

User manual

SeekOne™ DD Single Cell Full-length RNA Sequence Transcriptome-seq Kit

REF: K00801-02 (2 tests), K00801-08 (8 tests)

V1.2

K00202-0201 & K00801-0202 & K00801-0203 & K00202-0204 & K00202-0205 & K00801-0206
K00202-0801 & K00801-0802 & K00801-0803 & K00202-0804 & K00202-0805 & K00801-0806

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Beijing SeekGene BioSciences Co.,Ltd

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1. Introduction

1.1 Product Overview

SeekOne™ DD (Digital Droplet) **Single Cell Full-length RNA Sequence Transcriptome-seq Kit** (abbreviated as SeekOne™ DD scFAST-seq Kit) is independently developed by Beijing SeekGene BioSciences Co.,Ltd (abbreviated as SeekGene). This kit breaks through the limitation that the traditional 3' or 5' end transcriptome can only analyze one end sequence of mRNA, and uses microfluidic digital droplets and barcoded beads coupled with random primers to achieve random capture and detection of the whole transcriptome. This reagent kit needs to be used with our independently developed SeekOne™ Digital Droplet System (abbreviated as SeekOne™ DD, REF: M001A) to complete the entire process from single cell nucleic acid labeling to transcriptome library construction, and is equipped with single cell data analysis software SeekSoul tools, we provide you with one-stop-shop single cell transcriptome solutions.

The SeekOne™ DD Single Cell Full-length RNA Sequence Transcriptome-seq Kit includes: chip (SeekOne™ DD Chip S3, referred to as Chip S3), gasket, carrier oil, gel beads (SeekOne™ DD scFAST-seq Barcoded Beads, abbreviated as Barcoded Beads), amplification reagents, library construction reagents, and single cell data analysis software (SeekSoul Tools).

1.2 Intended Use

The SeekOne™ DD Single Cell Full-length RNA Sequence Transcriptome-seq Kit leverages microfluidic technology to isolate single cells within water-in-oil droplets. Nucleic acid modified Barcoded Beads uniquely label RNA from distinct cell sources. Random primers facilitate the capture of full-length coding and noncoding RNA, while innovative blocker technology effectively eliminates rRNA and mitochondrial contamination. Ultimately, a high-throughput single-cell transcriptome library, compatible with Illumina and GeneMind sequencers, is constructed. This kit finds application in diverse scientific research areas, such as tumor research, immunology studies, cellular development research, viral infection investigations, and targeted biomarker screening.

1.3 Intended User

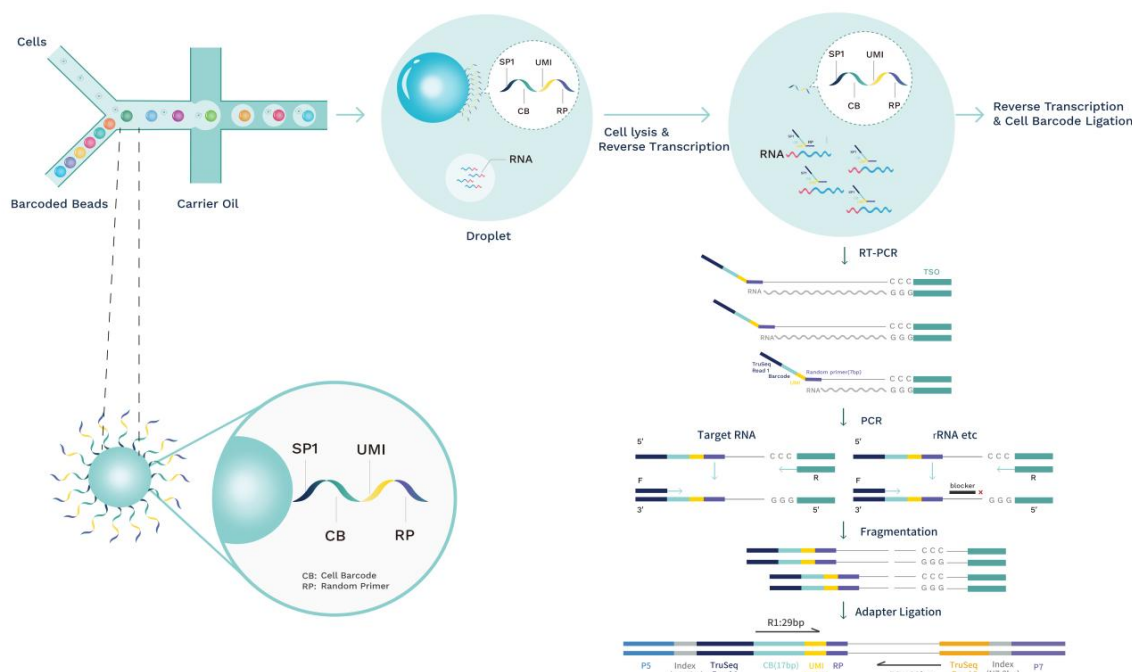
This kit is intended to be used by laboratory technicians or people with equivalent qualifications.

The requirements to use this kit include a certain level of theoretical knowledge and operational skills in molecular biology. After training and qualification by SeekGene, operators are capable of performing this reagent kit.

1.4 Limitations of Test Methods

This reagent kit is intended for use in the pre-processing of samples only and the results should NOT intended to be used directly as a result of an in vitro diagnostic tests.

1.5 Experimental Principles



1.6 Parameter Description

- 1) Sample throughput: Chip S3 is a single-channel chip that can flexibly run 1-8 samples in parallel as needed.
- 2) Cell capture range: A single channel can capture 500-12,000 cells.
- 3) Water-in-oil generation rate: 150,000 water-in-oil droplets generated within 3 minutes.
- 4) Doublet rate: Approximately 0.3% for 1,000 cells.

1.7 Sample Requirements

Fresh tissue should be dissociated into a single-cell suspension. For cultured cells or cells already in suspension, perform a cell washing protocol to remove the media. Large cells (>30 µm) and samples that are difficult to dissociate may require nuclei isolation.

1.7.1 Sample Type

❑ Single cell/ nucleus suspension: No large-particle precipitation should be present. If there is, filter using a 40 µm cell strainer. No calcium or magnesium ions should be present. FBS (Fetal Bovine Serum) concentration should be ≤2%. Higher FBS concentrations can

cause the SeekOne™ DD to clog. BSA (Bovine Serum Albumin) concentration should be $\leq 0.1\%$. Higher BSA concentration can lead to incomplete demulsification, affecting cell capture efficiency and gene count.

- ❑ Cell diameter: 5-40 μm .

1.7.2 Sample Quality

❑ **Cell count:** It is recommended to obtain a minimum of 50,000 viable cells. The minimum number of cells to be put into the assay must not be lower than 1,000 cells, resulting in a recovery of ~ 500 cells.

❑ **Cell viability:** For optimal analysis results, it is recommended to ensure cell viability $> 90\%$ (counting by cell counter). In cases of low cell viability, improve viability by removing dead cells. For single-nucleus experiments, cell viability should be below 5% as determined by staining with AO/PI. Additionally, the nuclei should exhibit intact and undamaged nuclear membranes when stained with Trypan Blue and observed under 40x magnification (confirmed by cell counter).

❑ Further requirements: **Cell aggregation rate** $< 10\%$, **Nucleated cell rate** $> 70\%$. For single-nucleus experiments, the percentage of nuclear impurities should be below 30% and the impurity diameter should not exceed 40 μm .

1.7.3 Sample Storage

For fresh single-cell suspensions, it is optimal to perform water-in-oil generation and barcode labeling (step 1) within 20 minutes of placing the sample on ice. If the operation time exceeds this limit, it is recommended to centrifuge and resuspend the cells in 4 ml of 1640 medium supplemented with 2% FBS.

***Note:** Before starting the experiment, it is essential to use a cell counter to count the cells and calculate the live cell rate of the single cell suspension.*

1.7.4 Sample Loading Volume Recommendations

Prior to loading into the SeekOne™ DD, resuspend cells in RPMI 1640 culture medium. The recommended concentration range for **live cells** is 700~1,200 cells/ μL . Please use the concentration of **live cells** only, not the total cell concentration.

