A Cost-Effective Single-Cell Workflow Advances Holistic Biological Views

A game-changing, microfluidics-free, sample-to-insights, single-cell workflow expands the breadth of single-cell sequencing experiments with minimal investment

An exceptionally useful tool for capturing critical behavior of specific cell types, single-cell sequencing is used to interrogate heterogeneous tissues, rare cells, and microenvironments in complex diseases like cancer. Any 'ome' can be studied at the single-cell level. The technique helps elucidate inter-cell genetic, epigenetic, transcriptomic, and proteomic heterogeneity. Analysis of a large number of single cells from a single tissue generates a unique holistic view, capturing diversity and identifying important differences between cell populations that increase the understanding of inherent tissue complexity.[1]

But many challenges limit the broad adoption of this approach. In fact, a meta-analysis of scRNA technologies revealed a growing demand for improvements to single-cell analysis accessibility, scalability, sensitivity, cell-capture efficiency, and and approachable tertiary analysis.[2]

A New Approach Meets the Demands

Truly revolutionary, the new Illumina single-cell solution provides researchers a better tool to characterize complex tissues. Based on particle-templated instant partition sequencing (PIPseq™), the approach allows massively scalable single-cell gene expression studies while eliminating the need for complex and expensive instrumentation, consumables and service contracts.

This game-changing single-cell solution contains new methods for cell lysis and capture, nucleic acid conversion, molecular counting, and sequencing library preparation. Its flexible workflows and sample compatibility have been demonstrated across an array of cell and tissue types.[3]

The technology allows simultaneous capture of a population of single cells using familiar benchtop tools. In just two minutes of vortexing, regardless of cell number, cells are instantaneously captured gently in an emulsion, potentially limiting expression changes, and nucleotides remain in emulsion and stable for days at room temperature.[4]

The Illumina solution decreases costs dramatically and also provides intuitive user interface-based informatics at no additional cost for an effective end-to-end workflow. The cost reduction means a researcher can prepare 5X more cells for sequencing at the same cost as other common methods, broadening experimental breadth and the ability to repeat experiments as needed.

The single-cell offering is designed with informatics in mind, including integration into Illumina Connected Software with access to DRAGEN™ secondary analysis and data interpretation and visualization capabilities powered by Partek™, along with scalable cloud-based storage on AWS.

"The ease which we have been able to generate large numbers of single cells for challenging neuronal samples is amazing. Sequencing quality, including genes detected, doublet errors, and mitochondrial contamination levels are exceptional, and on par with other methods. I have no doubt that this approach utilizing PIPseq will be an impactful addition to the laboratory repertoire for any researcher," said Shane Liddelow, PhD, Associate Professor, Department of Neuroscience and Physiology, NYU Langone, New York City.

A Stand Out Among Single-Cell Workflows

Single-cell workflows generally follow the same principles. Single cells are isolated from tissue by mechanical, enzymatic, or combinatorial approaches then encapsulated. Unique barcodes are added to the nucleotides from individual cells for identification during analysis. Library preparation amplifies and adds adapters to the nucleotides from individual cells to facilitate sequencer compatibility. Lastly, the cells are sequenced and analyzed.

Many methods abound to isolate single cells for library preparation. The emulsion-based, novel Illumina single-cell approach is instrument-free and easy-to-use. Similar to microfluidic methods, the technique uses water-in-oil droplets to compartmentalize lysis and RNA capture for individual cells. However importantly, in the Illumina method rapid emulsification occurs with a vortex mixer. The number of template particles used directly influences the quantity of cells that can be prepped and sequenced.

Four Straightforward Steps

First, cells are added to barcoded hydrogel templates. Next, the cells are lysed via precise micellar delivery of a chemical lysis reagent. mRNA is captured on polyacrylamide, poly(T)-coated beads and reverse transcribed. The remainder of the protocol is accomplished in a typical bulk reaction. Full-length cDNA is synthesized, amplified, and prepared for sequencing. Library preparation is accomplished using standard Illumina paired-end constructs.

It takes only 15 hours to go from cells to library (six-hour hands-on time) with safe stopping points throughout the protocol, ideal for cell sampling along time points for longitudinal studies. Kits are offered in four sizes—for 2K, 10K, 20K and 100K cells—for affordable prototyping through million-cell projects. Both fresh and fixed tissues are compatible along with a wide range of cell types, including brain cells, neurons, and immune cells.[5]

Of special note are the two cost-effective 2K and 10K capacity kits that are sized appropriately for smaller-scale experiments. The 2K kit is especially useful for pilot, organoid or stem cell studies, grant applications, and sorting-optimization studies.

