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User manual

SeekOne™ DD Single Cell 3' Transcriptome-seq Kit

REF: K00202-02 (2 tests), K00202-08 (8 tests)

V2.3

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

Envision the Future

Beijing SeekGene BioSciences Co.,Ltd

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1. Introduction

1.1 Product Overview

SeekOne™ DD Single Cell 3' Transcriptome-seq Kit (Digital Droplet) is a commercial single cell transcriptome library construction kit independently developed by Beijing SeekGene BioSciences Co., Ltd.(abbreviated as SeekGene), which uses microfluidic digital droplets and barcoded beads technology. 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. When equipped with single cell data analysis software SeekSoul Tools, we provide you with one-stop-shop single cell transcriptome solutions.

SeekOne™ DD Single Cell 3' Transcriptome-seq Kit includes: chip (SeekOne™ DD Chip S3, referred to as Chip S3), gasket, carrier oil, gel beads (SeekOne™ DD 3' Barcoded Beads, abbreviated as Barcoded Beads), amplification reagents, library construction reagents, and single cell data analysis software (SeekSoul Tools).

1.2 Intended Use

SeekOne™ DD Single Cell 3' Transcriptome-seq Kit is based on the principle of microfluidic technology. It realizes the separation and capture of single cells through water-in-oil droplets, and uses nucleic acid modified Barcoded Beads to mark the RNA from different cell sources, to finally obtain a high-throughput single-cell 3' transcriptome library compatible with GeneMind, Illumina, and MGI sequencers. The kit enables the analysis of single-cell gene expression. It can be applied to a variety of scientific research areas, including tumor research, immunology research, cellular development studies, viral infection research and targeted biomarker screening, etc.

1.3 Intended User

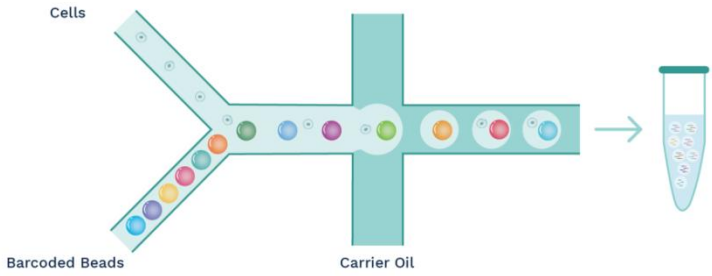
This kit is intended to be used by laboratory technicians or people with equivalent qualifications.

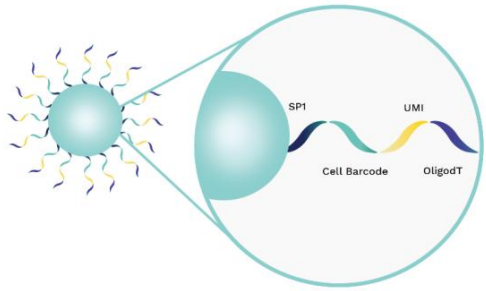
The requirements to use this kit include a certain level of theoretical knowledge and operational skills in molecular biology. After training and qualification by SeekGene, operators are capable of performing this reagent kit.

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 be used directly as the results of in vitro diagnostic tests.

1.5 Experimental Principles





1.6 Library Construction Process

1.7 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.

1.8 Sample Requirements

1.8.1 Sample Type

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.

□ 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 ⩽0.1%. Higher BSA concentration can lead to incomplete demulsification, affecting cell capture efficiency and gene count.

□ Cell diameter: 5-40 μm.

1.8.2 Sample Quality

□ Cell Count: It is recommended to obtain a minimum of 50,000 viable cells. The minimum amount 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.

1.8.3 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.*

1.8.4 Loading 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.

1.9 Product Components and Storage Conditions

SeekOne™ DD Single Cell 3' Transcriptome-seq Kit (K00202-02, 2 tests/K00202-08, 8 tests) is divided based on their reagent functions and storage conditions, including:

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

b. SeekOne™ DD Single Cell 3' Barcoded Beads Kit, K00202-0202 (2 tests) / K00202-0802(8 tests)

c. SeekOne™ DD Single Cell 3' Reverse Transcription Kit, K00202-0203 (2 tests) / K00202-0803(8 tests)

d. SeekOne™ DD Single Cell 3' cDNA Amplification Kit, K00202-0206 (2 tests) / K00202-0806(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)

