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User manual

SeekOne™ DD Single Cell 5' Transcriptome-seq Kit

SeekOne™ DD Single Cell BCR/TCR Enrichment Kit

V1.3

K00202-0201 & K00501-0202 & K00501-0203 & K00202-0204 & K00202-0205 & K00501-0206
K00202-0801 & K00501-0802 & K00501-0803 & K00202-0804 & K00202-0805 & K00501-0806

REF	Products
K00202-02	5' Transcriptome-seq, 2 tests
K00202-08	5' Transcriptome-seq, 8 tests
K00601-02	TCR, Human, 2 tests
K00601-08	TCR, Human, 8 tests
K00701-02	BCR, Human, 2 tests
K00701-08	BCR, Human, 8 tests
K01101-02	TCR, Mouse, 2 tests
K01101-08	TCR, Mouse, 8 tests
K01201-02	BCR, Mouse, 2 tests
K01201-08	BCR, Mouse, 8 tests

Envision the Future

Beijing SeekGene BioSciences Co.,Ltd

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1. Introduction

1.1 Product Overview

SeekOne™ DD Single Cell 5' 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.

The SeekOne™ DD Single Cell 5' Transcriptome-seq Kit consists of a chip (SeekOne™ DD Chip S3, Chip S3), gasket, carrier oil, gel beads (SeekOne™ DD 5' Barcoded Beads, abbreviated as Barcoded Beads), amplified reagent, library constructed reagent, and single cell data analysis software (SeekSoul Tools).

1.2 Intended Use

SeekOne™ DD Single Cell 5' 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, The labeled products are directly employed to construct a 5' gene expression library. The final result is a high-throughput single cell 5' transcriptome library that is compatible with GeneMind and Illumina sequencers. This kit is particularly suitable for research in various scientific areas, including the exploration of the tumor immune microenvironment, the investigation of immune cell regulation mechanisms, the screening of immunotherapeutic targets, as well as research on infectious diseases and vaccine screening.

1.3 Intended User

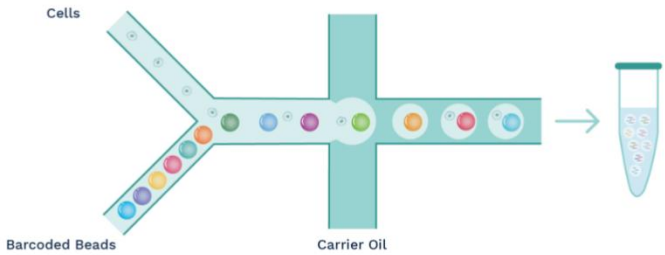
This kit is intended to be used by laboratory technicians or people with equivalent qualifications.

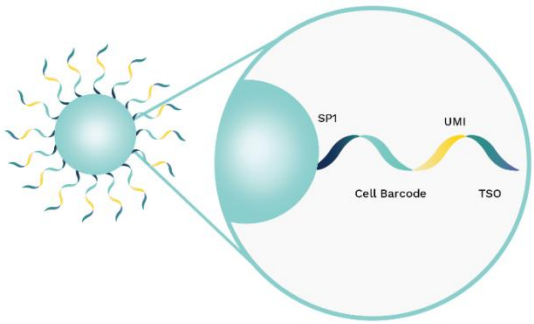
The requirements to use this kit include a certain level of theoretical knowledge and operational skills in molecular biology. After training and qualification by SeekGene, operators are capable of performing this reagent kit.