Unlike other single-cell methods that

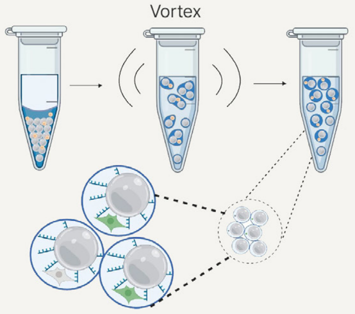
are expensive, limited in scale, have rigid workflows, and require significant expertise, the Illumina single-cell solution is simple, affordable, flexible, and scalable. The emulsion-based methodology requires no complex or expensive microfluidic equipment, only standard lab tools —a vortex, tube adapter and dry bath that are provided in a one-time starter kit purchase.

Since the emulsification process occurs simultaneously throughout the entire sample, the potential for sample degradation, which can occur during the sequential droplet creation and cell-capture process common in microfluidic methods, is reduced. The kits' performance is exceptional for identification of rare cells, well-correlated with microfluidic platforms, and top-tier cluster discrimination capability.[3]

Also in contrast to microfluidics, the number of droplets scales with the quantity of template particles, not emulsification time. And, since the technology is reagent- based, small quantities of cells can be prepped one sample at a time.

Once processed, samples are analyzed in the DRAGEN single cell pipeline and imported to Illumina Connected Multiomics, powered by Partek for intuitive point-and-click data exploration. This biologist friendly solution combines powerful statistics with information-rich, interactive visualizations to take your analysis from start-to-finish.

Using vortexing alone, Illumina Single-Cell Prep isolates single cells with barcoded template particles.



There are many benefits to this method, including:

5×

Value: For the same price, you can process 5X more cells using Illumina Single-Cell Prep than the leading single cell alternative[3]

Scalability: Kit sizes that scale with your experiment and provide a 75% capture rate and exceptionally low multiplet rate (2-8%)[2]

Flexibility: Compatible across species, with many different cell types, and from both fixed and frozen samples[2]

Results Matter

The innovative Illumina single-cell solution has proven its worth. The method was used to uncover cells and the signaling mechanisms driving intestinal tissue's regenerative response in hopes of finding new therapeutic avenues for intestinal regeneration. Using genetic mouse models, epigenetics, bulk, and single-cell RNA sequencing (scRNA-seq), and tissue/organoid culture approaches, the interplay among intestinal epithelial cells, mesenchymal cells, and a TGFB-induced regenerative circuit were explored that may have applications in cellular therapy.[6]

Other experiments have documented performance. Matched PBMCs processed using both the Illumina single-cell kit and the leading single-cell alternative kit produced highly comparable UMAP clustering and overall performance. In addition, early results suggest that the Illumina single-cell solution may detect cell types such as rare cells or cells that clog microfluidic devices that are missed via other approaches.[7]

Overall, gene expression patterns are well-correlated between the two kits. For example, an important application of single-cell sequencing is atlasing cell types in heterogeneous tissue. Both the Illumina and the leading alternative single-cell methods were applied to healthy breast tissue. After data was dimensionally reduced, similar clusters of expression resulted between the methods.[7]

Furthermore, the Illumina single-cell kit was shown to capture high quality RNA transcripts from single cells with low ambient RNA background in a study using a 50:50 mixture of HEK293T (human) and 3T3 (mouse) cultured cells. Using the same cell mixture, proof-of-concept experiments demonstrated mRNA expression clearly resolved human and mouse cells with an ~2% multiplet rate.[7]

The single-cell kits are immediately compatible with any properly tagged target such as CRISPR screening methods that include poly-adenylated guide RNAs (gRNAs) in library design. Results likewise suggest efficient use of the technology for CROP-seq experiments, which utilize a regular gRNA and a polyadenylated copy of the guide sequence from separate promoters, paving the way for routine million-cell experiments that map genotype-phenotype relationships at the genome scale.[7]

Now is the time, with minimal investment, to expand single-cell sequencing studies. The Illumina single-cell solution provides microfluidics-free, easy processing from cell capture to library preparation with stable preservation of mRNA. Added functionality through a simple full integration into existing Illumina workflows and the informatics ecosystem for analysis–along with AWS cloud storage capabilities–complete the workflow. Single-cell sequencing is now affordable, scalable and flexible. ■

References available online.

To learn more, download the FREE new single-cell ebook or call or email an Illumina single-cell expert at:

1-800-809-4566 *ext.* 1
insidesales@illumina.com

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