1.8 Product Components and Storage Conditions

The SeekOne™ DD Single Cell Full-length RNA Sequence Transcriptome-seq Kit (**abbreviated as SeekOne™ DD scFAST-seq Kit**) is categorized based on reagent functions and storage conditions. It includes:

- SeekOne™ DD Chip S3 Kit, K00202-0201 (2 tests) / K00202-0801 (8 tests)

- b. SeekOne™ DD scFAST-seq Barcoded Beads Kit, K00801-0202 (2 tests) / K00801-0802 (8 tests)
- c. SeekOne™ DD scFAST-seq Reverse Transcription Kit, K00801-0203 (2 tests) / K00801-0803 (8 tests)
- d. SeekOne™ DD scFAST-seq cDNA Amplification Kit, K00801-0206 (2 tests) / K00801-0806 (8 tests)
- e. SeekOne™ DD Library Construction Kit, K00202-0204 (2 tests) / K00202-0804(8 tests)
- f. SeekOne™ DD Single Cell Cleanup Kit, K00202-0205 (2 tests) / K00202-0805(8 tests)

1.8.1 SeekOne™ DD scFAST-seq Kit, 2 tests, REF: K00801-02

Name & PN & Storage	Tube lid color	Component	CN	2 tests
SeekOne™ DD Chip S3 Kit V1.0, K00202-0201, store at Room temperature	-	SeekOne™ DD Chip S3	R0003001	2 pieces
	-	Gasket	R0003101	2 pieces
	●	Carrier Oil	R0003201	0.6 mL
	●	Demulsion Agent	R0003301	0.5 mL
SeekOne™ DD scFAST-seq Barcoded Beads Kit V1.2 K00801-0202, store at -80℃	○	Single Cell Whole Transcriptome Barcoded Beads	R0008601	45 µL ×2 tubes
	○	TSO	R0003601	10 µL
SeekOne™ DD scFAST-seq Reverse Transcription Kit V1.2 K00801-0203, store at -20 ± 5℃	●	3×RT Buffer	R0008401	80 µL
	●	RT Enzyme	R0003801	15 µL
	●	Reducing Buffer	R0003901	100 µL
SeekOne™ DD scFAST-seq cDNA Amplification Kit V1.2 K00801-0206, store at -20 ± 5℃	●	2×PCR Master Mix	R0002101	60 µL ×2 tubes
	●	Blockers-HM	R0008501	10 µL
	●	cDNA Primers	R0004001	10 µL
SeekOne™ DD Library Construction Kit V1.0 K00202-0204, store at -20 ± 5℃	●	Fragmentation Buffer	R0004101	15 µL
	●	Fragmentation Enzyme	R0004201	24 µL
	●	Ligation Buffer	R0004301	60 µL
	●	DNA Ligase	R0004401	15 µL
	●	Adaptor	R0004501	15 µL
	●	2×PCR Master Mix	R0002101	60 µL
	●	N501	R0004601	25 µL
	●	N502	R0004701	25 µL
	●	N701	R0005001	25 µL
SeekOne™ DD Single Cell Cleanup Kit V1.0 K00202-0205, store at 2-8℃	○	N702	R0005101	25 µL
	○	Cleanup Beads	R0003401	0.5 mL

1.8.1 SeekOne™ DD scFAST-seq Kit, 8 tests, REF: K00801-08

Name & PN & Storage	Tube lid color	Component	CN	8 tests
SeekOne™ DD Chip S3 Kit V1.0, K00202-0801, store at Room temperature	-	SeekOne™ DD Chip S3	R0003001	8 pieces
	-	Gasket	R0003101	8 pieces
	●	Carrier Oil	R0003202	1.2 mL×2 tubes
	●	Demulsion Agent	R0003302	1.8 mL
SeekOne™ DD scFAST-seq Barcoded Beads Kit V1.2 K00801-0802, store at -80℃	○	Single Cell Whole Transcriptome Barcoded Beads	R0008601	45 µL ×8 tubes
	○	TSO	R0003602	20 µL
SeekOne™ DD scFAST-seq Reverse Transcription Kit V1.2 K00801-0803, store at -20 ± 5℃	●	3×RT Buffer	R0008402	280 µL
	●	RT Enzyme	R0003802	50 µL
	●	Reducing Buffer	R0003901	100 µL
SeekOne™ DD scFAST-seq cDNA Amplification Kit V1.2 K00801-0806, store at -20 ± 5℃	●	2×PCR Master Mix	R0002102	240 µL×2 tubes
	●	Blockers-HM	R0008502	30 µL
	●	cDNA Primers	R0004003	40 µL
SeekOne™ DD Library Construction Kit V1.0 K00202-0804, store at -20 ± 5℃	●	Fragmentation Buffer	R0004102	50 µL
	●	Fragmentation Enzyme	R0004202	100 µL
	●	Ligation Buffer	R0004302	240 µL
	●	DNA Ligase	R0004402	50 µL
	●	Adaptor	R0004502	50 µL
	●	2×PCR Master Mix	R0002102	240 µL
	●	N501	R0004601	25 µL
	●	N502	R0004701	25 µL
	●	N503	R0004801	25 µL
	●	N504	R0004901	25 µL
	●	N701	R0005001	25 µL
	●	N702	R0005101	25 µL
SeekOne™ DD Single Cell Cleanup Kit V1.0, K00202-0805, store at 2-8℃	○	Cleanup Beads	R0003402	1.75 mL

1.8.3 Storage Condition

Name	PN	Transportation	Storage
SeekOne™ DD Chip S3 Kit	K00202-0201/ K00202-0801	Room temperature	Room temperature
SeekOne™ DD Single Cell Cleanup Kit	K00202-0205/ K00202-0805	Room temperature	2-8℃
SeekOne™ DD scFAST-seq Barcoded Beads Kit	K00801-0202/ K00801-0802	Dry ice	-80℃
SeekOne™ DD scFAST-seq Reverse Transcription Kit	K00801-0203/ K00801-0803	Dry ice	-20 ± 5℃
SeekOne™ DD scFAST-seq cDNA Amplification Kit	K00801-0206/ K00801-0806	Dry ice	-20 ± 5℃
SeekOne™ DD Library Construction Kit	K00202-0204/ K00202-0804	Dry ice	-20 ± 5℃

1.8.4 Index Sequence

Index No.	Forward sequence	Reverse complementary sequence
● N501	ACTAGAGC	GCTCTAGT
● N502	TGCCTATA	TATAGGCA
● N503	GCAGCTGT	ACAGCTGC
● N504	ACGTTAAG	CTTAACGT
● N701	TCAAGTAT	
● N702	CACTTCGA	
● N703	GCCAAGAC	
● N704	AAACATCG	

Note 1: Forward sequence for the index refers to the direction consistent with the sequence provided by Illumina. If sequencing is performed on the HiSeq XTen platform, the reverse complementary sequence for N5 index should be provided.

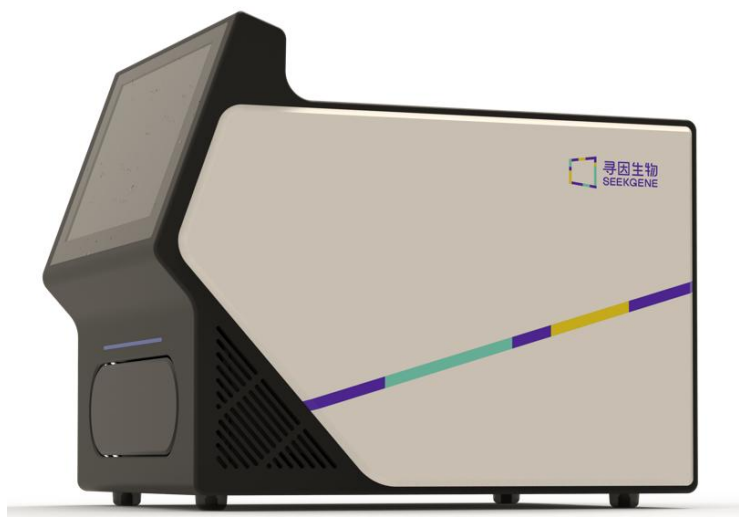
Note 2: The Index sequence provided by this kit can label up to 16 samples simultaneously.

Note 3: The adapter sequence of the library is as follows:

N5	5'AATGATACGGCGACCACCGAGATCTACAC[N5]ACACTCTTTCCCTACACGACGCTCTTCCGATCT 3'
N7	5'GATCGGAAGAGCACACGTCTGAACTCCAGTCAC[N7]ATCTCGTATGCCGTCTTCTGCTTG 3'

1.8.5 Compatible Instruments and Consumables

1. SeekOne™ Digital Droplet System(SeekOne™ DD, REF: M001A)



2. SeekOne™ DD Accessories: Each instrument is equipped with one set of this accessory, which includes the following two parts:

a) **SeekOne™ DD Chip Holder, Abbreviated as Chip Holder:** used in conjunction with SeekOne™ Digital Droplet System and Chip S3.

b) **Placed Chip, Abbreviated as Chip P:** Placed in the chip fixture (8 Chip Ps are included with each instrument). When the sample size is less than 8, the Chip P is used and placed at the position where no sample is added. It serves as a replacement for Chip S3.