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

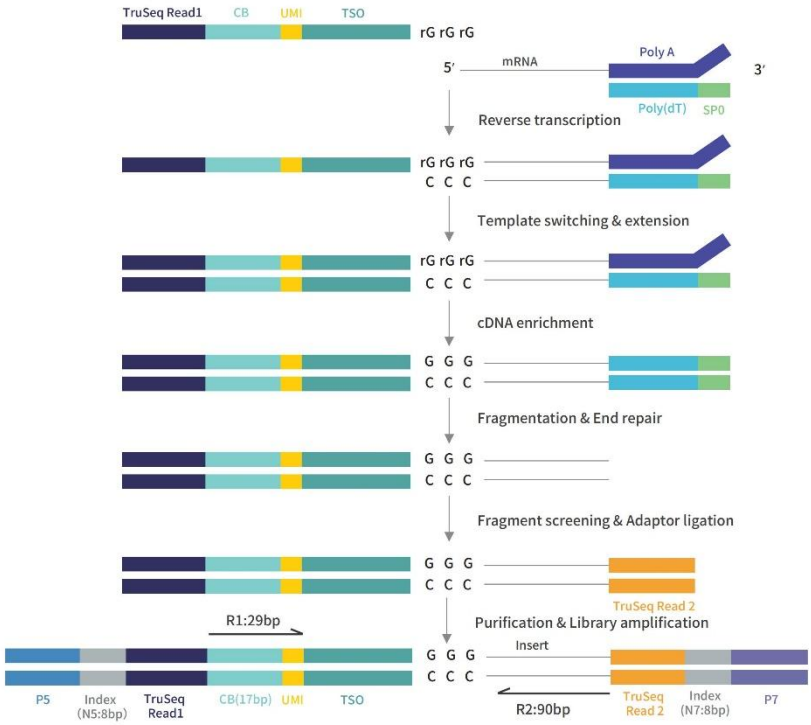
1.5 Experimental Principles



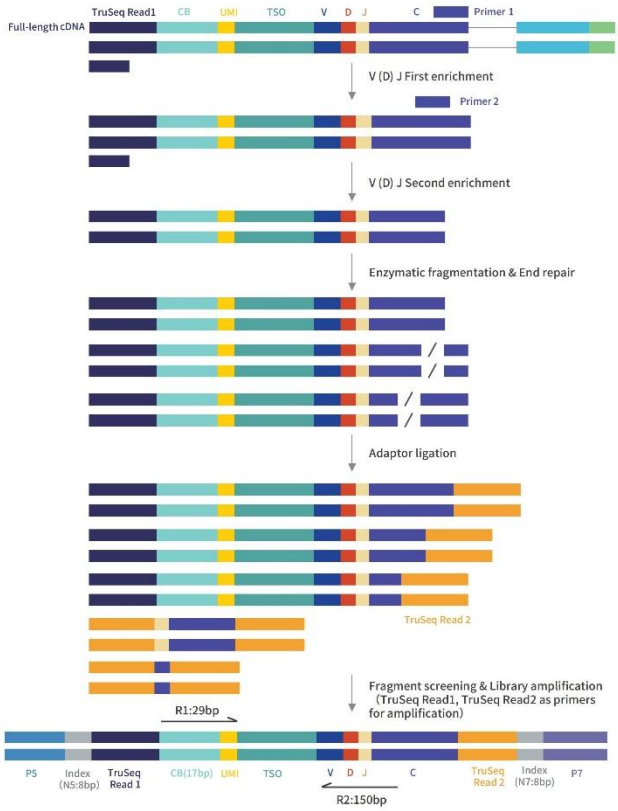


1.6 Library Construction Process

A. Single Cell 5' Transcriptome Library Construction



B. Single-cell Immune Profiling Library Construction



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.

□ Single cell 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.

- Further requirements: Cell aggregation rate ＜10%, Nucleated cell rate ＞70%.

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 5' Transcriptome-seq Kit (K00501-02, 2 tests/ K00501-08, 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 Cleanup Kit, K00202-0205 (2 tests) / K00202-0805 (8 tests)

c. SeekOne™ DD Single Cell 5' Barcoded Beads Kit, K00501-0202 (2 tests) / K00501-0802 (8 tests)

d. SeekOne™ DD Single Cell 5' Reverse Transcription Kit, K00501-0203 (2 tests) / K00501-0803 (8 tests)

e. SeekOne™ DD Single Cell 5' cDNA Amplification Kit, K00501-0206 (2 tests) / K00501-0806 (8 tests)

f. SeekOne™ DD Library Construction Kit, K00202-0204 (2 tests) / K00202-0804 (8 tests)

