SeekOne® DD Single Cell Full-length RNA Sequence Transcriptome-seq (scFAST-seq)

Product Overview

SeekOne® Digital Droplet (SeekOne® DD) High throughput Single Cell Full-length RNA Sequence Transcriptome-seq (scFAST-seq) Kit, self-developed by Beijing SeekGene BioSciences Co., Ltd., is a powerful commercial tool for **high-throughput whole transcriptome profiling**. The scFAST-seq method makes use of innovative techniques including semi-random primers, efficient reverse transcription, template swapping, and effective rRNA removal to build **full-length RNA libraries** of up to **12,000 cells.**

Compared to conventional 3' scRNA-seq, scFAST-seq has distinct advantages in detecting **non-polyadenylated transcripts, transcript coverage length, and identification of more splice junctions.** With target region enrichment, scFAST-seq can simultaneously detect somatic mutations and cell states in individual tumour cells, providing valuable information for **precision medicine**.

The kit is designed to be used in conjunction with our SeekOne® Digital Droplet System (SeekOne® DD) to complete the entire process from single-cell nucleic acid labelling to transcriptome library construction. When equipped with **"SeekSoul Tools"**, our single-cell data analysis software, we provide **a one-stop-shop single-cell transcriptome solution**.

Highlights

- Bring a powerful tool for studying tumour heterogeneity by **mutation + gene expression** profiling at the single cell level.
- Study isoforms generated by **alternative splicing** and **long non-coding RNA** in gene structure.
- Explore the **pathogenic mechanisms** associated with the expression characteristics of cell types mapped by mutant genes.
- Detect **polyA-tailed and non-polyA-tailed viral RNA transcripts** to analyze the specificity of virus-infected cell populations, virus-host cell interactions, and microenvironmental changes induced by viral infection.
- Facilitate basic R&D, **drug development**, non-clinical and clinical evaluation, manufacturing quality control, etc.
- **Break the single-omics limitation** of providing only one dimension of single cell information.