1.9 Additional Equipment & Kits, Reagents

1.9.1 Additional Equipment and Consumables

Name	Models	Manufacturer and Item No.
Cell counter equipment	CountStar Rigel S2, SeekMate Tinitan FL	Countstar, IN030101, SeekGene, M002C
24-well magnetic separator	24 x 200 µL	Mich Scientific, Magpow-24
Pipettes	0.1-2.5 µL, 0.5-10 µL, 2-20 µL, 20-200 µL, 100-1,000 µL	Eppendorf, RAININ
Thermal cyclers capable of uniformly heating 100 µl emulsion volumes	C1000 Touch™ Thermal Cycler with 96-Deep Well Fast Reaction Module	BioRad, 1851197
	MasterCycler® Pro	Eppendorf, North America 950030010 International 6321 000.019
	Veriti 96-Well Thermal Cycler	Thermo Fisher, 4375786
	LongGene, A300	LongGene, A300
DNA/RNA quality control equipment	Agilent 4200 TapeStation	Agilent, G2991AA
	Agilent 2100 Bioanalyzer	Agilent, G2939BA
	Bioptric, Qsep400	Bioptric, Qsep400
0.2 mL PCR tubes with flat caps	0.2 mL	Axygen, PCR-02-L-C
8-strip PCR tubes	0.2 mL	Axygen, PCR-0208-FCP-C
Qubit 4.0	Qubit 4.0 Fluorometer	Thermo Fisher Scientific, Q33238
Mini centrifuge	-	TIANGEN, OSE-MP25
Vortex mixer	IKA Shakers MS3 (MS3.4/MS3.5)	IKA, -
DNase/RNase-free Low- retention microcentrifuge tubes	1.5 mL	Axygen, MCT-150-L-C
Low retention pipette tips	0.5 - 10 µL/ 1 - 200 µL/	Axygen, T-300-L-R-S,
	100 - 1000 µL	Axygen, T-200-C-L-R-S, Axygen, T-1000-C-L-R-S

1.9.2 Additional Kits, Reagents

Name	Manufacturer and Item No.
RPMI 1640 Medium	Gibco, 11875093
HyClone phosphate buffered saline (PBS), 1X, 0.0067M PO ₄ , without calcium, magnesium	HyClone, SH30256.LS
Ethyl alcohol, Pure	Millipore Sigma, E7023-500ML
Nuclease-free Water	Thermo Fisher Scientific, AM9937
SPRIselect / AMPure XP(for Size Selection)	Beckman Coulter, B23318 or A63882
VAHTS DNA Clean Beads	Vazyme, N411
Qubit dsDNA Quantification Assay Kits	Thermo Fisher Scientific, Q32854
High Sensitivity D1000 ScreenTape/Reagents	Agilent, 5067-5592/ 5067-5593
High Sensitivity D5000 ScreenTape/Reagents	Agilent, 5067-5584/ 5067-5585,
S2-Standard Cartridge Kit,	Bioptric, C105201/C105801/C405101
S1-High Resolution Cartridge Kit	Bioptric, C105202/C105802/C405102
*Choose the kits based on the available equipment	

2. Experimental Operation Steps

Protocol Steps and Time

Steps		Timing
Cell Preparation		
~1-1.5 h	Dependent on cell type	~1-1.5 h
Step 1 Water-in-Oil Generation and Barcode Labeling		
~2 h	1-1 Prepare the Single-cell Mixture	10 min
	1-2 Add Reagent to Chip S3	5 min
	1-3 Run SeekOne™ DD	5 min
	1-4 Transfer the Resulting Water-in-oil	3 min
	1-5 Water-in-oil Reverse Transcription Reaction	95 min
○ STOPPING POINT: 4 °C ≤ 72 h or -20 °C ≤ 1 week		
Step 2 Sample Recovery and cDNA Amplification		
~3.5 h	2-1 Water-in-Oil Demulsification	45 min
	2-2 cDNA Amplification	40 min
	○ STOPPING POINT: 4 °C ≤ 72 h or -20 °C ≤ 1 week	
	2-3 cDNA Enrichment Products Purification	30 min
	○ STOPPING POINT: 4 °C ≤ 72 h or -20 °C ≤ 1 week	
	2-4 Secondary Enrichment of cDNA	35-40 min
	○ STOPPING POINT: 4 °C ≤ 72 h or -20 °C ≤ 1 month	
	2-5 Purification of cDNA Secondary Enrichment Products	30 min
	○ STOPPING POINT: 4 °C ≤ 72 h or -20 °C ≤ 1 month	
	2-6 Quality Control of cDNA Enrichment Products	30 min
Step 3 Library Construction		
~3 h	3-1 DNA Fragmentation and End Repair	40 min
	3-2 Adaptor Ligation	20 min
	3-3 Ligation Product Purification	20 min
	3-4 Library Amplification	40 min
	3-5 Fragment Purification	30 min
	○ STOPPING POINT: -20 °C ≤ 6 months	
	3-7 Library Quality Control	30 min

Step 1 Water-in-oil Generation and Barcode Labeling

Step 1-0 Preparation Before Experiment

- ❑ **Prepare ice:** Have an ice box ready in advance.
- ❑ **SeekOne™ DD Single Cell scFAST-seq Reverse Transcription Kit:**
 - Take out the **3x RT Buffer** and **Reducing Buffer** from -20°C in advance for thawing, after thawing vortex them thoroughly, centrifuge briefly, and place them on ice until use.
 - **RT Enzyme: Keep at -20°C until use.** Briefly centrifuge before pipetting, then return to -20°C immediately.
- ❑ **SeekOne™ DD Single Cell scFAST-seq Barcoded Beads Kit:**
 - Remove **Barcoded Beads** from the -80°C freezer in advance and equilibrate at room temperature for 30 minutes. Put back to -80°C freezer immediately after use. It should not stay at room temperature for more than 2 hours.
- ❑ Ensure that the **SeekOne™ Digital Droplet System** is placed horizontally, operating at room temperature, and free from vibration or collision.
- ❑ **Turn on** the SeekOne™ Digital Droplet System, place the Placed Chip (Chip P), and run the self-check program. Wait for the self-check to succeed before proceeding with the experiment.
- ❑ Pre-program the thermal cycler (with deep-well) to ramp at 1.5°C/s.

Note: If the experiment is canceled before proceeding to the next step, let the Barcoded Beads thaw completely for 30 minutes at room temperature before returning them to the -80 °C freezer. Avoid rapid freeze-thaw cycles, which can affect the density and viscosity of the beads.

Step 1-1 Prepare the Single-cell Mixture

1. **Prepare the Master Mix** on ice according to the table below. Mix by pipetting up and down 15 times, and then centrifuge briefly. (Make sure to prepare the Master Mix according to the table below before use). Keep on ice until use.

Component	Volume/sample
● 3 x RT Buffer	26.6µL
● RT Enzyme	5.2 µL
○ TSO	2.0 µL
● Reducing Buffer	1.6 µL
Total	35.4 µL

Note 1: 3×RT Buffer should be pink in color. Discard it if the color changes or if there is precipitation.

Note 2: RT Enzyme is highly viscous. Pipette slowly and avoid inserting the tip too deep to prevent loss of reagent.

2. Prepare the Single-cell Mix (final volume: 80 µL). It contains:

- a. 35.4 µL Master Mix (prepared above).
- b. Nuclease-free water.
- c. Cell suspension.

Note 1: Maintain the order of pipetting. Do not directly add nuclease-free water to the cell suspension.

Note 2: If working with multiple samples, add nuclease-free water to all samples before adding and mixing the single-cell suspension. This helps reduce the exposure time of cells in the mix, preventing prolonged exposure that may lead to reduced cell viability.

i. Calculation process:

a. Master Mix is always 35.4 µL. The remaining volume (44.6 µL) is made up of nuclease-free water and cells.

b. Subtract the calculated volume of cells from 44.6 µL to obtain the volume of nuclease-free water.

c. Determine the target number of cells (ranging from 500 to 12,000 cells). The final volume of required cell suspension can be found in the table below or calculated using the formula: Volume of Cell Suspension = (Targeted cell recovery number × 2) / Cell concentration. Consider the recommended cell concentration range of 700-1,200 cells/µL.

For example, if targeting 5,000 cells with a concentration of 900 cells/µL, the calculation would be: $(5,000 \times 2) / 900 = 11.1$ µL of cell suspension.

ii. Pipetting process: The final volume to be put onto the Chip S3 is 80 µL.

a. Pipette 35.4 µL of the Master Mix into a small tube(e.g. 500µL) on ice.

b. Subtract the calculated volume of cells from 44.6 µL to determine the volume of nuclease-free water to add. Mix the solution thoroughly using a pipette.

c. Pipette the calculated volume of cells into the mixture. Gently pipetting up and down to mix the single-cell suspension before adding to the mixture.

d. Finally, a total volume of 80 µL of the single-cell mix is ready.

In the example mentioned above, 11.1 µL of cells is required.

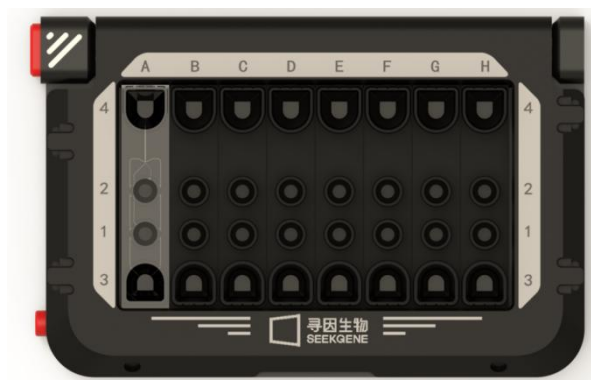
So first pipette 35.4 μL of the Master Mix. Then pipette 33.5 μL nuclease-free water (44.6 μL -11.1 μL =33.5 μL). Finally, pipette 11.1 μL of cells into the mixture.