1.9.1 SeekOne™ DD Single Cell 5' Transcriptome-seq Kit, 2 tests, REF: K00501-02

Name & PN & Storage	Cap 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 5' Barcoded Beads Kit V1.3, K00501-0202, **-80℃**	○	Single Cell 5' Barcoded Beads	R0006201	45 μL ×2 tubes
	○	Enhancer	R0011601	10 μL
SeekOne™ DD Single Cell 5' Reverse Transcription Kit V1.3, K00501-0203, **-20 ± 5℃**	●	3x RT Buffer	R0008401	80 μL
	●	RT Enzyme	R0003801	15 μL
	●	RT Primer	R0006301	10 μL
	●	Reducing Buffer	R0003901	100 μL
SeekOne™ DD Single Cell 5' cDNA Amplification Kit V1.3, K00501-0206, **-20 ± 5℃**	●	2×PCR Master Mix	R0002101	60 μL
	●	cDNA Primers	R0004001	10 μL
	●	Human Additive	R0011701	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

Table 1 SeekOne™ DD Single Cell 5' Transcriptome-seq Kit, 2 tests, REF: K00501-02

1.9.2 SeekOne™ DD Single Cell 5' Transcriptome-seq Kit, 8 tests, REF: K00501-08

Name & PN & Storage	Cap 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 5' Barcoded Beads Kit V1.3, K00501-0802, **-80℃**	○	Single Cell 5' Barcoded Beads	R0006201	45 μL ×8 tubes
	○	Enhancer	R0011602	20 μL
SeekOne™ DDSingle Cell 5' Reverse Transcription Kit V1.3, K00501-0803, **-20 ± 5℃**	●	3x RT Buffer	R0008402	280 μL
	●	RT Enzyme	R0003802	50 μL
	●	RT Primer	R0006302	20 μL
	●	Reducing Buffer	R0003901	100 μL
SeekOne™ DD Single Cell 5' cDNA Amplification Kit V1.3, K00501-0206, **-20 ± 5℃**	●	2×PCR Master Mix	R0002102	240 μL
	●	cDNA Primers	R0004002	20 μL
	●	Human Additive	R0011702	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

Table 2 SeekOne™ DD Single Cell 5' Transcriptome-seq Kit, 8 tests, REF: K00501-08

1.9.3 SeekOne™ DD Single Cell Immune Profiling Kits

(1) The SeekOne™ DD Single Cell TCR Enrichment Kit (Human) V1.3 (REF: K00601-02, 2 tests; K00601-08, 8 tests) contains:

a. SeekOne™ DD Single Cell TCR Amplification Kit (Human) V1.3, K00601-0201 (2 tests) / K00601-0801 (8 tests)

b. SeekOne™ DD Library Construction Kit, K00202-0204 (2 tests) / K00202-0804 (8 tests)

Name & PN & Storage	Cap Color	Component	CN	2 tests
SeekOne™ DD Single Cell TCR Amplification Kit (Human) V1.3, K00601-0201, store at **-20 ± 5℃**	🔴	2×PCR Master Mix	R0002101	60 µL x 2 tubes
	🟢	Human TCR Primers 1	R0006501	10 µL
	🟢	Human TCR Primers 2	R0006601	10 µL
SeekOne™ DD Library Construction Kit V1.0, K00202-0204, store at -20 ± 5℃. **Same as in Table 1.**				

Table 3 SeekOne™ DD Single Cell TCR Enrichment Kit (Human) , REF: K00601-02, 2 tests

Name & PN & Storage	Cap Color	Components	CN	8 tests
SeekOne™ DD Single Cell TCR Amplification Kit (Human) V1.3, K00601-0801, store at **-20 ± 5℃**	🔴	2×PCR Master Mix	R0002102	240 µL x 2 tubes
	🟢	Human TCR Primers 1	R0006502	30 µL
	🟢	Human TCR Primers 2	R0006602	30 µL
SeekOne™ DD Library Construction Kit V1.0, K00202-0804, store at -20 ± 5℃. **Same as in Table 2.**				

Table 4 SeekOne™ DD Single Cell TCR Enrichment Kit (Human) , REF: K00601-08, 8 tests

(2) The SeekOne™ DD Single Cell BCR Enrichment Kit (Human) V1.3 (REF: K00701-02, 2 tests; K00701-08, 8 tests) contains:

a. SeekOne™ DD Single Cell BCR Amplification Kit (Human) V1.3, K00701-0201 (2 tests) / K00701-0801 (8 tests)

b. SeekOne™ DD Library Construction Kit, K00202-0204 (2 tests) / K00202-0804 (8 tests)