1.9.1 SeekOne™ DD Single Cell 3' Transcriptome-seq Kit, 2 tests, REF: K00202-02

Name & PN & Storage	Tube lid color	Component	CN	2 tests
SeekOne™ DD Chip S3 Kit V1.0, K00202-0201, 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 Single Cell 3' Barcoded Beads Kit V2.3, K00202-0202, **-80℃**	○	Single Cell 3' Barcoded Beads	R0003501	45 μL×2 tube
	○	TSO	R0003601	10 μL
SeekOne™ DD Single Cell 3' Reverse Transcription Kit V2.3, K00202-0203, **-20 ± 5℃**	●	3x RT Buffer	R0008401	80 μL
	●	RT Enzyme	R0003801	15 μL
	●	Reducing Buffer	R0003901	100 μL
SeekOne™ DD Single Cell 3' cDNA Amplification Kit V2.3, K00202-0206, **-20 ± 5℃**	●	2×PCR Master Mix	R0002101	60 μL
	●	cDNA Primers	R0004001	10 μL
SeekOne™ DD Library Construction Kit V1.0, K00202-0204, **-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
	●	N702	R0005101	25 μL
SeekOne™ DD Single Cell Cleanup Kit V1.0, K00202-0205, **2-8℃**	○	Cleanup Beads	R0003401	0.5 mL

1.9.2 SeekOne™ DD Single Cell 3' Transcriptome-seq Kit, 8 tests, REF: K00202-08

Name & PN & Storage	Tube lid color	Component	CN	8 tests
SeekOne™ DD Chip S3 Kit V1.0, K00202-0801, 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 Single Cell 3' Barcoded Beads Kit V2.3, K00202-0802, **-80℃**	○	Single Cell 3' Barcoded Beads	R0003501	45 μL×8 tubes
	○	TSO	R0003602	20 μL
SeekOne™ DD Single Cell 3' Reverse Transcription Kit V2.3, K00202-0803, **-20 ± 5℃**	●	3x RT Buffer	R0008402	280 μL
	●	RT Enzyme	R0003802	50 μL
	○	Reducing Buffer	R0003901	100 μL
SeekOne™ DD Single Cell 3' cDNA Amplification Kit V2.3, K00202-0806, **-20 ± 5℃**	●	2×PCR Master Mix	R0002102	240 μL
	●	cDNA Primers	R0004002	20 μL
SeekOne™ DD Library Construction Kit V1.0, K00202-0804, **-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
	●	N703	R0005201	25 μL
	●	N704	R0005301	25 μL
SeekOne™ DD Single Cell Cleanup Kit V1.0, K00202-0805, **2-8℃**	○	Cleanup Beads	R0003402	1.75 mL

1.9.3 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 Single Cell 3' Barcoded Beads Kit	K00202-0202/ K00202-0802	Dry ice	-80℃
SeekOne™ DD Single Cell 3' Reverse Transcription Kit	K00202-0203/ K00202-0803	Dry ice	-20 ± 5℃
SeekOne™ DD Single Cell 3' cDNA Amplification Kit	K00202-0206/ K00202-0806	Dry ice	-20 ± 5℃
SeekOne™ DD Library Construction Kit	K00202-0204/ K00202-0804	Dry ice	-20 ± 5℃

1.9.4 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.*

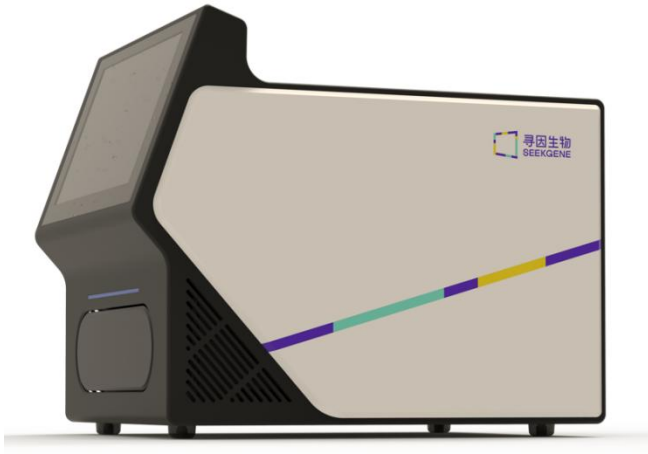
Note 2: *The Index sequence provided by this kit can label up to 16 samples simultaneously.*

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

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

1.9.5 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.

