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Illumina Single Cell 3' RNA Prep

Accessible and scalable scRNA-Seq

- Simple, affordable benchtop workflow that can be completed by users of any experience level
- High assay sensitivity to detect more genes and transcripts
- Cost-effective processing range of up to hundreds of thousands of cells to reveal rare cell types

Introduction

Single-cell RNA sequencing (scRNA-Seq) pairs single-cell capture and barcoding with next-generation sequencing (NGS) to provide a high-resolution view of complex tissues. Researchers can use scRNA-Seq to study heterogeneous cell populations, discover rare cell types, and characterize processes of disease and development at the cellular level. However, the need for specialized capital equipment, high reagent costs, and limited scalability have slowed widespread adoption of single-cell sequencing. This changes with Illumina Single Cell 3' RNA Prep.

Illumina Single Cell 3' RNA Prep* enables single-cell mRNA capture, barcoding, and library prep without complex workflows or microfluidics. Combined with Illumina sequencing and informatics solutions, Illumina Single Cell 3' RNA Prep provides an easy and scalable workflow (Figure 1) that makes high-performance scRNA-Seq accessible for more labs.

* Illumina Single Cell 3' RNA Prep was formerly the Fluent PIPseq V 3' Single Cell RNA Kit.

Accessible workflow

The Illumina Single Cell 3' RNA Prep workflow is easy to implement and does not require expensive microfluidic equipment or labor-intensive protocols.[1] Users can conduct single-cell studies on their benchtop using a simple manual workflow with flexible stopping points. For samples collected over a time course or that require transportation, the assay is compatible with DSP-methanol fixation† before sample processing, which expands the utility of scRNA-Seq experiments.

How the assay works

Illumina Single Cell 3' RNA Prep uses novel PIPseq™ chemistry for simple, scalable single-cell mRNA capture and barcoding (Figure 2).[1] Particle-templated instant partitions (PIPs) use emulsification with template particles that include barcoded oligonucleotides bound to hydrogel beads. During sample preparation, the cell suspension of interest is mixed with template particles and oil and segregated into templated emulsions by vortexing. Cells in the emulsions are then lysed and the mRNA is captured by the barcoded templates. The emulsion is broken and cDNA is generated from the captured mRNA via reverse transcription and amplified

† DSP, dithiobis (succinimidyl propionate).