Cell Stock Concentration (Cells/ μL)	Targeted Cell Recovery												
	500	1,000	2,000	3,000	4,000	5,000	6,000	7,000	8,000	9,000	10,000	11,000	12,000
300	3.3	6.7	13.3	20.0	26.7	n	n	n	n	n	n	n	n
400	2.5	5.0	10.0	15.0	20.0	25.0	30.0	n	n	n	n	n	n
500	2.0	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0	n	n	n	n
600	1.7	3.3	6.7	10.0	13.3	16.7	20.0	23.3	26.7	30.0	n	n	n
700	1.4	2.9	5.7	8.6	11.4	14.3	17.1	20.0	22.9	25.7	28.6	31.4	n
800	1.3	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	27.5	30.0
900	1.1	2.2	4.4	6.7	8.9	11.1	13.3	15.6	17.8	20.0	22.2	24.4	26.7
1,000	1.0	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	22.0	24.0
1,100	0.9	1.8	3.6	5.5	7.3	9.1	10.9	12.7	14.5	16.4	18.2	20.0	21.8
1,200	0.8	1.7	3.3	5.0	6.7	8.3	10.0	11.7	13.3	15.0	16.7	18.3	20.0
1,300	0.8	1.5	3.1	4.6	6.2	7.7	9.2	10.8	12.3	13.8	15.4	16.9	18.5
1,400	0.7	1.4	2.9	4.3	5.7	7.1	8.6	10.0	11.4	12.9	14.3	15.7	17.1
1,500	0.7	1.3	2.7	4.0	5.3	6.7	8.0	9.3	10.7	12.0	13.3	14.7	16.0
1,600	0.6	1.3	2.5	3.8	5.0	6.3	7.5	8.8	10.0	11.3	12.5	13.8	15.0
1,700	0.6	1.2	2.4	3.5	4.7	5.9	7.1	8.2	9.4	10.6	11.8	12.9	14.1
1,800	0.6	1.1	2.2	3.3	4.4	5.6	6.7	7.8	8.9	10.0	11.1	12.2	13.3
1,900	0.5	1.1	2.1	3.2	4.2	5.3	6.3	7.4	8.4	9.5	10.5	11.6	12.6
2,000	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0

Note : The blue background indicates the optimal single-cell suspension stock concentration range.

Step 1-2 Add Reagent to Chip S3

1. Prepare Chip S3: Take the required number of Chip S3 from the package (e.g. 4 chips for 4 samples). Place them into the slots of the Chip Holder by pressing the red button on the left to load each Chip S3. For unused positions, insert Chip P to ensure all 8 positions are filled. Then close the cover of the Chip Holder (as shown in the figure below).



Note 1: If you have fewer than 8 samples, fill empty slots with Chip P (no reagents are added to Chip P).

Note 2: Once opened, use Chip S3 within 24 hours to avoid contamination.

2. **Load Single-cell Mix:** Mix the single-cell mixture from above (total of 80 μ L) by gently pipetting up and down 15 times. Pipette 78 μ L of the mixture and carefully insert it into the **Well 1**, keeping the tip slightly above the bottom. Pipette slowly and avoid bubbles, and let the mix stand for 30 seconds.

3. **Add Barcoded Beads:** Thoroughly vortex the equilibrated Barcoded Beads for 30 seconds and centrifuge briefly for 5 seconds to ensure there are no air bubbles in the Barcoded Beads liquid. Pipette 38 μ L of the beads into **Well 2**, keeping the tip slightly above the bottom, and pipette slowly to avoid bubbles.

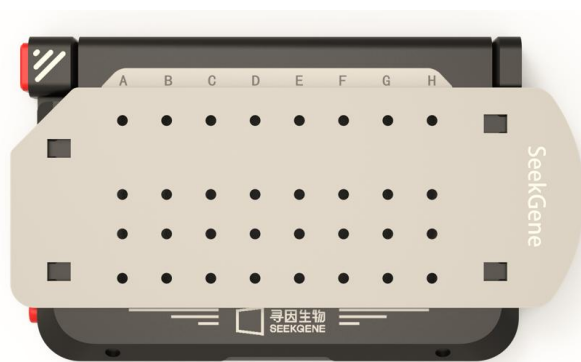
Note 1: When adding reagents, keep the pipette tip moving with the liquid level and always ensure that only the tip of the pipette is below the liquid surface by 3 mm to avoid generating bubbles.

Note 2: The Barcoded Beads are viscous. After pipetting the designated volume, leave the pipette tip in the reagent tube for 5 seconds before removing it for sample addition.

4. **Add Carrier Oil:** Pipette 120 μ L of Carrier Oil with a 200 μ L pipette into the **Well 3**, lean the tip against the inner wall, and pipette slowly without generating air bubbles. Repeat this step for a total of 240 μ L of Carrier Oil in Well 3.

Note: Adding Carrier Oil improperly may fail water-in-oil droplet generation or damage the instrument.

5. Attach the Gasket over the Chip Holder as shown in the illustration below, ensuring that the Gasket holes and the chip wells are aligned. The cut off corner should be on the left upper side.



Note: Do not touch the smooth surface of Gasket.

Step 1-3 Run SeekOne™ DD (Do not shake/move the device while it is running)

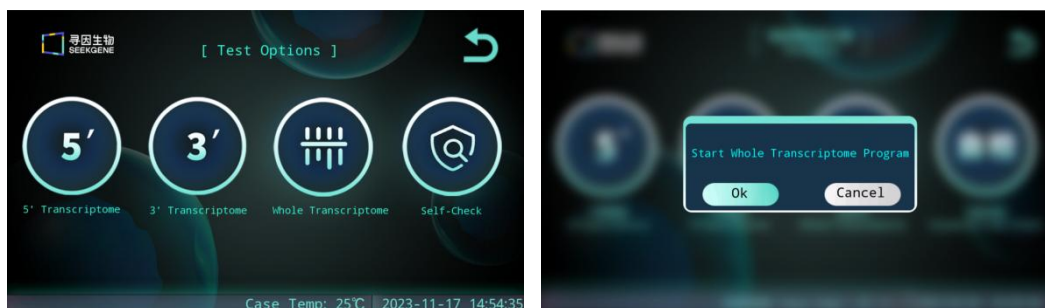
1. Click the "Open Chip Compartment" button on the SeekOne™ DD to eject the tray.



2. Put the Chip Holder with the covering Gasket into the tray according to the illustration, make sure the Chip Holder is placed horizontally, and click the "Close Chip Compartment" button to retract the fixture tray.



3. Click the "Whole Transcriptome" program and the "OK" button on the instrument screen to start the program.

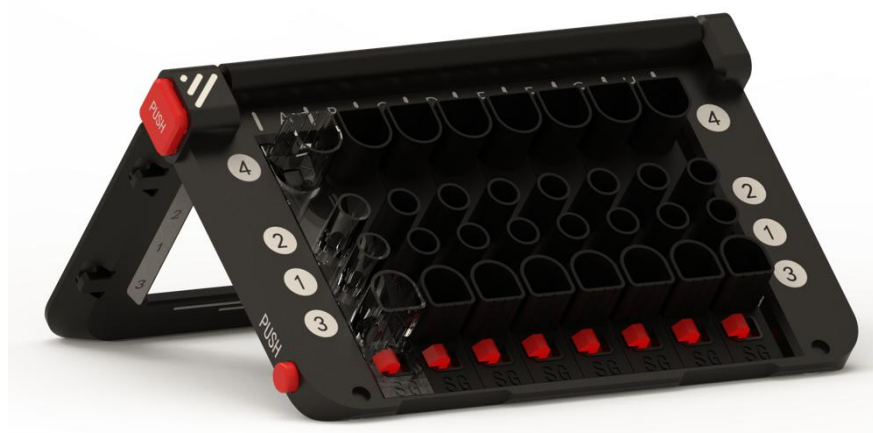


4. After the program is finished, click the "Run Completed" button and remove the Chip Holder. Immediately proceed to the next step.



Step 1-4 Transfer the Resulting Water-in-oil

1. **Prepare PCR Tube:** Place a new 0.2 mL PCR tube on ice.
2. **Open Chip Holder:** Discard the gasket, press and hold the square PUSH button, and fully open the Chip Holder, until the cover is at a 45° angle (as shown in the picture):



3. **Check Wells 1 and 2:** Inspect **Wells 1 (aqueous phase)** and **2 (adhesive bead phase)**. Any unusually high volume indicates a clog.

Note: Well 1 should have $\leq 10 \mu\text{L}$, and Well 2 should have $\leq 15 \mu\text{L}$. Larger volumes indicate a blocked chip.

4. **Aspirate Water-in-Oil:** Use a pipette to **slowly** aspirate at least 120 μL of the water-in-oil emulsion from the **Well 4**.

Note 1: Keep the pipette tip suspended in the liquid, avoiding the bottom of the well. If any clear excess Carrier Oil remains, use a small pipette to remove it without aspirating the pink water-in-oil.

Note 2: Bubbles may appear in Well 4 when running multiple chips simultaneously, but this does not affect the library preparation

5. **Inspect Pipette Tip:** The liquid (upper phase) should appear uniformly opaque and turbid. Excess clear Carrier Oil in the pipette tips indicates potential clogging.



Note: If the solution is like in the second tip from the left in the figure, it indicates a clog.

6. **Transfer to PCR Tube:** Slowly (~20 seconds) pipette the water-in-oil into the 0.2 mL PCR tube placed on ice, by pipetting it along the tube wall. . For each sample, use 2 PCR tubes if needed, ensuring you transfer at least 120 μL total. Do not exceed 100 μL per tube to remain within the volume limits set in the PCR program, which ensures uniform heating of the liquid within the thermal cycler's heated block.

Note : If the volume limit set in the PCR program allows for more than 120 μL , you may transfer the entire sample into a single tube and proceed with the PCR program without splitting into two tubes.

STOPPING POINT: Water-in-oil emulsion can be stored on ice for up to 1 hour once capped.