1.10 Additional Equipment & Kits, Reagents

1.10.1 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
	Bioptic, Qsep400	Bioptic, 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/ 100 - 1000 μL	Axygen, T-300-L-R-S, Axygen, T-200-C-L-R-S, Axygen, T-1000-C-L-R-S

1.10.2 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) VAHTS DNA Clean Beads	Beckman Coulter, B23318 or A63882 Vazyme, N411
Qubit dsDNA Quantification Assay Kits	Thermo Fisher Scientific, Q32854
High Sensitivity D1000 ScreenTape/Reagents High Sensitivity D5000 ScreenTape/Reagents S2-Standard Cartridge Kit, S1-High Resolution Cartridge Kit ***Choose the kits based on the available equipment**	Agilent,5067-5592/ 5067-5593 Agilent, 5067-5584/ 5067-5585, Bioptic, C105201/C105801/C405101 Bioptic, C105202/C105802/C405102

2. Experimental Operation Steps

Protocol Steps and Time

Steps		Time
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℃ ≤72 h or −20℃ ≤1 week***		
Step 2 Sample Recovery and cDNA Amplification		
~2.8 h	**2-1 Water-in-Oil Demulsification**	**45 min**
	2-2 cDNA Amplification	45~60 min
	○ ***STOPPING POINT: 4℃ ≤72 h or −20℃ ≤1 week***	
	2-3 cDNA Enrichment Products Purification	30 min
	○ ***STOPPING POINT: 4℃ ≤72 h or −20℃ ≤1 month***	
	2-4 Quality Control of cDNA Enrichment Products	30 min
Step 3 Library Construction		
~3.5 h	3-1 DNA Fragmentation and End Repair	40 min
	3-2 Fragment Sorting	40 min
	3-3 Adaptor Ligation	20 min
	3-4 Ligation Product Purification	20 min
	3-5 Library Amplification	40 min
	3-6 Fragment Sorting	40 min
	○ ***STOPPING POINT: -20℃ ≤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 3' Reverse Transcription Kit**:

■ Thaw **3x RT Buffer** and **Reducing Buffer** from -20 ℃. Once thawed, vortex well, centrifuge briefly, and keep on ice.

■ **RT Enzyme: Keep at -20° C until use.** Briefly centrifuge before pipetting, then return to -20℃ immediately.

☐ **SeekOne™ DD Single Cell 3' Barcoded Beads Kit**:

■ Take **Barcoded Beads** from the -80℃ freezer in advance and equilibrate at room temperature for 30 minutes. Put back to -80℃ freezer immediately after use. Keeping them at room temperature for no more than 2 hours.

■ Thaw **TSO** on ice from -80℃, after thawing, vortex thoroughly, and centrifuge briefly, then keep on ice.

☐ 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 complete before proceeding with the experiment.

☐ Prepare the deep-well thermal cycler program.

***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℃ 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 15 times with a pipette, and centrifuge briefly (**be sure to prepare the Master Mix according to the table below before use.**) Keep on ice until use.

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

***Note 1**: 3 x 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: Pipette in the correct order: Do not add nuclease-free water directly 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 (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× 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 tube(e.g. 500μL) on ice.

b. Subtract the calculated cell volume 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 (cell volume) = 33.5 μL.

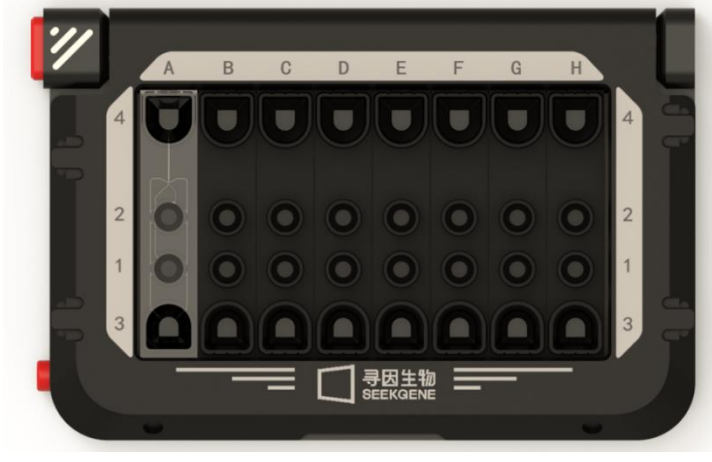
Finally, pipette 11.1 μL of cells into the mixture.

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 80 μL single-cell mixture (prepared in Step 1-1) by pipetting up and down 15 times. Pipette 78 μL of the mixture and carefully insert it into **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**: Vortex the Barcoded Beads well at room temperature for 30 seconds, briefly centrifuge for 5 seconds, ensure that there are no air bubbles in the Barcoded Beads liquid. Pipette 38 μL of the beads into **Well 2**, keeping the tip slightly above the bottom, and pipette slowly to avoid bubbles.