Figure 1: Illumina Single Cell 3' RNA workflow

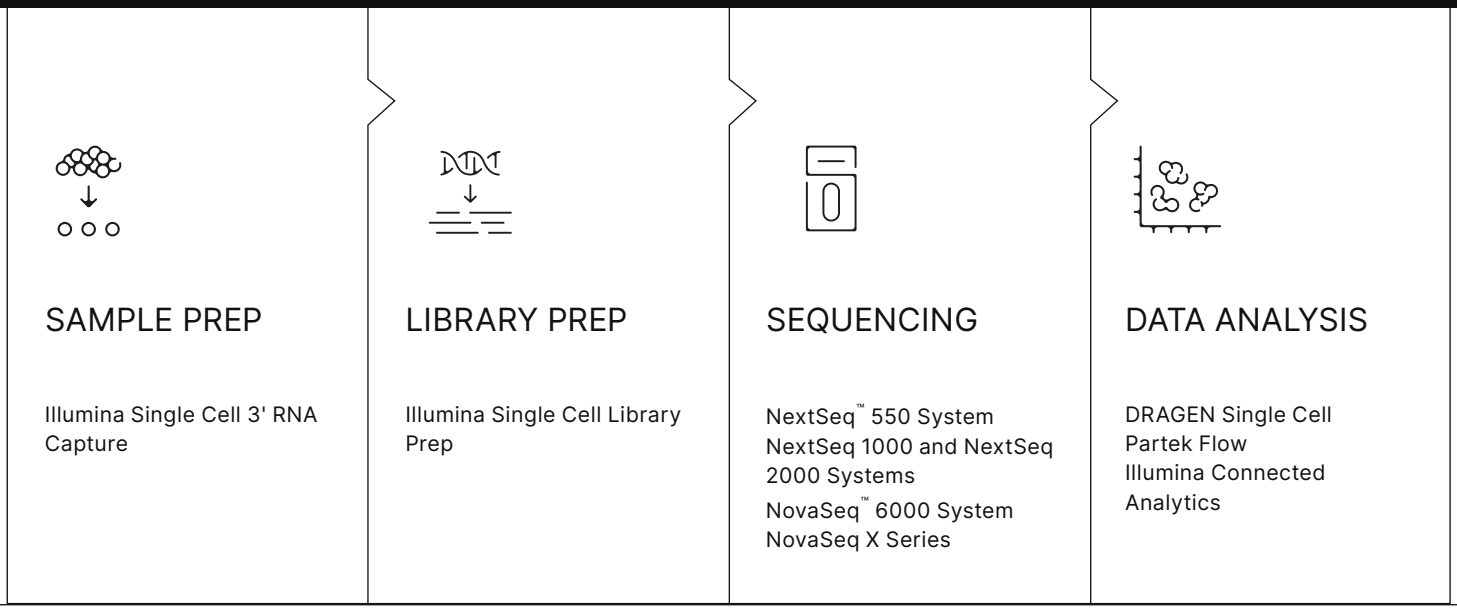
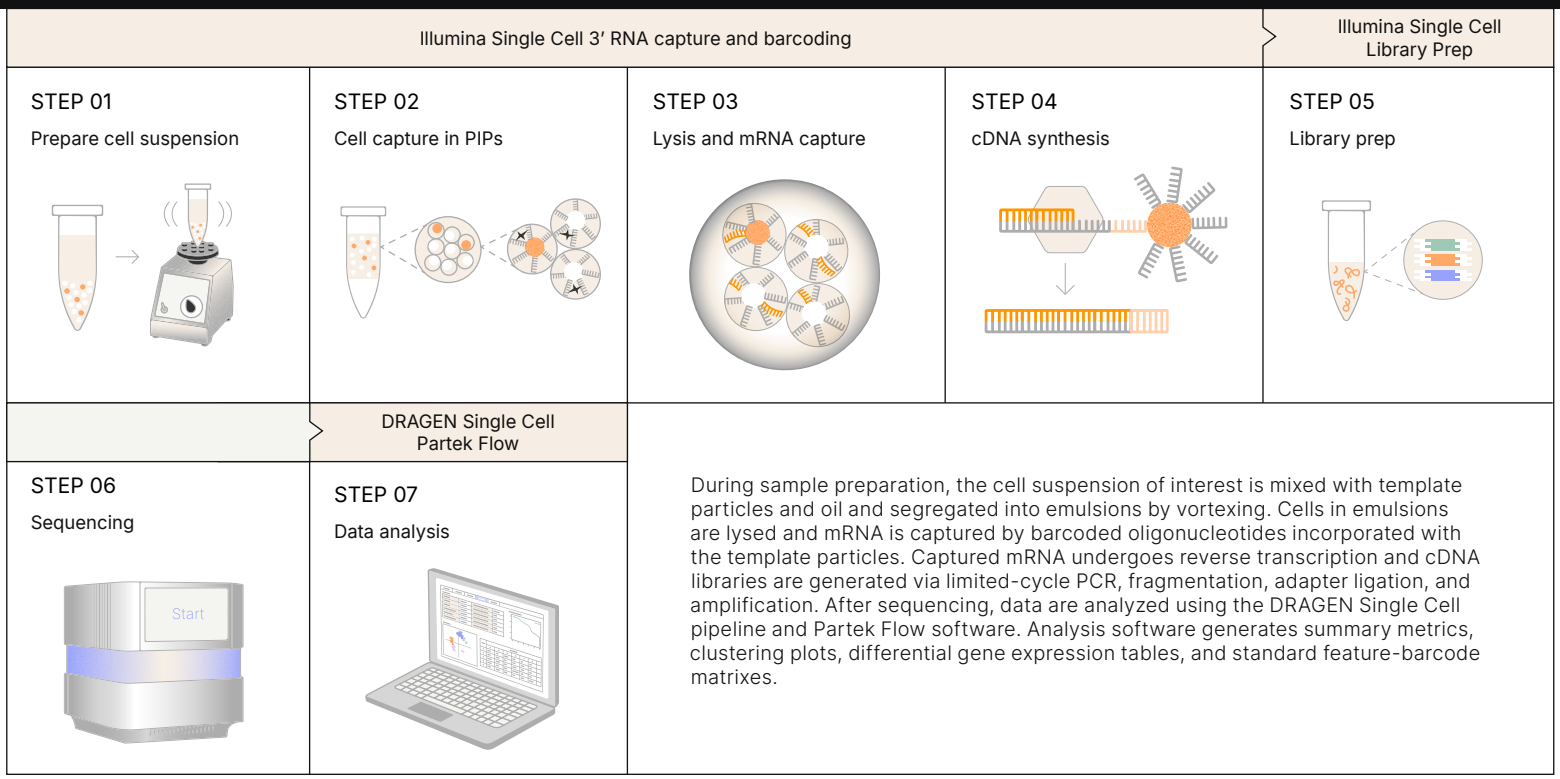


