csgenetics

SimpleCell™ 3′ Gene Expression Assay Data Sheet

Introduction

Single-cell RNA sequencing (scRNA-Seq) examines the transcriptomes of individual cells to identify and quantify cell types in a population, and capture gene expression differences in important subtypes.

Transcriptome profiling at the single-cell level is necessary to uncover biological complexities and bring added confidence to your results[1-3]. This Data Sheet provides a protocol overview for the SimpleCell™ 3′ Gene Expression Assay Kit.

The SimpleCell™ 3′ Gene Expression Assay Kit features a streamlined and scalable instrument-free workflow for single-cell gene expression studies. Cell pairing and indexing takes place within standard lab plasticware (i.e., single tube, 8-strip, and 96-well plates). SimpleCell™ kinetic confinement technology also features a fixation-free stopping point to let you freeze experiments asynchronously after cell priming and pairing.

Product Highlights

- **Simple, instrument-free workflow**
- **Scalable support for biological studies**
- **Flexible options for downstream analysis**

Simple Instrument-Free Workflow

The SimpleCell™ 3′ Gene Expression workflow is a transformative new technology for single-cell genomics, replacing complex hardware and manual workflows with a simple, instrument-free assay that plugs seamlessly into any molecular biology laboratory.

SimpleCell™ Technology employs a biophysical process known as kinetic confinement to perform high-fidelity single cell indexing in standard laboratory plasticware (**Figure 1**).

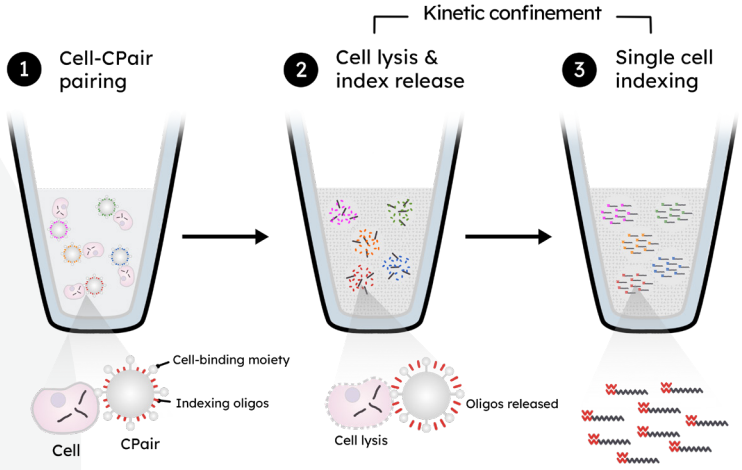

Figure 1. Single cell indexing is achieved by one-to-one pairing of cells with CPair, followed by temperature-dependent cell lysis (with simultaneous release of indexing oligos from CPair) and indexing occurring within the viscous Kinetic Confinement Buffer.

CPair is a bifunctional cell pairing reagent that delivers index sequences directly to single cells. The proprietary Kinetic Confinement Buffer (KCB) ensures efficient heat-activated cell lysis, and kinetically confined indexing prevents spatial diffusion of indexes and cellular mRNA. Cells can also be conveniently frozen and stored at this safe stopping point (**Figure 2**).

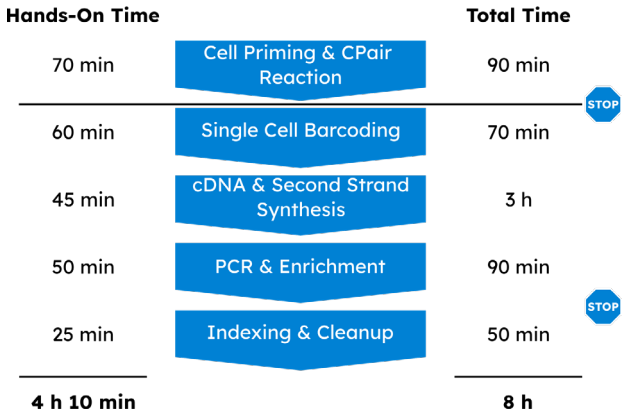

Figure 2. Hands-on and total time for the SimpleCell™ 3′ Gene Expression assay. Users can produce high quality, sequencing-ready libraries in just over 4h of hands-on time using standard molecular biology lab equipment.

After CPair and KCB enable cell lysis and single-cell indexing of cellular mRNA, first-strand synthesis begins with the extension of the indexing oligos to produce first-strand cDNA molecules containing a first PCR handle, a cell-specific index sequence, and the complement sequence of the cellular mRNA molecule (**Figure 3**).