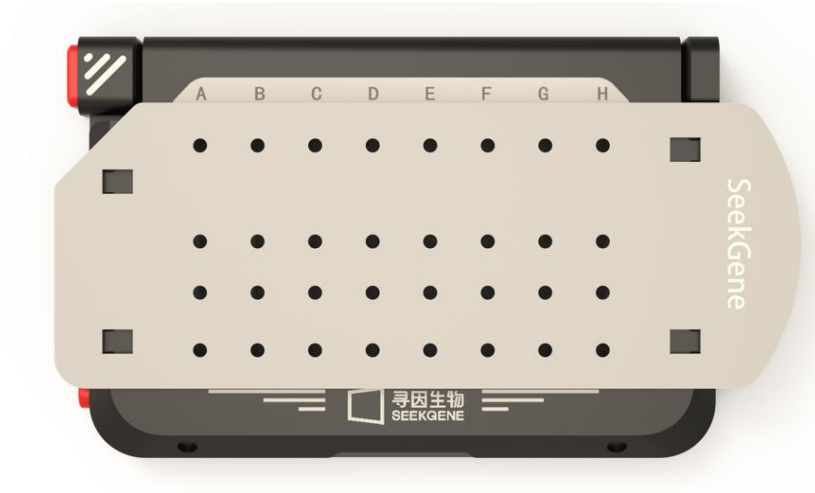
***Note 1:** When adding reagents, keep the pipette tip moving with the liquid level and always ensure that the pipette tip is below the liquid surface by 3 mm to avoid generating bubbles.*

***Note 2:** The Barcoded Beads are viscous. After dispensing, wait 5 seconds before removing the tip to avoid incomplete transfer.*

4. **Add Carrier Oil**: Using a 200 μL pipette, add 120 μL of Carrier Oil into **Well 3**. Repeat for a total of 240 μL, pipetting slowly against the inner wall to prevent bubbles.

***Note**: Improper addition of Carrier Oil may affect droplet formation or damage the instrument.*

5. **Attach the Gasket**: Place the Gasket over the Chip Holder as shown in the illustration below, ensuring the holes align with the wells. The cut-off corner should be at the top left.



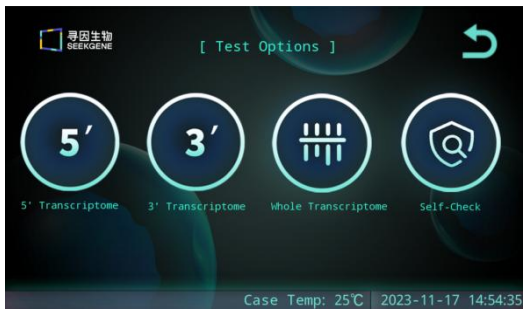
***Note**: Do not touch the smooth surface of Gasket.*

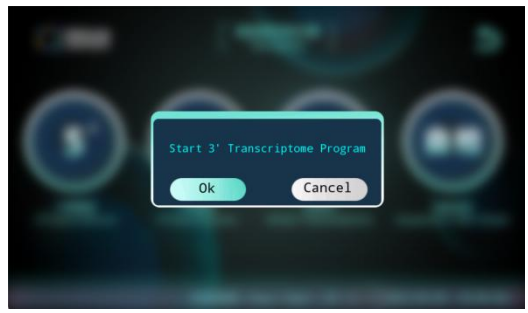
Step 1-3 Run SeekOne™ DD (Do not shake or move the device while it is running)

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

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

3. **Start the Program**: Click the "3' Transcriptome" program on the screen, then click "OK" to begin.



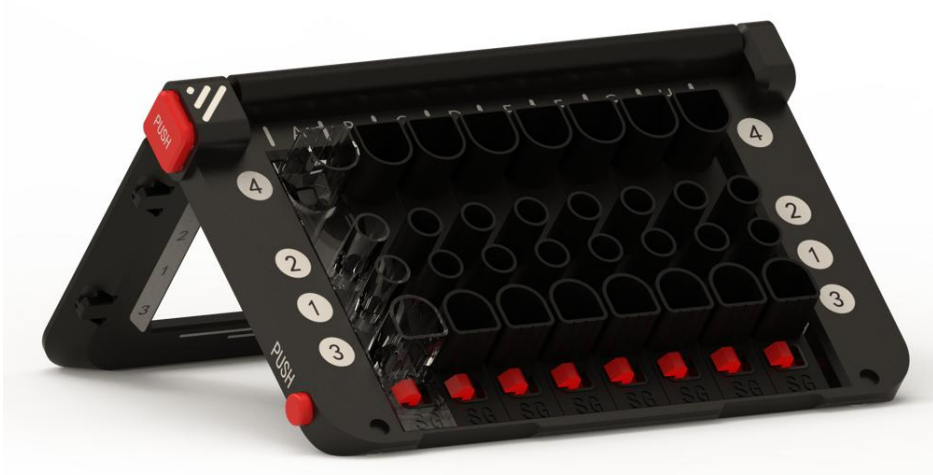


4. **Complete and Remove**: Once the program is finishes, click "Run Completed" and remove the Chip Holder. Immediately proceed to the next step.