Figure 2: Single-cell mRNA capture and barcoding with PIPseq chemistry

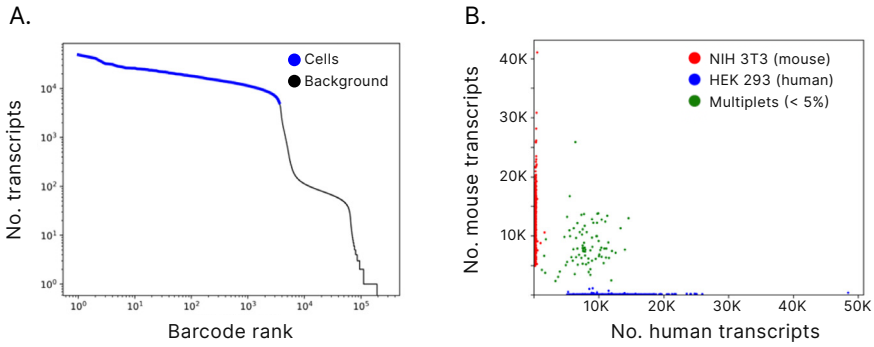


to create a cDNA library for each individual cell. Single-cell cDNA libraries are then processed into sequencing libraries using standard library preparation methods followed by NGS. Sequencing data are analyzed using the DRAGEN™ Single Cell pipeline and Partek™ Flow software.

High-quality performance

Illumina Single Cell 3' RNA Prep offers high-quality data with high transcript and gene sensitivity (Figure 3, Figure 4, Table 1). The gentle isolation technique helps detect fragile cells that are often missed by other methods.

Figure 3: High-resolution scRNA-Seq captures mRNA from single cells



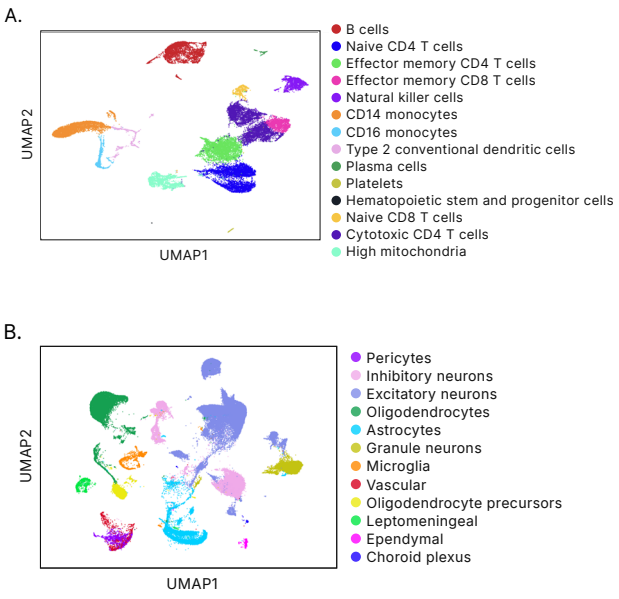
(A) Barcode rank plot and (B) barnyard scatter plot with mixed culture of NIH3T3 (mouse) and HEK 293 (human) cell lines, 4106 cells detected, 80% capture rate, and less than 5% multiplets (two or more cells in the same reaction) with the Illumina Single Cell 3' RNA Prep T2 kit.

The latest version of PIPseq chemistry lowers ambient RNA background, improving sensitivity by 30% for genes and transcripts in certain cell types[2] and reducing sequencing artifacts for more usable NGS data.

Flexible and scalable solution

The simple vortexer-based method for Illumina Single Cell 3' RNA Prep offers cost-effective scalability. For greater cell numbers, use larger volume PIP tubes.[1] The wide processing range from hundreds to hundreds of thousands of cells supports research application needs from pilot and low cell–diversity projects to complex tissue analysis.[1] The current kit configurations can profile up to 2000 cells per sample (T2 kit), 10,000 cells per sample (T10 kit), 20,000 cells per sample (T20 kit), or 100,000 cells per sample (T100 kit). The assay's capability for increased cell throughput can better reveal rare cell types (Figure 5). With 96 unique dual indexes available, sample multiplexing allows users to run many samples in parallel (Table 2).

Figure 4: High-resolution scRNA-Seq to fit experimental scale and applicaiton



(A) Uniform manifold approximation and projection (UMAP) of cryopreserved human peripheral blood mononuclear cells (PBMCs), 31,613 cells detected and 79% capture rate with Illumina Single Cell 3' RNA Prep T20 kit. (B) UMAP of mouse brain nuclei from frozen tissue, 155,000 nuclei detected and 78% capture rate with Illumina Single Cell 3' RNA Prep T100 kit.

