomes. For researchers working in functional genomics or drug development, PerturbView offers a **scalable, physiologically relevant alternative** to conventional screening methods for more precise discoveries.

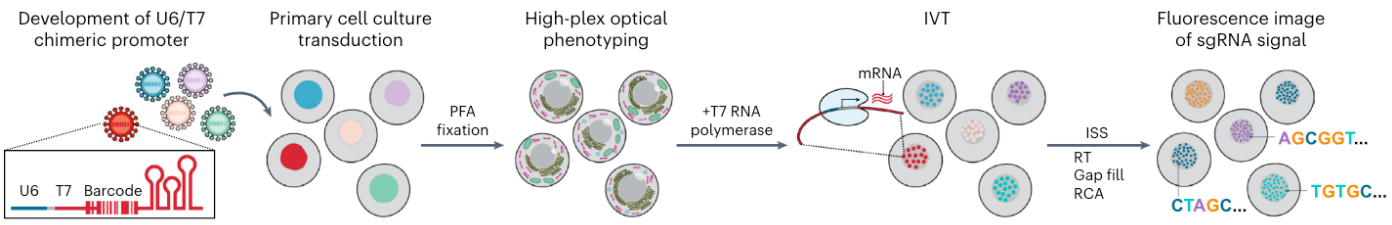


Figure 2. Method overview

Real-time and programmable transcriptome sequencing with PROFIT-seq

Zhang, J., Hou, L., Ma, L. et al. *Nature Cell Biology* (2024),
https://doi.org/10.1038/s41556-024-01537-1

PROFIT-seq (Programmable Full-Length Isoform Transcriptome Sequencing) is a novel RNA sequencing method developed by the Chinese Academy of Sciences. This tool **enhances RNA sequencing accuracy by selectively enriching target RNAs** while capturing a comprehensive view of the transcriptome. Traditional RNA sequencing often struggles with enriching specific targets without sacrificing complete coverage, but PROFIT-seq overcomes this with a **combinatorial reverse transcription process that captures diverse RNA types**, including circular RNAs and non-polyadenylated transcripts. A programmable control system further ensures that only relevant transcripts, such as those linked to disease mutations, are enriched.