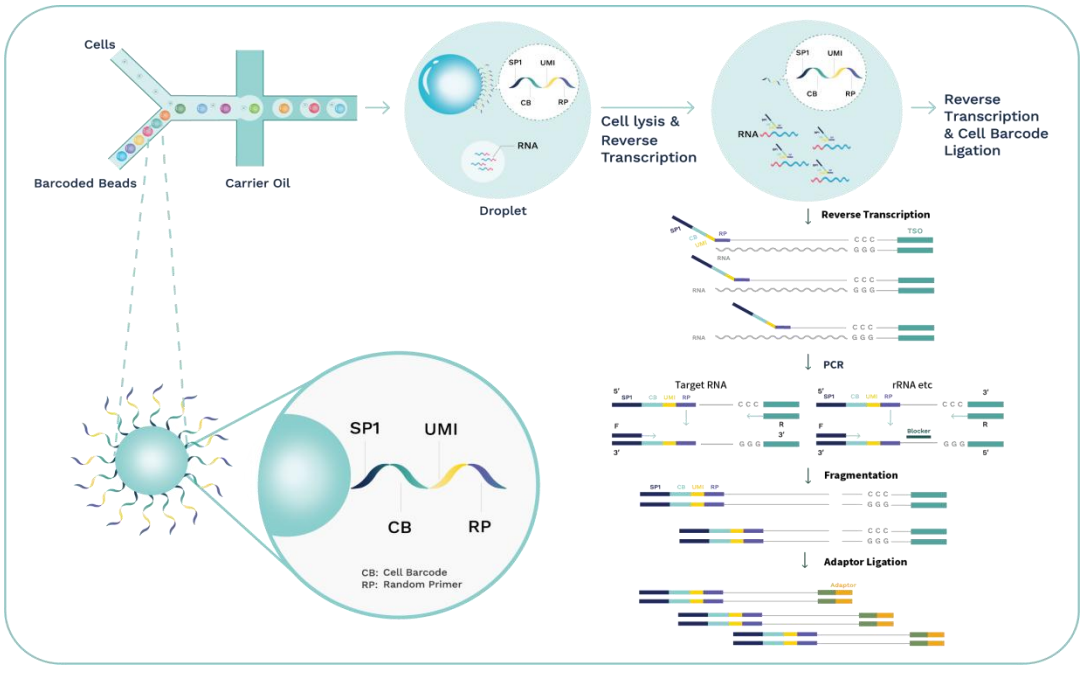


Figure 1. The core technology for Single Cell Full-length mRNA Sequence Transcriptome Sequencing (scFAST-seq).

Core Technology

Single Cell Full-length RNA Sequence Transcriptome-seq (scFAST-seq) allows full-length transcriptome analysis of thousands of fresh or fixed cells in a single experiment. In contrast to the current single-cell transcriptome assays based on oligo-dT reverse transcription, the method used in scFAST-seq provides coverage of the whole transcriptome. The sensitivity of the scFAST-seq technique assays depends primarily on the efficiency of reverse transcription, cDNA enrichment, and ribosome depletion.

First, cells and barcoded beads are added and react to generate emulsion droplets in the ✈-shaped channel of the SeekOne® Digital Droplet System **(Figure 1).** Reverse transcription is then performed by encapsulating the microbeads coupled to cell labels and semi-random primers with individual cells in a single droplet, resulting in cell-labelled cDNA fragments. Once the reverse transcription of each cDNA is complete, a template transition sequence (template switch oligo, TSO) is added to the 3' end of the cDNA using the end-transferase activity of the reverse transcriptase. The barcoded cDNA is then amplified in vitro, where the ribosomal RNA (rRNA) is depleted. Finally, a portion of the cDNA is fragmented, end-repaired, and ligated to a sequencing adaptor. The library is constructed and amplified using the unique dual-indexed polymerase chain reaction (PCR) primers for sequencing on Illumina or MGI platforms. In this way, the cDNA amplified by PCR would reflect both polyA and non-polyA RNA sequences.

Product Features

Accurate single-cell transcriptome	Achieve full-length transcriptome detection, without being limited by the 3’ or 5’ end.
Genotype to phenotype	Simultaneously obtain single-cell mutation and corresponding gene expression information, establishing the relationship between genotype and phenotype
Breaking the species limits	In addition to mRNA, non-coding RNA without 3’ polyA tails can also be captured.
Crossing the species barrier	Enables the detection of viruses and prokaryotes.
Improving analysis accuracy	Allows the detection of isoform switching events of mRNA and lncRNA

Product Specifications

- Currently applicable species: human and mouse
- Fraction of Reads Mapped to Middle Genebody is greater than **55%**
- Fraction over **0.2** mean coverage depth of ACTB gene is higher than **70%**
- Rapid generation of **150,000** water-in-oil droplets in **3** minutes
- Efficiently capturing **500-12,000** cells per channel
- Flexible running of **1~8** samples in parallel
- Cell size flexibility: cell diameter of **5~40** μm
- High cell capture rates of up to **65%**
- Low doublet rates of under **0.3%** per **1,000** cells