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.

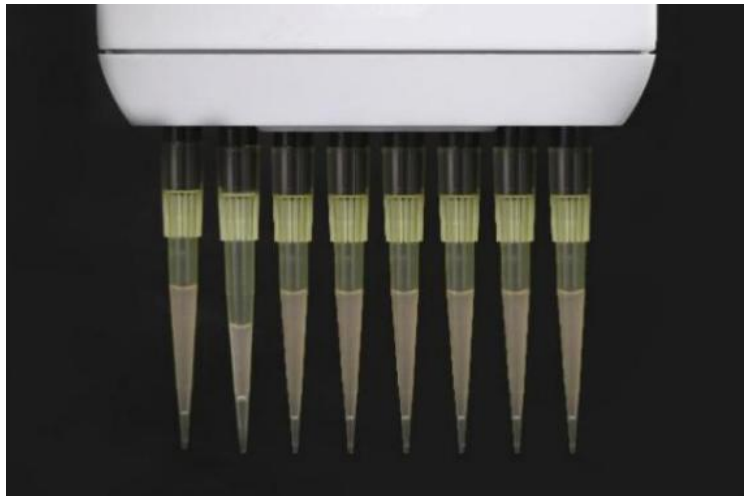
***Note**: Well 1 should have ≤ 10 μL, and Well 2 should have ≤ 15 μL. Larger volumes indicate a blocked chip.*

4. **Aspirate Water-in-Oil**: Use a pipette to **slowly** aspirate at least 120 μL of the water-in-oil emulsion from **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 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	~95 min
Step	**Temperature**	**Time**
1	42˚C	90 min
2	85˚C	5 min
3	4˚C	Hold

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

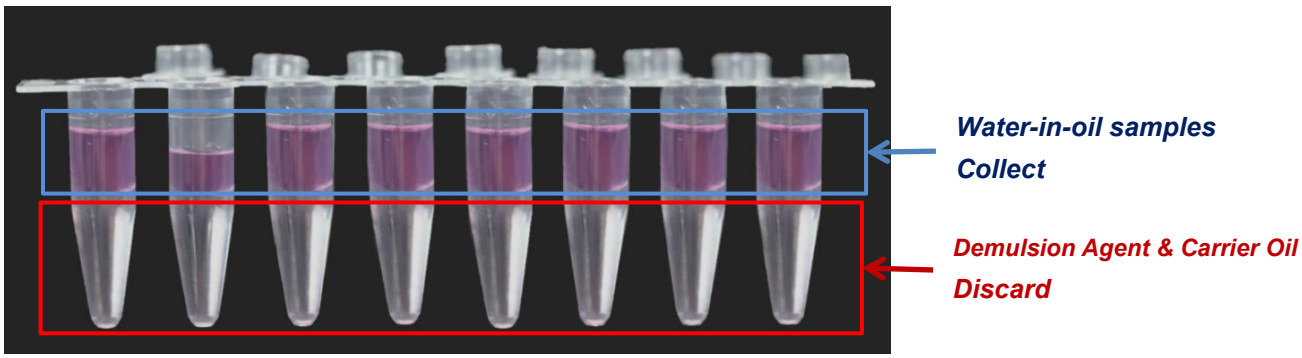
☐ **Thaw Reagents**: Remove **2× PCR Master Mix**, **cDNA Primers**, and **Reducing Buffer** from -20℃, put on ice in advance to thaw. Once thawed, vortex thoroughly, centrifuge briefly, and place on ice until use.

☐ **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.



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:

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

Note: *Vortex the Cleanup Beads for 2 minutes and mix well before use.*

3. **Remove Demulsion Mixture**: Carefully **remove and discard** 130 µL of the Demulsion Agent/Carrier Oil mixture by pipetting from the bottom of the PCR tube, leaving 2-5 µL 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 µL 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 (still on the magnetic rack). Add 24 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 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
cDNA Primers	2 μL
Total	**27 μL**

2. **Mix and Start PCR**: Add the prepared 27 μL cDNA amplification mix to the 23 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℃	50 μL	~42-53 min
Total Cycles	**Temperature**	**Time**
	98℃	3 min
10-13 (See table below)	98℃	10 sec
	63℃	15 sec
	72℃	3 min
	72℃	5 min
	4℃	Hold

Cell diameter	Number of loaded cells	Recommended Cycles
⩽10 μm	500-5,000	13
	5,000-15,000	12
	15,000-24,000	11
＞10 μm	500-5,000	12
	5,000-15,000	11
	15,000-24,000	10

Centrifuge the samples after the PCR run is completed.