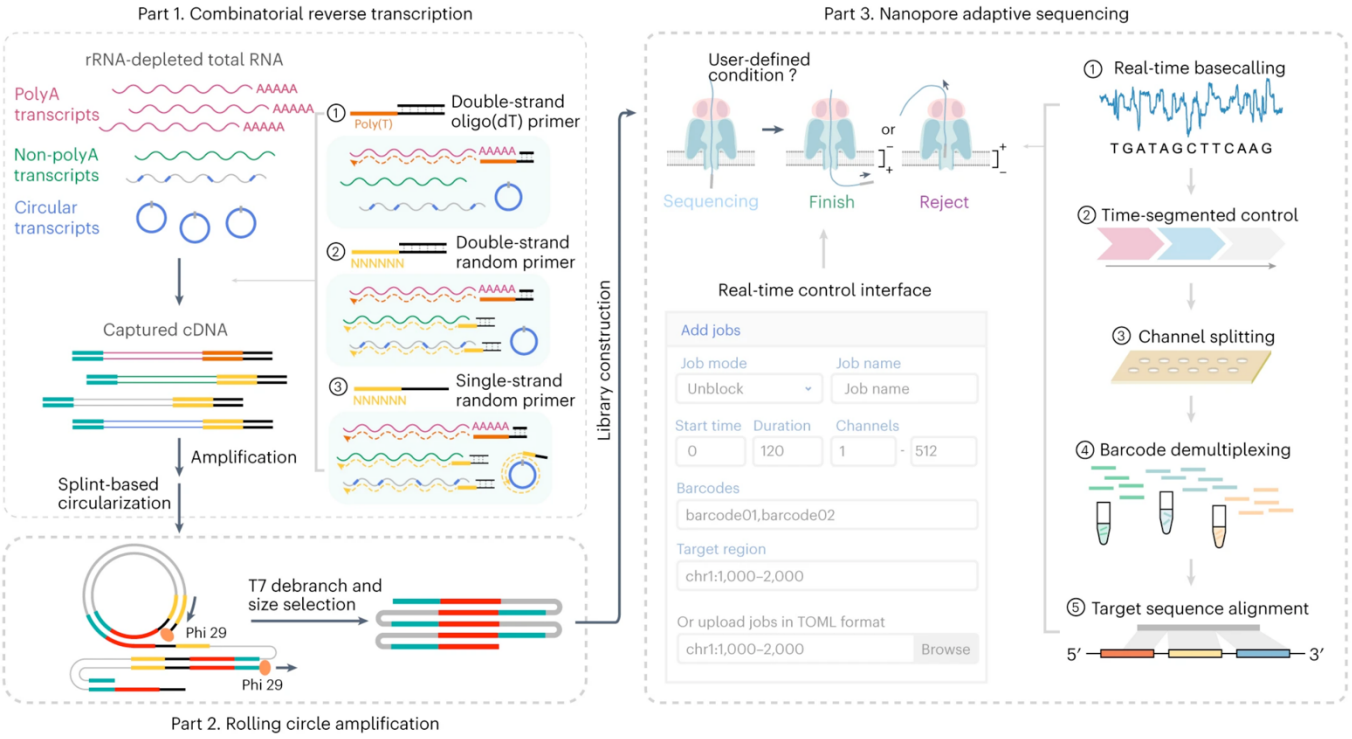


Figure 3. A schematic overview of the PROFIT-seq method.

Using a real-time, adaptive sequencing method, PROFIT-seq adjusts sequencing based on collected data, allowing it to focus on critical transcripts. The method **can reduce sequencing time by 75%** and increase data efficiency threefold, which was demonstrated in a study of colorectal polyp development, revealing complex host-microbiome interactions potentially linked to cancer risk. PROFIT-seq's ability to combine target-specific insights with whole-transcriptome coverage has broad applications in clinical research, from identifying pathogens to investigating disease mechanisms. This technology holds promise for advancing diagnostics, precision medicine, and targeted disease research.

Multiplexed profiling of intracellular protein abundance, activity, interactions and druggability with LABEL-seq

Simon, J.J., Fowler, D.M. & Maly, D.J. *Nature Methods* (2024),
https://doi.org/10.1038/s41592-024-02456-7

LABEL-seq is an advanced tool for multiplexed protein profiling, **designed to assess protein abundance, activity, interactions, and druggability** directly within live cells. Unlike traditional methods, which often require complex sample preparation or lack functional insights, LABEL-seq **enables researchers to analyze thousands of proteins simultaneously,** providing a comprehensive view of cellular dynamics in real-time. This innovation is particularly valuable in drug discovery and systems biology, where understanding the activity and interactions of proteins within their native environment can lead to breakthroughs in identifying and validating therapeutic targets.

The core of LABEL-seq's methodology involves selective in situ labeling of proteins of interest. This is achieved using a **library of chemical probes or affinity tags** that bind specifically to targeted proteins or protein modifications. After labeling, these protein complexes are sequenced, allowing researchers to quantify their abundance and evaluate changes in activity or interactions under different conditions. This labeling also **makes it possible to monitor post-translational modifications** and other protein activities that reflect functional states critical for cellular processes.