During the second-strand synthesis step, first-strand cDNA molecules are converted into PCR-amplifiable molecules using a primer that hybridizes and primes at a random position along the first-strand cDNA molecule and contains the second PCR handle.

Next, PCR amplification and enrichment of the second strand synthesis products are performed using standard amplification and sample-barcoding primer sets.

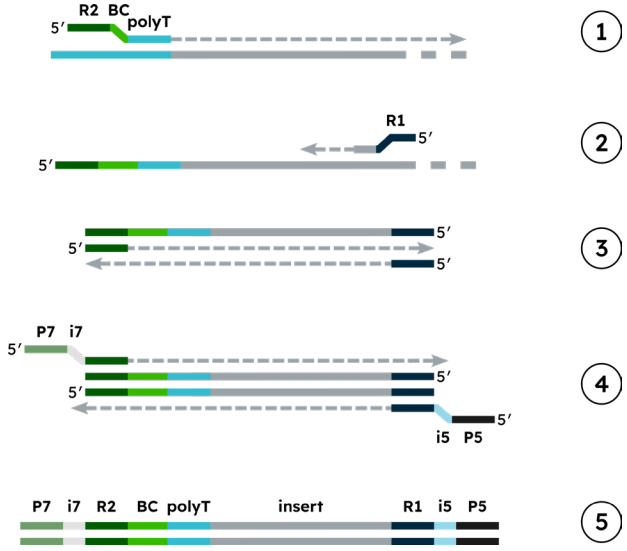

Figure 3. Single-cell barcoding steps involved in the SimpleCell™ 3′ Gene Expression assay. Single cell indexing of mRNA molecules is followed by (1) reverse transcription, (2) second strand synthesis and library preparation steps (3 and 4) to generate DNA libraries (5) that can be sequenced using short-read NGS technologies.

To understand the effect of sequencing depth on sensitivity, 1:1 mix of Hela and NIH3T3 and fresh PBMCs were processed according to the SimpleCell™ 3′ Gene Expression kit instructions. FASTQs were randomly downsampled to different

Typical Performance

Performance Criteria*	Mixed Species‡	PBMCs**
Total Cells per Sample	2,000-4,000	1,500-3,000
Sensitivity, i.e. median genes per cell	2,200-3,000	900-1,200
Capture Rate %	40-80%	20-35%
Multiplet Rate %	6-9%	N/A

*Based on performance with sequencing read depth of 30,000 raw reads per cell ‡Tissue types tested: HeLa:NIH3T3 (1:1 mix)
**Tissue types tested: Fresh Human PBMCs

numbers of raw reads (**Figure 4**). At 30,000 raw reads per cell, the median number of nuclear genes is above 1,200 for PBMCs and above 3,200 for mixed cell lines.

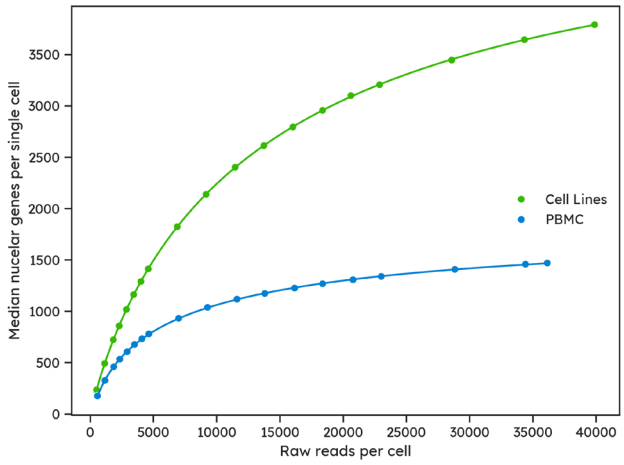

Figure 4. Sensitivity curve for representative cell lines (Hela:NIH3T3) and fresh PBMCs

As shown in **Figure 5**, single cells are efficiently called with a low multiplet rate using a mixed-species experiment. For each CPair barcode, the number of counts associated with the mouse genome and the human genome are determined and the cell caller thresholds are used to classify each barcode as noise (grey points), a single cell (green points), or a mixed-species multiplet (blue points).