Workflow Steps

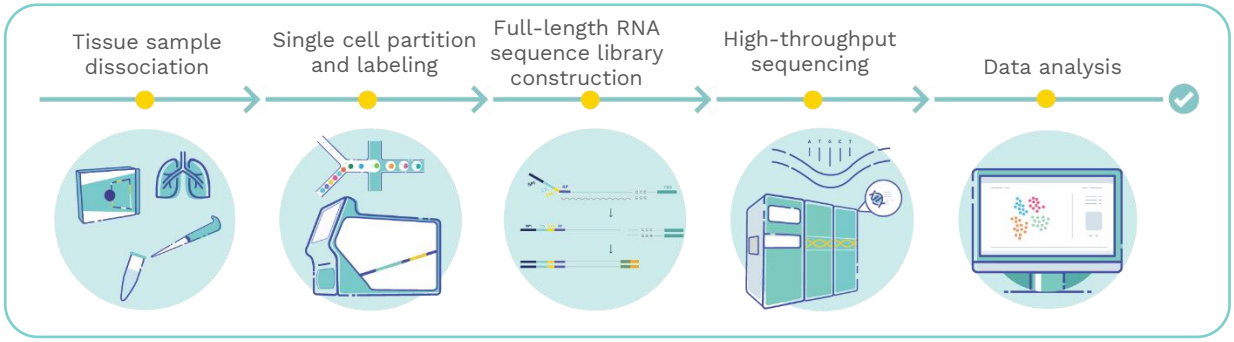


Figure 2. SeekOne® DD Single Cell Full-length mRNA Sequence Transcriptome Sequencing (scFAST-seq) Workflow. SeekOne® DD scFAST-seq overcomes the restriction of 3' or 5' scRNA-seq that only sequences at the ends of mRNA can be amplified. By using microfluidic digital droplets and barcoded beads coupled with semi-random primers, the scFAST-seq kit can achieve random capture of the full-length RNA sequence transcriptome, allowing the detection of both coding and non-coding RNA. ScFAST-seq data can be processed with SeekSoul® Tools ——our efficient data analysis software for comprehensive information on mutations, fusions, and alternative splicing.

Data Presentation

Table 1. Representative data presentation from human PBMCs, lung cancer, and K562 cell line samples targeting 10,000 cells. This data can be used to evaluate assay performance.

Species	Tissue	Estimated Number of Cells	Mean Reads per Cell	Median Genes per Cell	Sequencing Saturationn	Fraction reads in cell	Total Genes Detected	Median UMI Counts per Cell	rRNA%	Reads coverage middle part of genebody	Over 0.2X mean coverage of ACTB
Homo	Blood	1,1645	38,737	1,547	87.65%	94.42%	24,084	3,102	1.34%	59.88%	83.22%
Homo	Lung cancer	8,970	36,737	2,487	63.41%	96.22%	27,791	6,775	2.12%	56.75%	80.32%
Homo	Cell line	8,137	53.816	4,712	53.35%	90.13%	28,448	14,546	1.54%	58.62%	82.14%

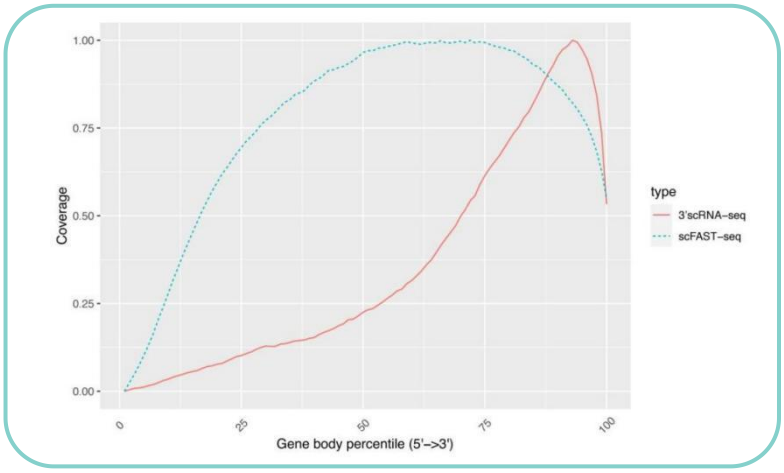


Figure 3. Comparison of transcriptome coverage between 3' scRNA-seq and scFAST-seq. The coverage of 3' scRNA-seq is significantly skewed to the left of the 3' end. In contrast, the scFAST-seq is evenly distributed across the gene body percentile, indicating a full-length coverage of the entire gene body and the capture of unspliced and splice junction regions with less bias.