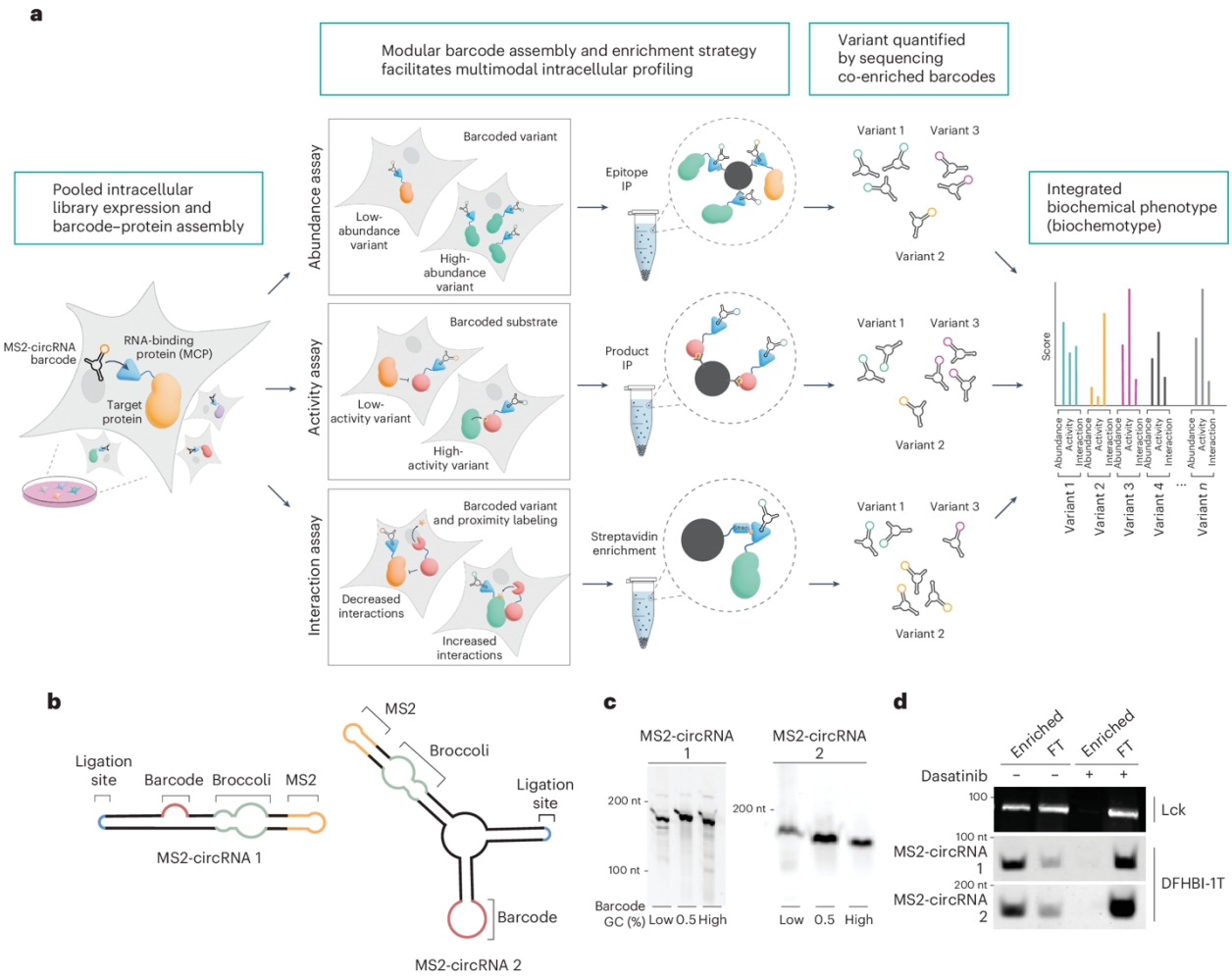


Figure 4. A schematic overview of the LABEL-seq platform.

LABEL-seq combines these labels with next-generation sequencing technology to produce a high-throughput, multiplexed readout of the labeled proteins. This sequencing-based approach distinguishes LABEL-seq from traditional proteomics, which often rely on mass spectrometry and can be less sensitive to protein interactions or activities. By directly sequencing labeled proteins, LABEL-seq **can detect low-abundance proteins and subtle activity changes**, making it well-suited for applications where sensitivity and specificity are paramount.

One of LABEL-seq's most promising applications is in drug discovery, particularly for mapping the interactions between drugs and their protein targets within live cells. By allowing for **detailed profiles of cellular responses to drugs**, LABEL-seq can identify how drugs affect protein functions and interactions. This information is invaluable in evaluating potential side effects, mechanisms of action, and therapeutic efficacy, accelerating the pipeline from discovery to clinical development. Through its high sensitivity and multiplexed capability, LABEL-seq provides a powerful framework for identifying new therapeutic targets, screening drug candidates, and understanding disease-related protein dysfunction.

If you would like to announce anything single cell related, e.g. job announcement, event, your published paper, technology development etc., please contact us.

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