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

Step 2-3 cDNA Enrichment Products 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. 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 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. Add 41 μL of nuclease-free water to fully resuspend 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 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.**

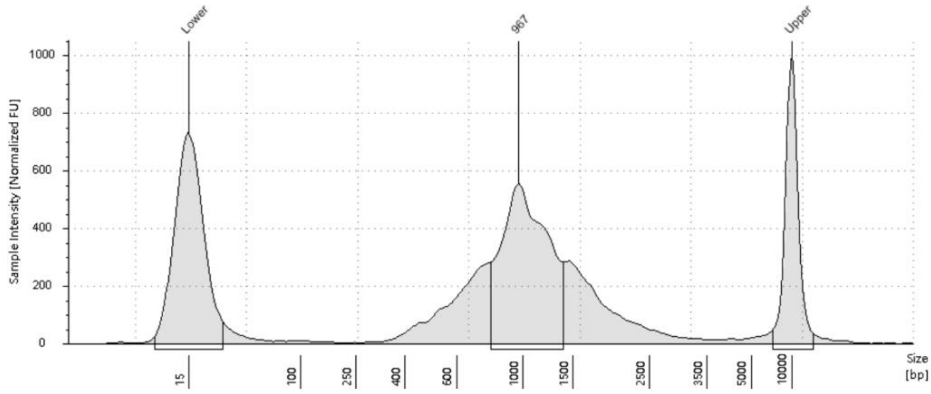
Note 1*: 0.6× refers to the volume ratio of added DNA sorting magnetic beads to PCR products, which is 30μL / 50μ L=0.6×.*

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

STOPPING POINT*: The products generated in step 2-3 can be stored at -20℃ for up to 1 month or 4℃ for up to 72 h.*

Step 2-4 Quality Control of cDNA Enrichment Products

1. Qualified enrichment product sizes range from 250-5,000 bp with major peaks in the 750-2,000 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/μL**. 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/μL**. 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/μL and <1 ng/μL, 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/μL and the fragment size range is between 250 bp and 5000 bp, but the main peak not within the 750-2500 bp range, it suggests a potential risk.

4. If the concentration (Qubit) is <0.5 ng/μL, 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 ready.

☐ **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:** Mix absolute ethanol (e.g., 99.8%) with nuclease-free water to make 1.5 mL of 80% ethanol per sample. Prepare 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
70˚C	50 µL	~35 min
Steps	**Temperature**	**Time**
1	4˚C	Hold
2	32˚C	5 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 following table.

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

Calculate the required cDNA volume and add 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 fragmentation reaction.

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

Any remaining cDNA can be stored at 4˚C (up to 72 hours) or at −20˚C (up to 1 month) for generating additional 3' transcriptome libraries.

Example: *If the cDNA concentration is 5 ng/μl, to get 50 ng, use 10 μL (50 ng ÷ 5 ng/μL = 10 μL).*

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 the 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 the PCR program.

Step 3-2 Fragment Sorting

1. **First Selection:** Immediately after the reaction, centrifuge briefly and add 30 μL of well-mixed DNA selection beads (0.6×) to the mixture. Mix by pipetting up and down 10 times or by vortexing, then centrifuge briefly. Incubate the mixture at room temperature for 5 minutes, centrifuge briefly, and place it on a magnetic rack until the solution is clear.

Note 1*: Gently pipette up and down 5 times during separation to enhance bead adhesion.*

Note 2*: 0.6× means 30 μL of beads for a 50 μL PCR product, i.e., 30 μL / 50 μL = 0.6×.*

2. **Second Selection: Transfer the supernatant (do NOT discard it)** to a new PCR tube containing 10 μL of DNA selection beads (0.8×). Mix by pipetting 10 times. Incubate the mixture at room temperature for 5 minutes, then centrifuge briefly. Place the tube on the magnetic rack until the solution appears clear. **Remove and discard the supernatant.**

Note 1*: Gently pipette up and down 5 times during separation to enhance bead adhesion.*

Note 2*: 0.8× refers to the total bead volume ratio (30 μL+ 10 μL) to the PCR product i.e., (30 μL + 10 μL) / 50 μL = 0.8×.*

Note 3: *During the first round of DNA selection, larger fragments bind to the beads and are discarded, while the supernatant containing smaller fragments is kept and moves on to the second round. In the second round, the smaller fragments in the supernatant are discarded, and the larger fragments bound to the beads are kept. As a result of removing both larger and smaller fragments, the final sample exhibits a tight fragment size distribution.*

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

4. **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.

5. **Dry Beads**: Let the tubes stand on the magnetic rack at room temperature for 3-5 minutes to air-dry. The magnetic beads should appear dull and no crack. Adjust drying time based on your beads status and environment.

6. **Resuspend Beads:** Add 51 µL of nuclease-free water to fully resuspend the beads and incubate for 2 minutes at room temperature on a normal rack (not a magnetic rack), centrifuge briefly.