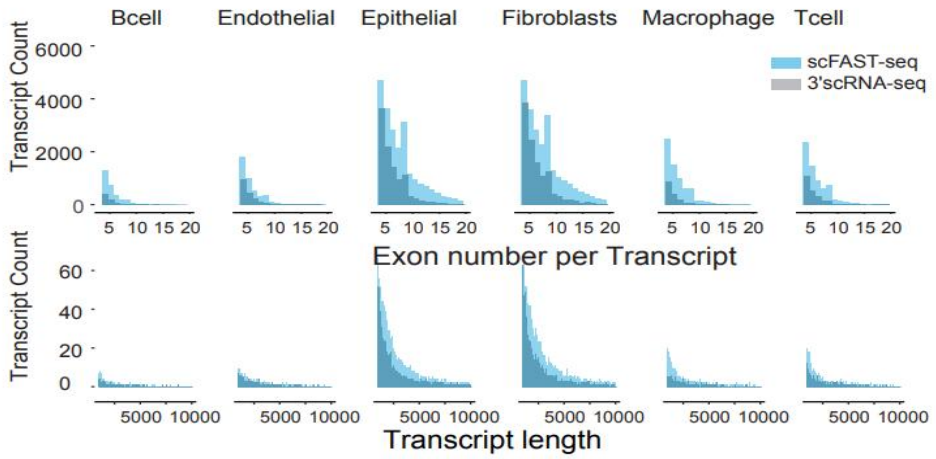
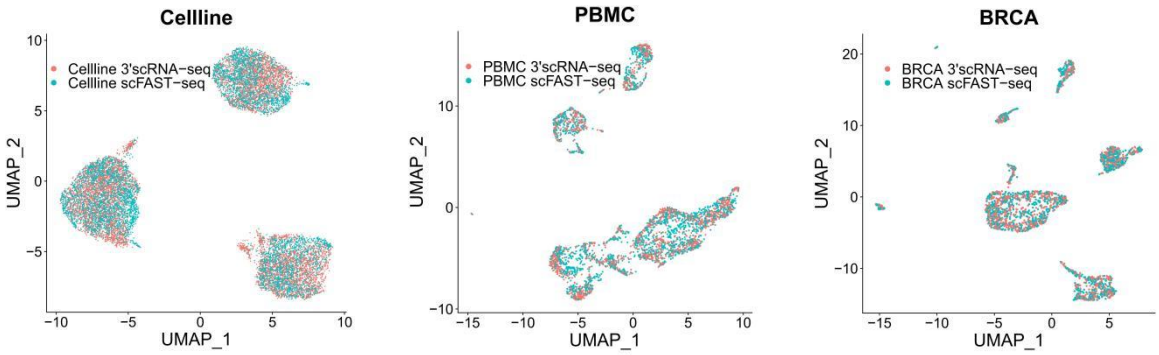


Figure 4. The number of transcripts of each cell in different cancer and PBMC samples. Blue represents the full-length technique scFAST-seq and grey denotes the 3 ' scRNA-seq. scFAST-seq detected significantly more transcripts than 3' scRNA-seq in glioma and breast cancer tissue.

A.



B.