Step 1-5 Water-in-oil Reverse Transcription Reaction

1. **Prepare for Reverse Transcription:** Use a thermal cycler that can accommodate at least 100 μL volume. Run the following procedure by placing the PCR tube containing the water-in-oil emulsion from the previous step into a thermal cycler. If the water-in-oil emulsion is split into two tubes for reverse transcription, combine the contents of both tubes after the PCR program is complete to achieve approximately 120 μL for each sample. Briefly centrifuge to ensure proper mixing.

Lid Temperature		Reaction Volume	Run Time
85°C		100 µL	~83 min
Total Cycles	Temperature	Time	Ramp Rate
15 cycle	8°C	12 s	
	15°C	45 s	1.5°C/s
	20°C	45 s	1.5°C/s
	30°C	30 s	1.5°C/s
	42°C	3 min	1.5°C/s
85°C		5 min	
4°C		Hold	

STOPPING POINT: The products generated in step 1-5 can be stored at 4 °C for up to 72 hours or at -20 °C for up to 1 week.

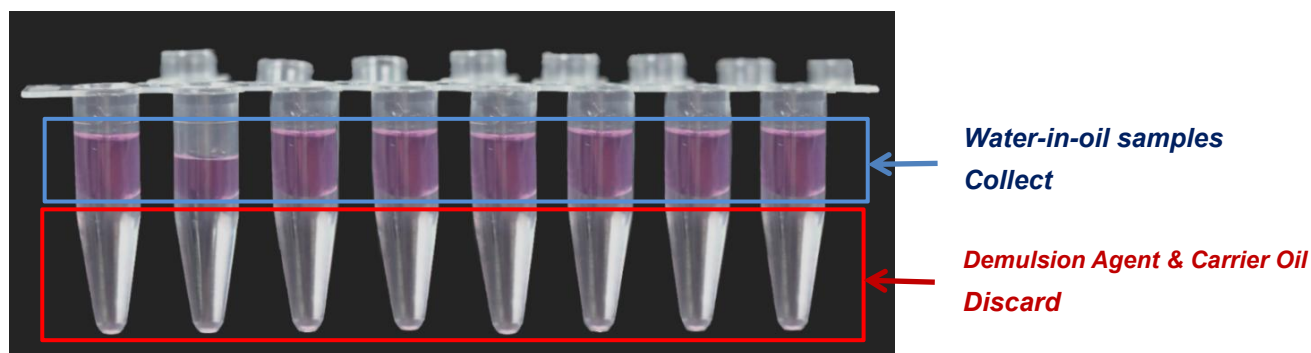
Step 2 Sample Recovery and cDNA Amplification

Step 2-0 Prepare Before the Experiment

- ❑ **Prepare Ice:** Prepare a box with ice in advance.
- ❑ **Thaw Reagents:** Remove **2× PCR Master Mix**, **cDNA Primers**, **Blockers-HM**, and **Reducing Buffer** from -20°C, put on ice in advance to thaw. Once thawed, vortex thoroughly, centrifuge briefly and place on ice until use.
- ❑ **Make 80% Ethanol:** Prepare 80% ethanol by using absolute ethanol (e.g., 99.8%) and nuclease-free water. Prepare 1.5 mL of 80% ethanol per sample. Make this fresh right before use and use it within 24 hours.
- ❑ **Warm Cleanup Beads:** Equilibrate the Cleanup Beads (from the Kit) and the DNA selection beads (you supply) to room temperature in advance (at least 30 minutes).

Step 2-1 Water-in-oil Demulsification

1. **Add Demulsion Agent:** Add 100 µL of Demulsion Agent to each tube of water-in-oil liquid at room temperature and let the tubes stand for 2 minutes at room temperature.



Note 1: The resulting mixture (shown above) includes Demulsion Agent/Carrier Oil (clear) and aqueous phase reaction solution (pink).

Note 2: A smaller aqueous phase volume indicates a clog during water-in-oil generation (as shown in the second left tube of the figure above).

2. **Prepare the Cleanup Mix** according to the table below (vortex the Cleanup Beads for 2 minutes and mix well before use):

Component	Volume/Sample
○ Cleanup Beads	175.5 μ L
● Reducing Buffer	4.5 μ L
Total	180 μL

3. **Remove Demulsion Mixture:** Carefully **remove and discard** 130 μ L of the Demulsion Agent/Carrier Oil mixture by slowly pipetting from the bottom of the PCR tube, leaving 2-5 μ L clear mixture at the bottom of the tube to avoid removing any of the pink aqueous phase.

Note: If a cloudy pink aqueous phase remains, repeat steps 1 and 3.

4. **Add Cleanup Mix:** Add 180 μ L of vortex-mixed Cleanup Mix to each sample tube, Gently pipette up and down at least 15 times without creating bubbles. Incubate at room temperature for a total of 10 minutes with the lid open. After 5 minutes, gently pipette up and down 10-15 times and continue incubating for another 5 minutes.

Note: Adjust pipette movement with the liquid level to avoid spills.

5. **Beads Adsorption:** Place the tubes on a magnetic rack for 1-2 minutes until the beads adhere and the solution is clear. **Remove and discard the supernatant.**

Note: During this step, gently pipette up and down 5 times to mix and enhance magnetic bead adhesion, tilt the tip away from the beads.

6. **Ethanol Wash:** Add 300 μL of 80% ethanol to the tubes while still on the magnetic rack. Leave for about 30 seconds then **carefully remove the supernatant and discard it. Repeat this step once.**

Note: The tube should be kept open until this step to prevent the cap from sticking to the liquid inside the tube (the tube is too full to close), which may affect subsequent experiments.

7. **Collect Beads:** Briefly centrifuge, return the tubes to the magnetic rack, and **remove any remaining liquid with a 10 μL pipette.** During centrifugation, position the beads side of the tube outward, away from the rotor's central axis.

8. **Dry Beads and Resuspend Beads:** Keep the tubes open and allow the ethanol to evaporate by leaving the mixture at room temperature for about 1 minute until the beads appear dull and matte. Add 23 μL of nuclease-free water to fully suspend the beads, vortex for ~20 seconds to make sure the beads do not stick, and leave them at room temperature for another 2 minutes on a normal rack (not on the magnetic stand).

Note 1: Beads typically dry to a dull and matte appearance within 1-1.5 minutes. The time depends on the environment. Adjust drying time based on room temperature to prevent over-drying and clumping.

Note 2: For complete bead suspension, vortex for 10-15 seconds, followed by a brief centrifuge, and pipette up and down 15 times.

9. **Collect Supernatant:** Place the tubes onto the magnetic rack until the beads are stuck to the magnet and the solution appears clear, **transfer the 23 μL of supernatant to a new 0.2 mL PCR tube.** This now contains nucleic acid, put the tubes on ice and pipette the next steps on ice. The tube with the beads can be discarded.

Step 2-2 cDNA Amplification

1. Preparation of cDNA amplification mix:

Component	Volume/Sample
● 2×PCR Master Mix	25 μL
● Blockers-HM	1.5 μL
● cDNA Primers	2 μL
Total	28.5 μL

2. **Mix and Start PCR:** Add the prepared 28.5 μL cDNA amplification mix to the 22 μL of purified cDNA sample from Step 2-1. Pipette up and down 10 times to mix. Briefly centrifuge the mixture, then proceed to run the PCR program. Set up the PCR program as follows:

Lid Temperature	Reaction Volume	Run Time
105°C	50 µL	~32 min
Total Cycles	Temperature	Time
7	98°C	3 min
	98°C	10 sec
	63°C	15 sec
	72°C	3 min
	72°C	5 min
	4°C	Hold

Centrifuge the samples after the PCR run is completed.

STOPPING POINT: The products generated in step 2-2 can be stored at 4 °C for up to 72 h or -20 °C for up to 1 week.

Step 2-3 cDNA Enrichment Product Purification

1. Add DNA Selection Beads: Vortex to resuspend the DNA selection beads. Pipette 30 µL (0.6×) of DNA selection beads into each sample, mix by pipetting 10 times or vortex to mix well, and centrifuge briefly.

2. Incubate and Remove Supernatant: After incubating at room temperature for 5 minutes, briefly centrifuge and place the tubes onto the magnetic rack. The beads will stick to the magnet and the solution will appear clear after 1-2 minutes. **Then the supernatant should be removed and discarded.**

Note: During adsorption, gently pipette up and down 5 times with the pipette tip positioned away from the beads to help the beads settle.

3. Ethanol Wash: Keep the sample on the magnetic rack and add 200 µL of 80% ethanol. After 30 seconds, **carefully remove the ethanol supernatant and discard it. Repeat this step once.**

4. Remove Ethanol: Close the lids and briefly centrifuge them. Put the tubes back onto the magnetic stand, and **remove any residual supernatant with a 10 µL pipette.** During centrifugation, ensure that the beads side of the tubes is facing away from the centrifuge's center.

5. Dry and Resuspend Beads: Keep the tubes open and allow the ethanol to evaporate for about 3-5 minutes. Monitor the beads closely—they should turn dull and matte but not dry out completely (avoid cracks on the bead surface). Adjust drying time based on your beads status and environment. Add 23 µL of nuclease-free water to fully suspend the beads, vortex for ~20 seconds. Incubate at room temperature for 2 minutes on a normal rack (not on the magnetic stand) and then briefly centrifuge.