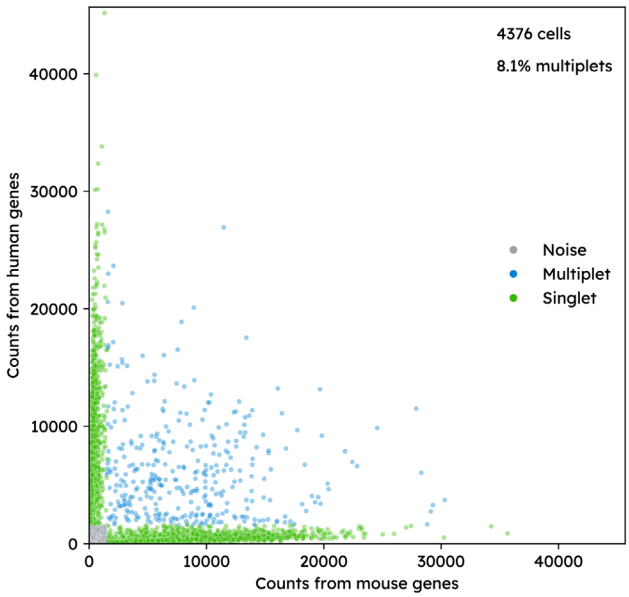

Figure 5. Assessment of single cell indexing using mixed species input (Hela and NIH3T3).

The SimpleCell™ Assay can be used to examine the composition and gene expression of heterogeneous primary cell populations. As a demonstration of this, 96 frozen whole blood samples that had undergone red blood cell lysis and dead cell removal were processed, and high-resolution annotation of cell types was possible (**Figure 6**).

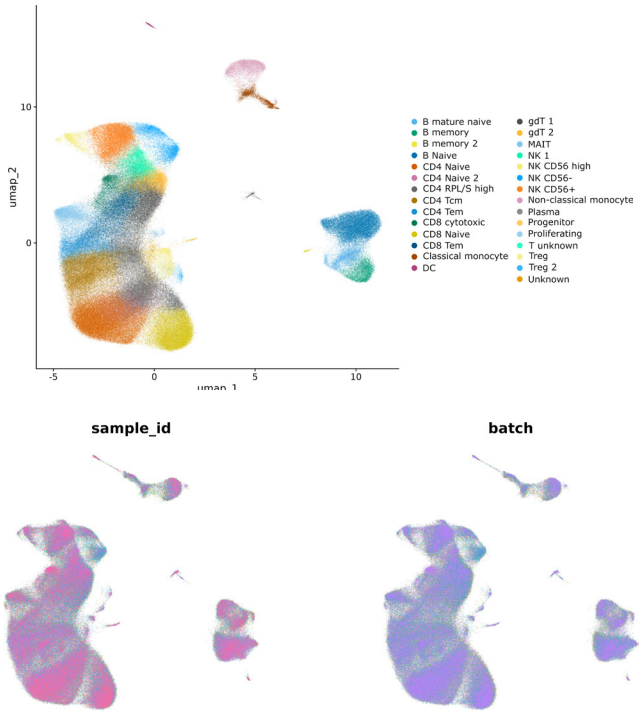

Figure 6. Top: Graph-based clustering and cell-type annotation of cells within all 96 whole-blood samples. Bottom: Dimensionality reduction plots colored according to possible causes of batch effect within the dataset.

Scalable Support for Biological Studies

SimpleCell™ technology features a fixation-free, asynchronous sample collection point. This is useful because increasing sample number will improve statistical significance of many studies and enable previously-impossible large-scale experiments. The assay is scalable and amenable to automation enabling an affordable price point so researchers will no longer have to limit the scale of their studies.

Flexible Options for Downstream Analysis

Primary processing of sequencing data is performed by a custom, open-source pipeline that can be installed on your own compute environment. This produces count matrices that are compatible with all existing secondary and tertiary analysis tools for single cell gene expression, such as Seurat or Scanpy[4]. Alternatively, customers will be able to use cloud-based informatics providers to process data from sequencing files through to downstream analysis.

Ordering Information

SimpleCell™ 3′ Gene Expression 8 Samples	1000393
SimpleCell™ 3′ Gene Expression 16 Samples	1000394
Accessory Starter Kit 8 Samples	1000396
Accessory Starter Kit 16 Samples	1000406

References

1. Regev, A. & Human Cell Atlas Meeting Participants 2017 *eLife* 6:e27041.
2. Lindeboom, R.G.H., et al. 2024 Nature 631, 189–198.
3. Vento-Tormo, R., et al. 2018 Nature 563, 347–353.
4. Marafini, P., et al. 2024 *bioRxiv*

Customer Support

Please contact support@CSGenetics.com or visit www.csgenetics.com