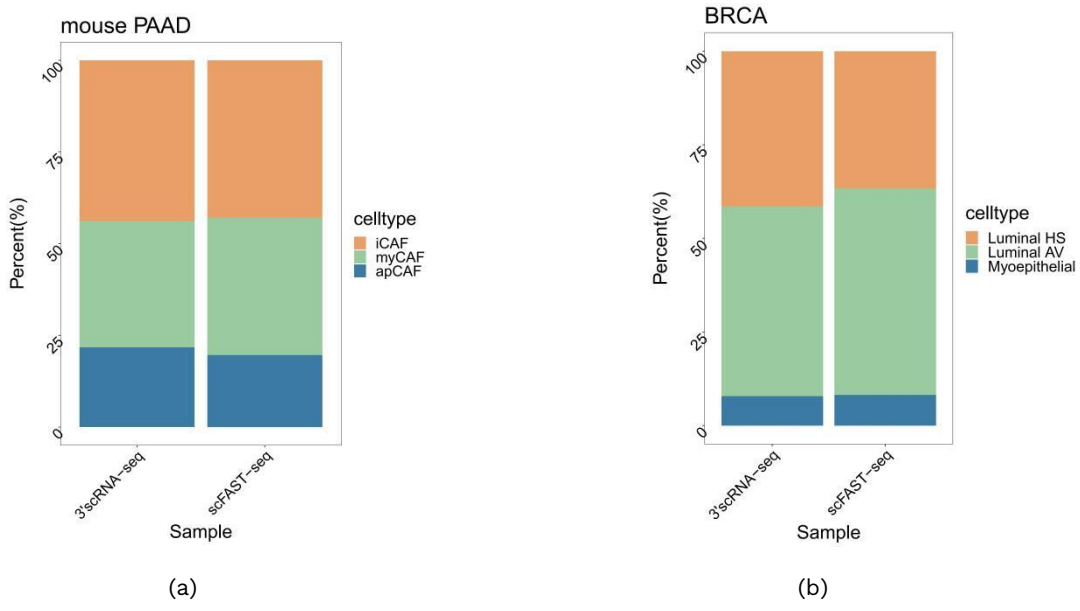


Figure 5. (A) UMAP plots of K562, A549, HCC827 cell line mixture, PBMC, and breast epithelial cells using scFAST-seq and 3' scRNA-seq. By applying the typical correlation analysis method in the Seurat package to integrate and visualize the scFAST-seq and 3' scRNA-seq data, it was found that the clustering and relative positions of cells in the two types of data in the three samples were highly overlapping in the two-dimensional UMAP. **(B) Cell subtype proportions in mouse fibroblasts (a) and epithelial (b) detected by 3' scRNA-seq and scFAST-seq.** The subtype proportions of fibroblasts in pancreatic cancer and epithelial cells in breast cancer indicate a high consistency in cell proportions between scFAST-seq and 3' scRNA-seq data, such as Luminal HS, Luminal AV, and Myoepithelial.

Applications

The single-cell FAST-seq allows access to more dimensions of information:

✓ Mutation

SeekOne® scFAST-seq provides two dimensions (mutation & gene expression) of single cell information, overcoming the restrictions of single-omics **(Figure 6)**. The simultaneous two separate profiling of mutations + expression at the single cell level creates a novel tool for a wide range of studies, including precise identification of tumour cells, clonal evolution, mutation mapping, and therapeutic evolution. In terms of co-mutation, it helps to evaluate the different tumour co-mutation strategies and to investigate the mechanisms of co-mutation-driven molecular dependencies. Furthermore, it can be used to explore the expression characteristics of cell types with mutant genes or changes in signaling pathways. With scFAST-seq, differences in mutation and gene expression profiles between primary and metastatic tumour sites can also be revealed.