6. Transfer Supernatant: Place on the magnetic rack for 1-2 minutes until the solution appears clear. **Transfer 22 μ L of the supernatant (containing the purified cDNA) to a new tube.**

Note 1: 0.6X refers to the volume ratio of added DNA selection beads to PCR products, which is $30 \mu\text{L} / 50 \mu\text{L} = 0.6\text{X}$

Note 2: Gently pipette up and down 5 times during adsorption to enhance bead adhesion.

Step 2-4 Secondary Enrichment of cDNA

1. Preparation of cDNA amplification mix:

Component	Volume/sample
● 2×PCR Master Mix	25 μ L
● Blockers-HM	1.5 μ L
● cDNA Primers	2 μ L
Total	28.5 μL

2. Mix and Start PCR: Add 28.5 μ L of the prepared cDNA amplification mix to the 22 μ L cDNA sample purified from Step 2-3. Pipette up and down 10 times to mix thoroughly, briefly centrifuge, and then proceed with PCR. Set up the PCR program as follows:

Lid Temperature	Reaction Volume	Run Time
105°C	50 μ L	~29-32 min
Total Cycles	Temperature	Time
6-7	98°C	3 min
	98°C	10 sec
	63°C	15 sec
	72°C	3 min
	72°C	5 min
	4°C	Hold
Number of loaded cells	Recommended cycles	
500-12,000	7	
12000-24,000	6	

STOPPING POINT: The products generated in step 2-4 can be stored at -20 °C for no more than 1 month or 4 °C for no more than 72 h.

Step 2-5 Purification of cDNA Secondary Enrichment Products

1. **Add DNA Selection Beads:** Vortex to resuspend the DNA selection beads. Pipette 30 μL (0.6 \times) of DNA selection beads into each sample, mix by pipetting 10 times or vortex to mix well, and centrifuge briefly.

Note: 0.6X refers to the volume ratio of added DNA selection beads to PCR products, i.e., 30 μL / 50 μL =0.6X

2. **Incubate and Remove Supernatant:** After incubating at room temperature for 5 minutes, briefly centrifuge and place the tubes onto the magnetic rack. After 1-2 minutes (when the beads stick and the solution is clear), carefully **remove and discard the supernatant**.

Note: During adsorption, gently pipette up and down 5 times with the pipette tip positioned away from the beads to help the beads settle.

3. **Ethanol Wash:** Keep the sample on the magnetic rack and add 200 μL of 80% ethanol. After 30 seconds, **carefully remove the ethanol supernatant and discard it. Repeat this step once.**

4. **Remove Ethanol:** Close the lids and briefly centrifuge them. Put the tubes back onto the magnetic stand, and **remove any residual supernatant with a 10 μL pipette**. During centrifugation, ensure that the beads side of the tubes is facing away from the centrifuge's center.

5. **Dry and Resuspend Beads:** Keep the tubes open and allow the ethanol to evaporate by leaving the mixture at room temperature for about 3-5 minutes. Monitor the beads closely—they should turn dull and matte but not dry out completely (avoid cracks on the bead surface). Adjust drying time based on your beads status and environment. Add 41 μL of nuclease-free water to fully suspend the beads, vortex for ~20 seconds to make sure the beads do not stick and leave them at room temperature for another 2 minutes on a normal rack (not on the magnetic stand).

6. **Transfer Supernatant:** Place the sample on the magnetic rack for 1-2 minutes until the solution becomes clear. **Transfer 40 μL of the supernatant (containing the purified cDNA) to a new tube.**

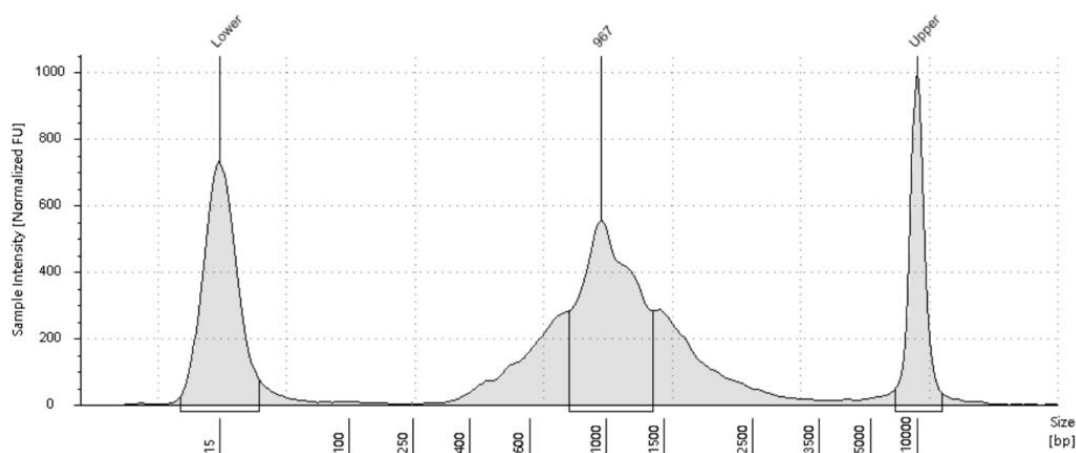
Note 1: 0.6X refers to the volume ratio of added DNA selection beads to PCR products, which is 30 μL / 50 μL =0.6X.

Note 2: Gently pipette up and down 5 times during adsorption to enhance bead adhesion.

STOPPING POINT: The products generated in step 2-5 can be stored at -20 $^{\circ}\text{C}$ for no more than 1 month or 4 $^{\circ}\text{C}$ for no more than 72 h.

Step 2-6 Quality Control of cDNA Enrichment Products

1. Qualified enrichment product sizes range from 250-5,000 bp with major peaks in the 750-20,00 bp range without small fragments. If there are small fragments, perform another 0.6 × purification until there are no small fragments (Agilent 4200 TapeStation/ Qsep).



2. Qubit 4.0 measures cDNA enrichment product concentration.

cDNA Quality Control Criteria:

1. For capturing 500-5000 cells, the concentration of cDNA (Qubit) should be ≥ 1 ng/ μ L. The fragment range should be between 250 bp and 5000 bp, with the main peak falling within the 750-2500 bp range, indicating a pass.
2. For capturing 6000-10000 cells, the concentration of cDNA (Qubit) should be ≥ 3 ng/ μ L. The fragment range should be between 250 bp and 5000 bp, with the main peak falling within the 750-2500 bp range, indicating a pass.
3. If the concentration (Qubit) is between 0.5 ng/ μ L and <1 ng/ μ L, and the fragment size range is between 250-5,000 bp with the main peak within the 750-2,500 bp range, or if the concentration (Qubit) is ≥ 1 ng/ μ L and the fragment size range is between 250-5,000 bp, but the main peak not within the 750-2,500 bp range, it suggests a potential risk.
4. If the concentration (Qubit) is <0.5 ng/ μ L, or if there are no target fragments detected within the 250-5000 bp range, or if there is no apparent main peak, it is considered a failure.

Step 3 Library Construction

Step 3-0 Prepare Before the Experiment

- ❑ **Prepare Ice:** Have a box with ice in advance.
- ❑ **Thaw Reagents:** Take out in advance the **Fragmentation Buffer**, **Ligation Buffer**, **Adaptor**, **2×PCR Master Mix** from -20°C put onto ice to thaw, vortex thoroughly, centrifuge briefly, and place on ice until use.
- ❑ **Handle Enzymes:** **Fragmentation Enzyme** and **DNA Ligase** should only be taken out from -20°C directly before use, immediately briefly centrifuge, then pipetted and put back at -20°C, because enzymes are temperature sensitive.
- ❑ **Prepare 80% ethanol:** Prepare 80% ethanol by using absolute ethanol (e.g., 99.8%) and nuclease-free water. Prepare 1.5 mL of 80% ethanol per sample. Make sure to prepare it just before use and use it within 24 hours.
- ❑ **Warm DNA Selection Beads:** Let the DNA selection beads reach room temperature for at least 30 minutes before use.

Step 3-1 DNA Fragmentation and End Repair

1. **Set up the Thermal Cycler:** Set up the program according to the table below and start the thermal cycler.

Lid Temperature	Reaction Volume	Run Time
65°C	50 µL	~32 min
Steps	Temperature	Time
1	4°C	Hold
2	32°C	2 min
3	65°C	30 min
4	4°C	Hold

2. **Prepare the Reaction Mix:**

For each sample, prepare the reaction system according to the table below.

Use 50-100 ng of total cDNA as the template.

Calculate the required cDNA volume, and add the corresponding volume of nuclease-free water to complete the reaction.

If the total cDNA amount is less than 50 ng, take 15 µL of cDNA enrichment product for each sample for the subsequent fragmentation reaction.

Vortex to mix, briefly centrifuge, and keep on ice.

Any remaining cDNA can be stored at 4°C for up to 72 hours or at -20°C for up to 1 month for generating additional libraries.

Example: If the cDNA concentration is 5 ng/µl, to get 50 ng, use 10 µL ($50 \text{ ng} \div 5 \text{ ng}/\mu\text{L} = 10 \mu\text{L}$).

Component	Volume/Sample
cDNA enrichment products	X µL
Nuclease-free Water	(35-X) µL
● Fragmentation Buffer	5 µL
Total	40 µL

3. **Add Fragmentation Enzyme:** Add 10 µL of Fragmentation Enzyme to each reaction system, pipette up and down 15 times on ice, centrifuge briefly and put back onto ice.