7. Collect Supernatant: Place the tube on the magnetic rack until the solution becomes clear, then **transfer 50 µL of the supernatant to a new 0.2 mL PCR tube.**

***Note**: Gently pipette up and down 5 times during magnetic separation to help the beads settle.*

Step 3-3 Adaptor Ligation

1. **Prepare the Reaction Mix**: Prepare 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 3-4 Ligation Product Purification

1. **Add DNA Selection Beads**: After PCR, centrifuge briefly and add 80 μL of well-mixed DNA selection beads (0.8×), mix 10 times with a pipette, or mix by vortexing and centrifuge briefly.

***Note**: 0.8× means 80 μL of beads for 100 μL of PCR product, i.e., 80 μL / 100 μL = 0.8×.*

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). After 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 (containing the purified product) 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 3-5 Library Amplification

1. **Prepare the Reaction Mix**: Prepare 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℃	50 μL	~22-30 min
Total Cycles	**Temperature**	**Time**
	98℃	3 min
11-16 (See table below)	98℃	20 sec
	54℃	30 sec
	72℃	20 sec
	72℃	5 min
	4℃	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 3-6 Fragment Sorting

1. **First Selection:** After the reaction, briefly centrifuge the sample. Add 25 μL of the well-mixed DNA selection beads (0.5×) and mix by pipetting up and down 10 times or vortex. Incubate the mixture at room temperature for 5 minutes. Briefly centrifuge, place the tube on a magnetic rack until the solution clears.

Note 1*: 0.5× means 25 μL of beads for 50 μL of PCR product, i.e., 25 μL / 50 μL = 0.5×.*

Note 2: *During adsorption, gently pipette up and down 5 times to enhance bead adhesion.*

2. **Second Selection: Transfer the supernatant** (do **NOT** discard) to a new PCR tube with 15 μL DNA selection beads (0.8×). Pipette up and down 10 times to mix thoroughly. Incubate the mixture at room temperature for 5 minutes. Briefly centrifuge and place on the magnetic rack until clear. **Remove and discard the supernatant.**

Note 1: *0.8× refers to the volume ratio of added DNA selection beads to PCR products, i.e., (first 25 μL + second 15 μL) / 50 μL = 0.8×.*

Note 2: *During adsorption, gently pipette up and down 5 times to enhance bead adhesion.*

3. Ethanol Wash: Add 200 μL of 80% ethanol (keep placed on the magnetic rack), wait for about 30 seconds. **Carefully discard the ethanol. Repeat this wash 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 to let the ethanol evaporate completely (the magnetic beads should appear dull but no crack). 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 back on the magnetic rack, let the solution clear and **transfer 30 μL of the supernatant (containing the final library) to a new tube**. Take 1 μL for Quibit measurement.

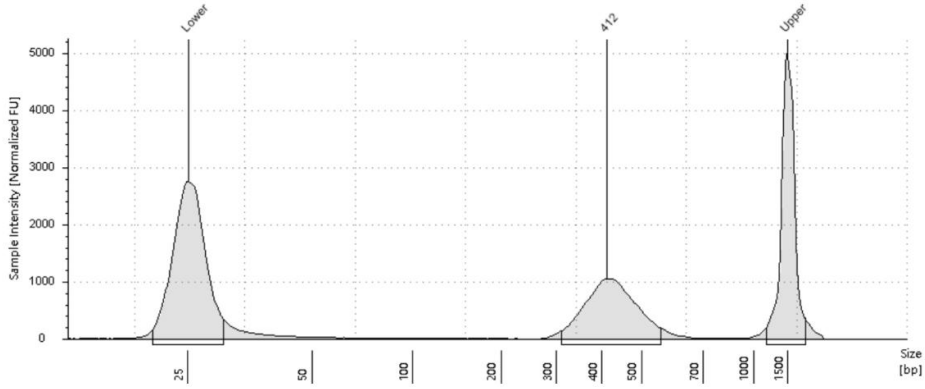
Note 1*: During adsorption, gently pipette up and down 5 times to enhance bead adhesion.*

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

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

Step 3-7 Library Quality Control

1. The qualified library has a main peak fragment size ranging from 350-750 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. Qubit 4.0 measures library concentration.

Library Quality Control Criteria:

(1) Library concentration (Qubit): ⩾5 ng/μL, no contamination by small fragments, and the main peak within the range of 350-750 bp (Agilent 4200 TapeStation/ Qsep). Considered qualified.

(2) Library concentration: 1 ng/μL ⩽ concentration < 5 ng/μL, no contamination by small fragments, and the main peak within the range of 350-750 bp. Can proceed with some risk.