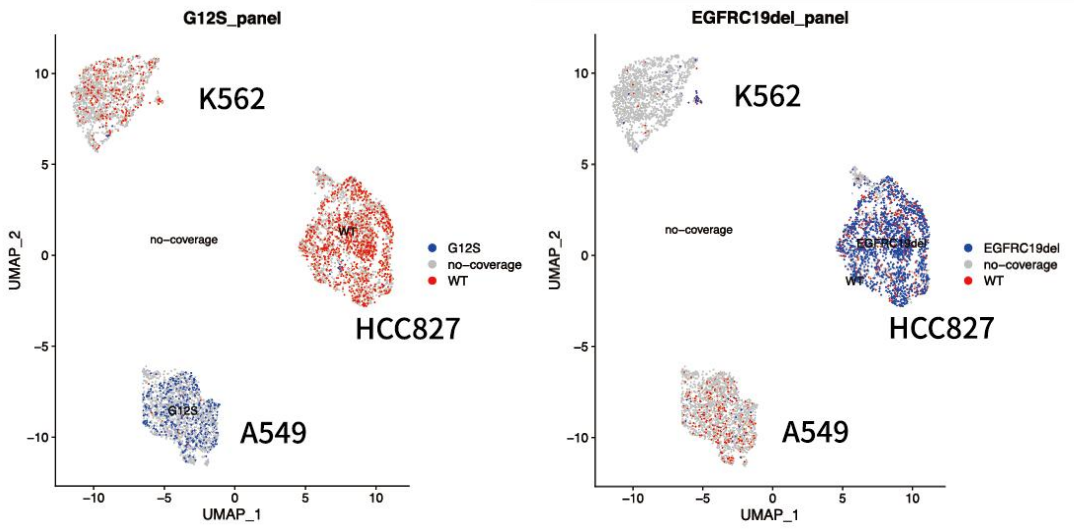


Figure 6. Mutation detection in a hybrid sample of K562 (BCR-ABL1 fusion), HCC827 (EGFR 19del), and A549 (KRAS G12S) cell lines. Data from the scFAST-seq and targeted enrichment libraries allowed clear differentiation of each cell type and accurate detection of the targeted mutant genes in each cell line.

A549: KRAS G12S (mRNA 5' end); HCC827: EGFR 19del (At the middle of the gene); K562: BCR-ABL1 fusion (At the middle of the gene)

✓ Alternative splicing

Compared to 3' scRNA-seq, scFAST-seq yields a significantly higher number of alternative splicing sites for novel and known mRNA/lncRNA, which expands our understanding of transcriptome and proteome diversity, the complexity of gene expression and the underlying mechanisms of RNA regulation **(Figure 7)**. As alternative splicing mediates diverse biological processes throughout the life span of organisms, scFAST-seq analysis can be applied to cellular differentiation and organism development.

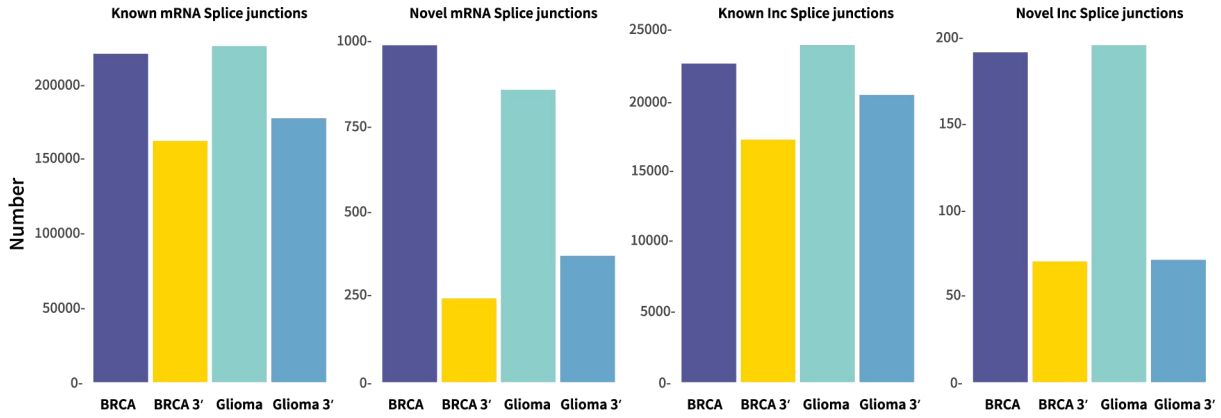


Figure 7. A bar chart plot of the number of known and unknown splice junctions of recognized mRNAs and lncRNAs in BRCA and glioma cells. scFAST-seq achieves even coverage of the entire gene body, thus allowing unbiased capture of unspliced regions and splice junctions. Compared to 3' scRNA-seq, the scFAST-seq provides more alternative splicing detection of known or novel mRNAs and lncRNAs.

Alternative splicing sites for novel and known mRNA/lncRNA: scFAST-seq > 3' scRNA-seq.