4. **Run the PCR Program:** Place the prepared reaction into the thermal cycler (set at 4°C) immediately. Select "Next" or "Skip Step" (depending on the thermal cycler) to continue running the PCR program.

Step 3-2 Adaptor Ligation

1. **Prepare the Reaction Mix:** Prepare the reaction system according to the following table, fully vortex, and centrifuge briefly.

Component	Volume/Sample
● Ligation Buffer	20 µL
● DNA Ligase	5 µL
● Adaptor	5 µL
Nuclease-free Water	20 µL
Total	50 µL

2. **Add Mix to Samples:** Add 50 µL of the reaction system to the fragmentation product, mix 15 times with a pipette, and briefly centrifuge.

3. **Run the PCR Program:** Set up the PCR program according to the table below and perform the reaction with a thermal cycler.

Lid Temperature	Reaction Volume	Run Time
30°C	100 µL	~15 min

Steps	Temperature	Time
1	20°C	15 min
2	4°C	Hold

Step 3-3 Ligation Product Purification

1. **Add DNA Selection Beads:** After the reaction is completed, centrifuge briefly and add 60 µL of well-mixed DNA selection beads (0.6×), mix 10 times with a pipette, or mix by vortexing and centrifuge briefly.

Note: 0.6× means 30 µL of beads for 50 µL of PCR product, i.e., $30 \mu\text{L} / 50 \mu\text{L} = 0.6\times$.

2. **Incubate and Remove Supernatant:** After 10 minutes of incubation at room temperature, centrifuge briefly and place the tube on the magnetic rack to allow the solution to clear. Carefully **remove the supernatant and discard it**.

Note: Gently pipette up and down 5 times during adsorption to improve bead adhesion.

3. **Ethanol Wash:** Add 200 µL of 80% ethanol (keep on the magnetic rack) for approximately 30 seconds. **Carefully remove the supernatant and discard it. Repeat this step once.**

4. **Remove Ethanol:** Close the lids and briefly centrifuge them. Put the tubes back onto the magnetic stand, and **remove any residual supernatant** with a 10 µL pipette. During centrifugation, ensure that the beads side of the tubes is facing away from the centrifuge's center.

5. **Dry Beads:** Leave the tubes open for 3-5 minutes until the ethanol evaporates and the beads appear dark and matte but not over-dry (avoid cracks on the bead surface). Adjust drying time based on your beads status and environment.

6. **Resuspend Beads:** Add 24 µL of nuclease-free water to fully resuspend the beads and let the tubes stand for 2 minutes at room temperature on a normal rack (not a magnetic rack), then centrifuge briefly.

7. **Transfer Supernatant:** Place the tubes onto the magnetic rack until the solution appears clear, **transfer 23 µL of the supernatant to a new 0.2 mL PCR tube**. Ensure the supernatant is clear and bead-free.

Note: During adsorption, gently pipette up and down 5 times to improve bead adhesion.

Step 3-4 Library Amplification

1. **Prepare the Reaction Mix:** Prepare the reaction system according to the following table, fully vortex and centrifuge briefly.

Component	Volume/Sample
● 2×PCR Master Mix	25 µL
● N5	1 µL
● N7	1 µL
Total	27 µL

2. **Add Mix to Samples:** Add 27 µL of the reaction system to the ligated purification product, mix 15 times with a pipette, and centrifuge briefly.

3. **Run PCR:** Set up the PCR program according to the following conditions and perform the reaction.

Lid Temperature	Reaction Volume	Run Time
105°C	50 µL	~22-30 min
Total cycles	Temperature	Time
11-16 (See table below)	98°C	3 min
	98°C	20 sec
	54°C	30 sec
	72°C	20 sec
	72°C	5 min
	4°C	Hold

cDNA input amount	Recommended cycles
≤ 10 ng	16
10-25 ng	14
25-50 ng	13
50-75 ng	12
75-100 ng	11

Step 3-5 Fragment Purification

1. **Add Beads:** After the reaction is completed, centrifuge briefly. Then add 26 µL of the well-mixed DNA selection beads (0.52×) and pipette up and down 10 times or mix by vortexing.

Note: 0.52× means 26 µL of beads for 50 µL of PCR product, i.e., $26 \mu\text{L} / 50 \mu\text{L} = 0.52\times$

2. **Incubation:** Incubate the mixture at room temperature for 5 minutes. Briefly centrifuge, place the tube on a magnetic rack until the solution clears. **Remove the supernatant and discard it.**

***Note:** During adsorption, gently pipette up and down 5 times to enhance bead adhesion.*

3. **Ethanol Wash:** Add 200 μL of 80% ethanol (keeping placed on the magnetic rack), and wait for about 30 seconds. Carefully **remove the supernatant ethanol and discard it. Repeat this step once.**

4. **Remove Ethanol:** Close the lids and briefly centrifuge them. Put the tubes back onto the magnetic stand, and **remove any residual supernatant with a 10 μL pipette.** During centrifugation, ensure that the beads side of the tubes is facing away from the centrifuge's center.

5. **Dry Beads:** Leave them standing on the magnetic rack at room temperature for 3-5 minutes. Monitor the beads closely—they should turn dull and matte but not dry out completely (avoid cracks on the bead surface). Adjust drying time based on your beads status and environment.

6. **Resuspend Beads:** Add 31 μL of nuclease-free water to fully resuspend the beads and let the tubes stand for 2 minutes at room temperature on a normal rack (not a magnetic rack), then centrifuge briefly.

7. **Transfer Supernatant:** Place the tube on the magnetic rack until the solution becomes clear, then **transfer 30 μL of the supernatant to a new 0.2 mL PCR tube.** Take 1 μL for Qubit measurement.

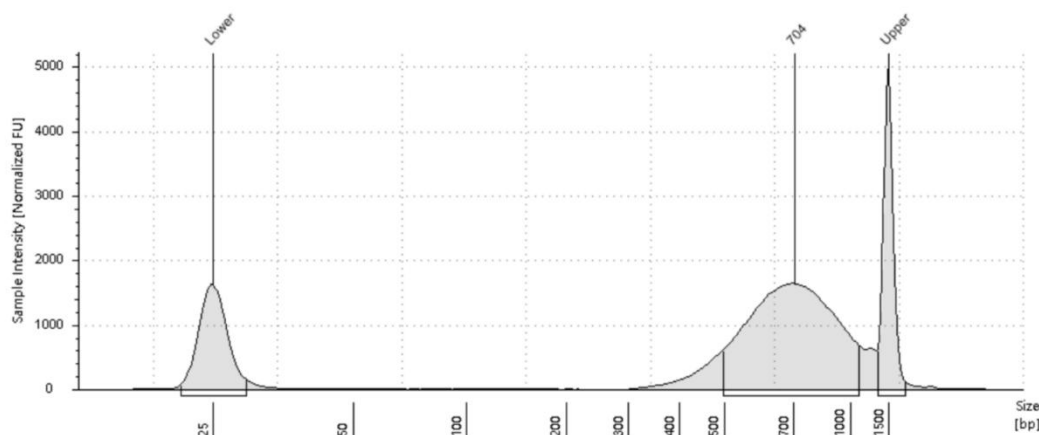
***Note 1:** During adsorption, gently pipette up and down 5 times to enhance bead adhesion.*

***Note 2:** Record the library concentration, sample name, and index number on the tube wall simultaneously.*

STOPPING POINT: The products generated in step 3-5 can be stored at -20°C for up to 6 months.

Step 3-6 Library Quality Control

1. The qualified library has a main peak fragment size ranging from 450-900 bp, with no small fragments present. If small fragments are present, perform an additional 0.75x purification until no small fragments are present (Agilent 4200 TapeStation/ Qsep).



2. Library concentration not less than 1 ng/μL (Qubit 4.0 measurement).

Library Quality Control Criteria:

1. Concentration (Qubit) ≥ 5 ng/μL, with fragment size range between 250 bp and 1500 bp, no small fragment contamination, and the main peak within the range of 450-900 bp, is considered qualified.
2. Concentration (Qubit) between 1 ng/μL and <5 ng/μL, with fragment size range between 250 bp and 1500 bp, no small fragment contamination, and the main peak falling within the 450-900 bp range, may be considered acceptable for sequencing with some risks.
3. Concentration (Qubit) ≥ 5 ng/μL, with fragment size range between 250 bp and 1500 bp, and the main peak falling within the 450-900 bp range, but showing some small fragment contamination with their peak lower than the target fragment peak, may be considered acceptable for sequencing with some risks.
4. Concentration (Qubit) <1 ng/μL, or detection of no fragments within the 250-1500 bp range, no clear main peak, or small fragment peak higher than the target fragment peak, is considered unqualified for sequencing.