Table 1: Validated performance of Illumina Single Cell 3' RNA Prep on Illumina sequencing systems

Cell type	Kit size	Sequencing system	Size	Reads	Cells	Reads per cell
HEK/3T3	T2	NextSeq 2000 System	9.8 Gb	140M	3611	38,633
HEK/3T3	T10	NextSeq 2000 System	23.6 Gb	333M	10,723	31,140
PBMC	T10	NovaSeq 6000 System	390 Gb	2.7B	14,307	190,526
PBMC	T20	NovaSeq X Plus System	81.3 Gb	674M	31,613	21,314
Mouse lung nuclei	T20	NextSeq 2000 System	8.9 Gb	131M	2768	47,276
Mouse brain nuclei	T20	NextSeq 2000 System	10.4 Gb	155M	2019	76,784
Mouse brain nuclei (fixed)	T20	NextSeq 2000 System	39.5 Gb	590M	34,596	17,041
Mouse brain nuclei	T100	NextSeq 2000 System	176.6 Gb	2.6B	155,000	17,068

Single-cell applications

The accessibility, precision, and sensitivity of Illumina Single Cell 3' RNA Prep enables new research and discoveries. Processing higher cell numbers will accelerate cell atlas projects for normal and disease states and power genome-scale functional genomics screens. Wider adoption of scRNA-Seq will especially benefit labs studying cancer, immunology, neuroscience, and other complex diseases.[3–9]

scRNA-Seq for neuroscience

Neuronal tissues comprise a complex organization of diverse cell types that continuously reorganize and remodel throughout an organism's lifetime. scRNA-Seq is a valuable tool to catalog the true diversity of neuronal cell populations with transcriptional sensitivity, providing insight into:

- Neurodevelopmental and neurodegenerative diseases[9]
- Previously unknown roles of specific neuronal populations[9]
- The effect of neuroimmune cells on disease and development[10]

scRNA-Seq for cancer research

Cancer is a dynamic and diverse disease involving complex cell populations. Understanding the cell types and mutations that drive cancer requires sophisticated methods. scRNA-Seq has been a vital tool to:

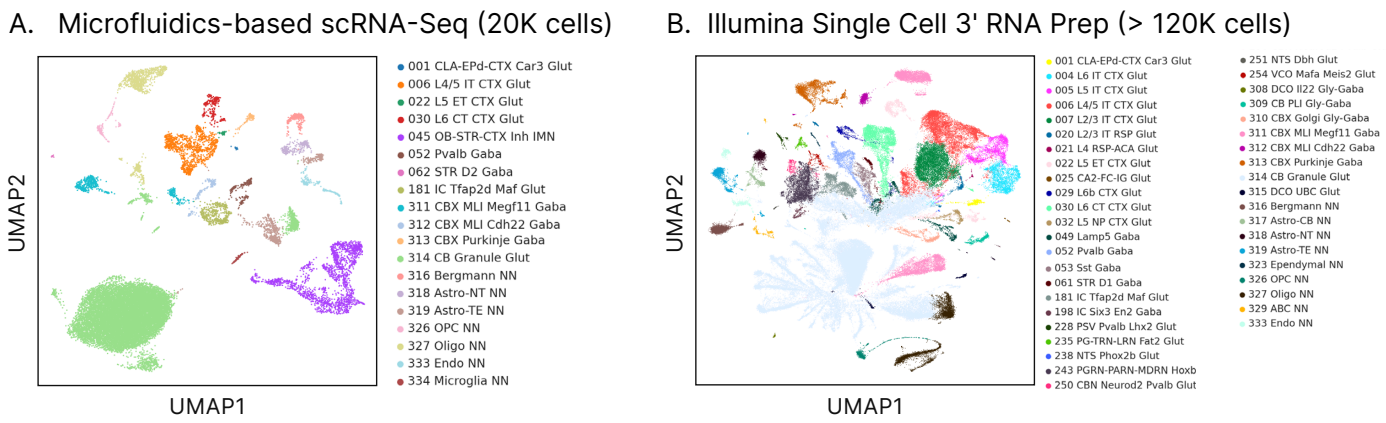
- Detail heterogeneity of the tumor microenvironment[4,5,11,12]
- Identify novel cancer biomarkers[13,14]
- Understand the mechanisms of immunotherapy and drug resistance[3]

scRNA-Seq for immunology

The immune system is composed of a complex hierarchy of diverse cell types that work in concert to identify, target, and eliminate pathogens. scRNA-Seq allows researchers to:

- Understand developmental and differentiation pathways in immune cell populations[15]
- Evaluate the roles and functions of immune cell populations[12,16]
- Study the immune response to pathogens[6]

Figure 5: Increased experimental scale with Illumina Single Cell 3' RNA Prep reveals more cell types



(A) Microfluidics-based scRNA-Seq on 20K cells identified 19 distinct cell types from mouse brain nuclei. (B) Illumina Single Cell 3' RNA Prep on > 120K cells from the same mouse brain nuclei sample prep identified 42 distinct cell types: 17 of the 19 identified by microfluidics-based scRNA-seq plus 25 additional cell types.

Table 2: Example sample throughput per flow cell for Illumina Single Cell 3' RNA Prep[a]

System	NextSeq 550		NextSeq 1000/2000				NovaSeq 6000				NovaSeq X		
Flow cell	Mid	High	P1	P2	P3[b]	P4[b]	SP	S1	S2	S4	1.5B	10B	25B
Output per flow cell (reads)	130M	400M	100M	400M	1.2B	1.8B	800M	1.6B	4.1B	10B	1.6B	10B	26B
Kit size	No. of samples per flow cell												
T2	1	4	1	4	12	18	8	16	41	100[c]	16	100[c]	260[c]
T10	-	1	-	1	3	5	2	4	12	29	4	29	76
T20	-	-	-	-	1	2	1	2	5	12	2	12	32
T100	-	-	-	-	-	-	-	-	1	2	-	2	6

a. Calculations based on 20K reads per input cell. T2 requires 5K input cells and 100M reads per sample. T10 requires 17K input cells and 340M reads per sample. T20 requires 40K input cells and 800M reads per sample. T100 requires 200K input cells and 4B reads per sample.
b. P3 and P4 flow cells only available on the NextSeq 2000 System.
c. Illumina Single Cell Unique Dual Indexes offers 96 indexes for 96 samples. Running over 96 samples per flow cell is possible with individual lane loading. Use the NovaSeq 6000 Xp workflow for individual lane loading on the NovaSeq 6000 System.

Multiomics applications

Illumina Single Cell 3' RNA Prep is compatible with other multiomic measurements.[1,17] The assay allows for reinterrogation of captured mRNA for custom single-cell applications. cDNA generated during reverse transcription remains bound to the hydrogel beads enabling reuse for supplemental enrichment or amplification reactions. This experimental versatility can help researchers undertake larger and more varied multiomics studies without the budget or technology constraints of other single-cell approaches.

Summary

Single-cell NGS analysis opens doors to new areas of discovery in cancer research, immunology, neuroscience, and more. Illumina Single Cell 3' RNA Prep is an accessible and highly scalable scRNA-Seq solution that will expand discovery power for both new and experienced single-cell researchers. The simple manual workflow includes mRNA capture, barcoding, and library prep that integrates seamlessly with Illumina sequencing systems and data analysis software. Illumina Single Cell 3' RNA Prep offers high performance and ease of use to bring scRNA-Seq capabilities to more labs.

LEARN MORE →

Illumina Single Cell 3' RNA Prep

Single-cell RNA sequencing

Ordering information

Product	Catalog no.
Illumina Single Cell 3' RNA Prep, T2 (8 samples, 2000 cells/sample)	20135689
Illumina Single Cell 3' RNA Prep, T10 (8 samples, 10,000 cells/sample)	20135691
Illumina Single Cell 3' RNA Prep, T20 (4 samples, 20,000 cells/sample)	20135692
Illumina Single Cell 3' RNA Prep, T100 (2 samples, 100,000 cells/sample)	20135693
Illumina Single Cell Unique Dual Indexes (96 indexes, 96 samples)	20132788
Illumina Single Cell Library Prep (8 reactions)	20132789
Ilumina Single Cell 3' RNA Capture, T2 (8 samples, 2000 cells/sample)	20132790
Illumina Single Cell 3' RNA Capture, T10 (8 samples, 10,000 cells/sample)	20132791
Illumina Single Cell 3' RNA Capture, T20 (4 samples, 20,000 cells/sample)	20132792
Illumina Single Cell 3' RNA Capture, T100 (2 samples, 100,000 cells/sample)	20132793
Illumina Single Cell Nuclei Isolation Kit (4 samples)	20132795
Illumina Single Cell Supplemental Enrichment and Amplification Kit	20132794
Illumina Single Cell Prep Starter Equipment	20132796

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