✓ Non-polyA tailed RNA

The semi-random primers of scFAST-seq enable the capture of viral RNA genes with polyA tails or non-polyA tails to identify non-polyA viral infected cell clusters **(Figure 8)**. This detection overcomes the species limitations and facilitates studies in viral immunology research, including the identification of virus-infected cell populations, virus-host cell interactions, changes in the microenvironment induced by virus infections, exploration of viral therapeutic targets, and characterization of gene expression at different infection or treatment stages.

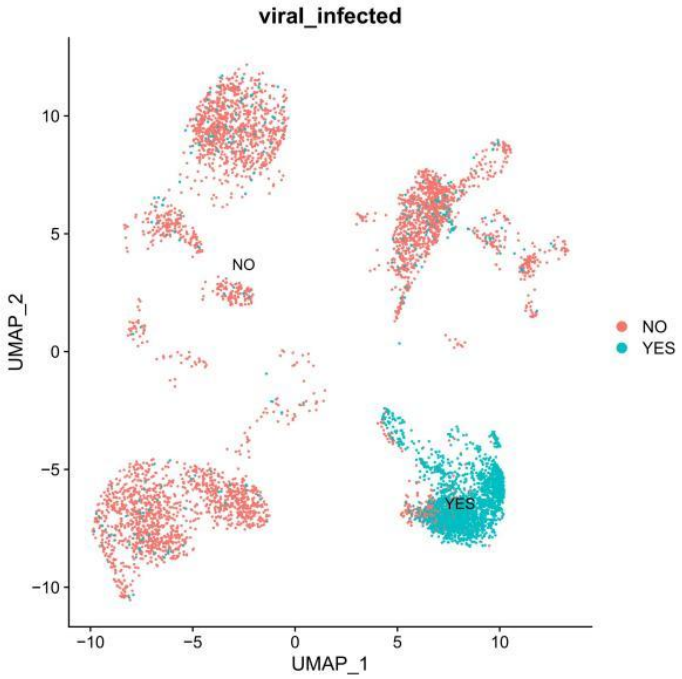


Figure 8. Identification of non-polyA viral infected cell clusters. For viral gene detection, the semi-random primers used in scFAST-seq can capture viral RNA genes with polyA tails or non-polyA tails. The viral UMI in individual cells obtained by scFAST-seq can be linked back to the cell type annotation plot.

SeekOne® DD Single Cell Full-length RNA Sequence Transcriptome-seq (scFAST-seq) Kit

The SeekOne® DD Single Cell Full-length RNA Sequence Transcriptome-seq (scFAST-seq) Kit includes SeekOne® DD Chip S3 (Chip S3), Gasket, Carrier Oil, SeekOne® DD scFAST-seq Barcoded Beads, amplification reagents, library construction reagents, and single-cell data analysis software (SeekSoul® Tools).

SeekOne® DD Single Cell Full-length RNA Sequence Transcriptome-seq (scFAST-seq) Kit

Product	Product code
SeekOne® DD Single Cell Full-length RNA Sequence Transcriptome-seq (scFAST-seq), 2 tests/8 tests	K00801-02/K00801-08
Product Components	**Component code**
SeekOne® DD Chip S3 Kit, 2 tests/8 tests	K00202-0201/K00202-0801
SeekOne® DD scFAST-seq Barcoded Beads Kit, 2 tests/8 tests	K00801-0202/K00801-0802
SeekOne® DD scFAST-seq Reverse Transcription Kit, 2 tests/8 tests	K00801-0203/K00801-0803
SeekOne® DD Library Construction Kit, 2 tests/8 tests	K00202-0204/K00202-0804
SeekOne® DD scFAST-seq Cleanup Kit, 2 tests/8 tests	K00202-0205/K00202-0805

Compatible Instrument

Compatible Instrument	Product code
SeekOne® Digital Droplet System	M001A

Compatible Software

Compatible Software
SeekSoul®Tools single-cell data analysis software

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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