(3) Library concentration: ≥ 5 ng/μL, the main peak within the range of 350-750 bp, but contamination by small fragments with lower heights than the target fragment. Can proceed with some risk.

(4) Library concentration: < 1 ng/μL, no distinct main peak, or small fragment heights higher than the target fragment. Considered not qualified.

【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

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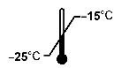
【Explanations for Symbols】

	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℃		CE mark
	Store at 2~8℃		Store at -80℃

【Edition】

V2.3

【Revised date】

2024/12/20

Appendix 1 High-throughput Sequencing

1. Sequencing Library: SeekOne™ DD Single-Cell 3' Transcriptome sequencing library starts with P5 and ends with P7 sequences. The cell barcode (CB) contains 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 GeneMind sequencing platforms, Illumina sequencing platforms, and MGI sequencing platforms.

GeneMind sequencing platforms: SURFSeq 5000

Illumina platforms: NextSeq 500/550, NextSeq 1000/2000, HiSeq 2500 (Rapid Run), HiSeq 3000/4000, NovaSeq.

MGI platform: DNBSEQ-T7RS

***Note**: For use with the MGI sequencing platform, the libraries generated in steps 3-6 require additional conversion to circular ssDNA libraries.*

3. Library Sequencing Depth and Run Parameters:

Sequencing Depth	Minimum 20,000 reads per cell, recommended ≥50,000 reads per cell
Sequencing Type	Paired-end, dual indexing
Sequencing Read Length	Read 1：29 bp N7 Index：8 bp N5 Index：8 bp Read 2：90 bp

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

***Note 2**: Recommended read length for paired-end sequencing. Read 1 should be at least 29 bp to capture complete Cell Barcode and UMI sequences, and Read 2 should be at least 90 bp for downstream single-cell transcriptome data analysis.*

4. **Library Loading Concentration**:

Platform	Instrument	Loading Concentration (pM)	PhiX（%）
Illumina	NextSeq500/550	1.8	1
	NextSeq1000/2000	650	1
	HiSeq2500(RR)	11	1
	HiSeq 4000	240	1
	NovaSeq	150*/300	1
MGI	DNBSEQ-T7RS	1	5

***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, make sure that the sequencing libraries used for pooling do not have the same indexes, as samples with the same index cannot be demultiplexed for subsequent data analysis.

Appendix 2. Bioinformatics Analysis

Analysis Software: Single-cell data analysis utilizes SeekSoul Tools, a proprietary software 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.

1) Input Files: FASTQ

2) Output Files: BAM, HTML, CSV, Matrix (filtered_feature_bc_matrix, raw_feature_bc_matrix)

3) Operating System: Linux

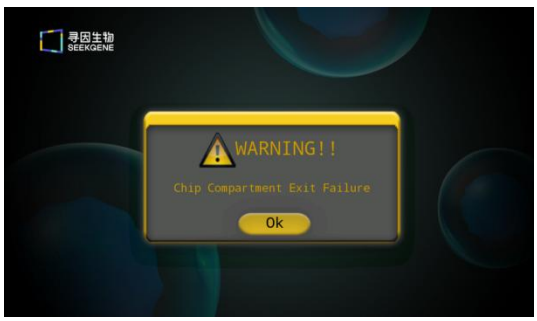
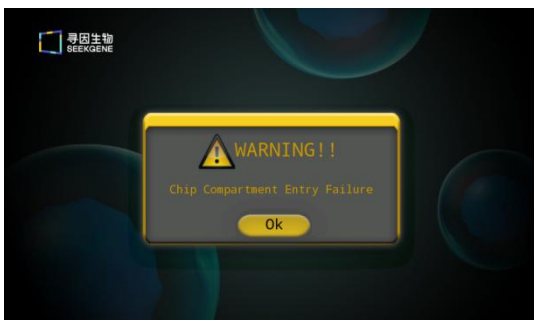
Software Access: To obtain the software package and installation instructions, please visit http://seeksoul.seekgene.com/en/index.html

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
	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.
	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.
	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.
	Please try again or restart the operation. If the message appears repeatedly, please contact (info@seekgene.com) for further assistance.

	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.
	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 V2.1	Cell line change, gel bead process modification, kit splitting	2022/07/21
3	V2.1 to V2.2	V2.2: Component change, process optimization	2023/02/28
4	V2.2 to V2.3	Library reagent replacement, library amplification process optimization	2023/12/19
5	Revised	Revision of details	2024/04/22
6	Revised	Updated link to SeekSoul Tools	2024/05/29
7	Revised	Updated the recommendations for the number of library amplification cycles in Step 3-5.	2024/7/31
8	Revised	Revised for language and term consistency.	2024/12/20