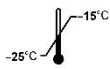



【Manufacturer/After-sales service unit】

Manufacturer: Beijing SeekGene BioSciences Co.,Ltd
Address: Room 201, Floor 2, Tower A Building 9, Zone 1, 8 Life Science Parkway, Changping District, Beijing, China
Zip code: 102206
Tel: +86- (0) 10 56918048

【Information on EU representatives】

EU Name: Medpath GmbH
EU Address: Mies-van-der-Rohe-Strasse 8,80807 Munich, Germany
DIMDI No: DE/0000047823
SRN Code: DE-AR-000000087
Tel: +49 (0) 89 189174474
Fax: +49 (0) 89 5485 8884
Email: info@medpath.pro

【Explanations for Symbols】

	Manufacture		European union representative
	In vitro diagnostic medical device		Use-by date
	Batch code		Catalogue number
	Unique device identifier		Consult instructions for use
	Keep dry		Keep away from sunlight
	Do not use if package is damaged and consult instructions for use		Cautions
	Biological risks		Fragile, handle with care
	Store at -25~-15°C		CE mark
	Store at 2~8°C		Store at -80°C

【Edition】

V1.2

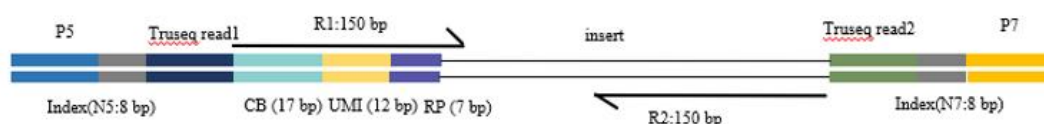
【Revised date】

2024/12/20

Appendix 1 High-throughput Sequencing

1. Sequencing Library

The SeekOne™ DD Single-Cell Full-Transcriptome Sequencing Library starts with P5 and ends with P7 sequences. The cell barcode consists of 17 bp, UMI is 12 bp, and sample dual-end indexes are N5 (8 bp) and N7 (8 bp). Sequencing the library will yield basic FASTQ data for standard single-cell analysis.



2. Sequencing Platforms

Single-cell libraries constructed with this kit are compatible with both GeneMind sequencing platforms and Illumina sequencing platforms.

- GeneMind sequencing platforms: SURFSeq 5000
- Illumina platforms: MiSeq, NextSeq 500/550, NextSeq 1000/2000, HiSeq 2500 (Rapid Run), HiSeq 3000/4000, NovaSeq

3. Library Sequencing Depth and Run Parameters

Sequencing Depth	Minimum 20,000 reads per cell, recommended $\geq 50,000$
Sequencing Type	Paired-end, dual indexing
Recommended	Read1: 150 cycles N7 Index: 8 cycles
Number of Cycles	N5 Index: 8 cycles Read2: 150 cycles

Note 1: Recommended sequencing depth is $\geq 50,000$ reads per cell to ensure the accuracy of single-cell sequencing data analysis.

Note 2: Recommended read length for paired-end sequencing, with Read1 and Read2 both having a length of 150 bp.

4. Library Loading Concentration

Platform	Instrument	Loading concentration(pM)	PhiX(%)
Illumina	MiSeq	11	1
	NextSeq 500/550	1.8	1
	HiSeq 2500(RR)	11	1
	HiSeq 4000	240	1
	NovaSeq	150*/300	1

Note: Use a loading concentration of 150 pM for Illumina XP workflow. For additional sequencing platform information please contact us (info@seekgene.com) for further assistance.

5. Library Pooling

Considering that gene expression libraries may be pooled into a single lane for sequencing, it is important to note that the sequencing libraries for pooling should not use the same indexes. Samples with the same index cannot be demultiplexed in subsequent data analysis.

Appendix 2 Bioinformatics Analysis

1. Analysis Software

Single-cell data analysis utilizes SeekSoul Tools, developed independently by Beijing SeekGene BioSciences Co.,Ltd.. SeekSoul Tools can identify cell barcode labels, perform quantitative alignment, and generate downstream analysis results in the form of a cell expression matrix, which is used for subsequent cell clustering and differential analysis.

Input Files: FASTQ

Output Files: BAM, HTML, CSV, matrices (filtered_feature_bc_matrix, raw_feature_bc_matrix)

Operating System: Linux




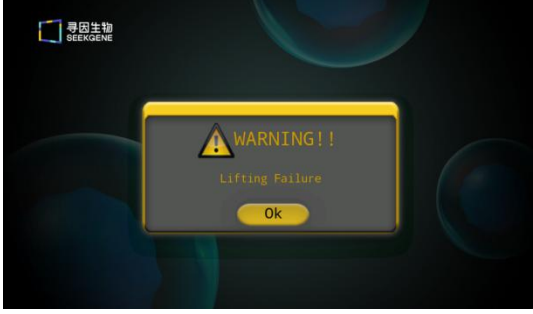

Software Access: To obtain the software package and installation instructions, please visit <http://seeksoul.seekgene.com/en/index.html>

Appendix 3: SeekOne™ Digital Droplet System User Manual

Refer to *SeekOne™ Digital Droplet System User Manual*

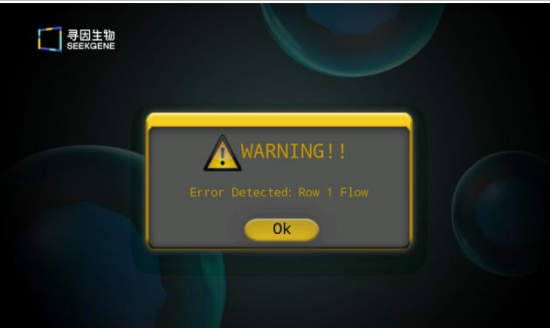
Appendix 4: SeekOne™ Digital Droplet System Troubleshooting

Problems may occur during the operation of the equipment. The following table describes the fault types and how to deal with them. When the equipment malfunctions occur, the user can first troubleshoot and deal with it according to the following table, if the issue cannot be solved, please contact our company in time.

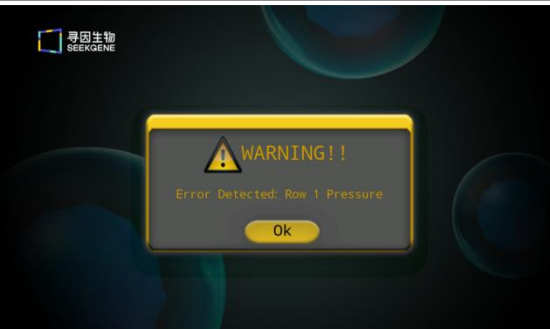
Failure type	Solution
 <p>The image shows a warning dialog box with a yellow border and a warning icon. The text inside reads: "WARNING!!", "Controller Communication Failure", and "Ok". The background is dark with a blue circular pattern.</p>	<p>Please make sure the device is installed correctly, Click “OK” to perform a self-check, or restart the device. If this message appears repeatedly, it may indicate an internal hardware issue. Continued use under these circumstances can result in damage to the instrument. Please contact (info@seekgene.com) for further assistance.</p>
 <p>The image shows a warning dialog box with a yellow border and a warning icon. The text inside reads: "WARNING!!", "Chip Compartment Exit Failure", and "Ok". The background is dark with a blue circular pattern.</p>	<p>The operation in and out of the warehouse may be blocked. Please confirm that no objects are blocking the running path and click the "OK" button on the prompt window. The instrument will proceed to the next step. If the message appears repeatedly, please contact (info@seekgene.com) for further assistance.</p>
 <p>The image shows a warning dialog box with a yellow border and a warning icon. The text inside reads: "WARNING!!", "Chip Compartment Entry Failure", and "Ok". The background is dark with a blue circular pattern.</p>	<p>The operation in and out of the warehouse may be blocked. Please confirm that no objects are blocking the running path and click the "OK" button on the prompt window. The instrument will proceed to the next step. If the message appears repeatedly, please contact (info@seekgene.com) for further assistance.</p>
 <p>The image shows a warning dialog box with a yellow border and a warning icon. The text inside reads: "WARNING!!", "Lifting Failure", and "Ok". The background is dark with a blue circular pattern.</p>	<p>Please try again or restart the operation. If the message appears repeatedly, please contact (info@seekgene.com) for further assistance.</p>
 <p>The image shows a warning dialog box with a yellow border and a warning icon. The text inside reads: "WARNING!!", "Plate Setting Down Failure", and "Ok". The background is dark with a blue circular pattern.</p>	<p>Please verify if the gasket is properly seated on the Chip Holder and reposition the Chip Holder. Check if there are any foreign objects on the surface of the chip compartment and clean the surface. If the message appears repeatedly, please contact (info@seekgene.com) for further assistance.</p>



Restart the device, if it recurs, contact (info@seekgene.com) for further assistance.



Please check if the sealing gasket is clean, if the chip has any damage on its surface, and if the Chip Holder is installed correctly. If there is dirt in the sealing gasket or damage on the chip's surface, please replace the gasket or chip and try again. If the error message recurs, contact (info@seekgene.com) for further assistance.



Please check if the sealing gasket is clean, if the chip has any damage on its surface, and if the Chip Holder is installed correctly. If there is dirt in the sealing gasket or damage on the chip's surface, please replace the gasket or chip and try again. If the error message recurs, contact (info@seekgene.com) for further assistance.

Appendix 5: Revision

No.	Revision	Modified Content	Effective Date
1	New Creation	New file	2022/02/21
2	V1.1 to V1.2	Change cell lines, change gel bead process, split kits.	2022/07/21
3	Revised	Updated the operational details. Revised for language and term consistency.	2023/12/19
4	Revised	Optimize the detail description	2024/04/22
5	Revised	The volume of cDNA Primers in the kit for 8-tests was adjusted from 20 µL to 40 µL.	2024/05/29
6	Revised	Updated the recommendations for the number of library amplification cycles in Step 3-5.	2024/07/31
7	Revised	Revised for language and term consistency.	2024/12/20