Name & PN & Storage	Cap Color	Components	CN	2 tests
SeekOne™ DD Single Cell BCR Amplification Kit (Human) V1.3, K00701-0201, store at **-20 ± 5℃**	🔴	2×PCR Master Mix	R0002101	60 µL x 2 tubes
	🔵	Human BCR Primers 1	R0006701	10 µL
	🔵	Human BCR Primers 2	R0006801	10 µL
SeekOne™ DD Library Construction Kit V1.0, K00202-0204, store at -20 ± 5℃. **Same as in Table 1.**				

Table 5 SeekOne™ DD Single Cell BCR Enrichment Kit (Human) , REF: K00701-02, 2 tests

Name & PN & Storage	Cap Color	Components	CN	8 tests
SeekOne™ DD Single Cell BCR Amplification Kit (Human) V1.3, K00701-0801, store at **-20 ± 5℃**	🔴	2×PCR Master Mix	R0002102	240 µL x 2 tubes
	🔵	Human BCR Primers 1	R0006702	30 µL
	🔵	Human BCR Primers 2	R0006802	30 µL
SeekOne™ DD Library Construction Kit V1.0, K00202-0804, store at -20 ± 5℃. **Same as in Table 2.**				

Table 6 SeekOne™ DD Single Cell BCR Enrichment Kit (Human) , REF: K00701-08, 8 tests

(3) The SeekOne™ DD Single Cell TCR Enrichment Kit (Mouse) V1.3 (REF: K01101-02, 2 tests; K01101-08, 8 tests) contains:

a. SeekOne™ DD Single Cell TCR Amplification Kit (Mouse) V1.3, K01101-0201 (2 tests) / K01101-0801 (8 tests)

b. SeekOne™ DD Library Construction Kit, K00202-0204 (2 tests) / K00202-0804 (8 tests)

Name & PN & Storage	Cap Color	Components	CN	2 tests
SeekOne™ DD Single Cell TCR Amplification Kit (Mouse) V1.3, K01101-0201, store at **-20 ± 5℃**	🔴	2×PCR Master Mix	R0002101	60 µL x 2 tubes
	🟢	Mouse TCR Primers 1	R0007601	10 µL
	🟢	Mouse TCR Primers 2	R0007701	10 µL
SeekOne™ DD Library Construction Kit V1.0, K00202-0204, store at -20 ± 5℃. **Same as in Table 1.**				

Table 7 SeekOne™ DD Single Cell TCR Enrichment Kit (Mouse) , REF: K01101-02, 2 tests

Name & PN & Storage	Cap Color	Components	CN	8 tests
SeekOne™ DD Single Cell TCR Amplification Kit (Mouse) V1.3, K01101-0801, store at **-20 ± 5℃**	🔴	2×PCR Master Mix	R0002102	240 µL x 2 tubes
	🟢	Mouse TCR Primers 1	R0007602	30 µL
	🟢	Mouse TCR Primers 2	R0007702	30 µL
SeekOne™ DD Library Construction Kit V1.0, K00202-0804, store at -20 ± 5℃. **Same as in Table 2.**				

Table 8 SeekOne™ DD Single Cell TCR Enrichment Kit (Mouse) , REF: K01101-08, 8 tests

(4) The SeekOne™ DD Single Cell BCR Enrichment Kit (Mouse) V1.3 (REF: K01201-02, 2 tests; K01201-08, 8 tests) contains:

a. SeekOne™ DD Single Cell BCR Amplification Kit (Mouse) V1.3, K01201-0201 (2 tests) / K01201-0801 (8 tests)

b. SeekOne™ DD Library Construction Kit, K00202-0204 (2 tests) / K00202-0804 (8 tests)

Name & PN & Storage	Cap Color	Components	CN	2 tests
SeekOne™ DD Single Cell BCR Amplification Kit (Mouse) V1.3, K01201-0201, store at **-20 ± 5℃**	🔴	2×PCR Master Mix	R0002101	60 µL x 2 tubes
	🔵	Mouse BCR Primers 1	R0007801	10 µL
	🔵	Mouse BCR Primers 2	R0007901	10 µL
SeekOne™ DD Library Construction Kit V1.0, K00202-0204, store at -20 ± 5℃. **Same as in Table 1.**				

Table 9 SeekOne™ DD Single Cell BCR Enrichment Kit (Mouse) , REF: K01201-02, 2 tests

Name & PN & Storage	Cap Color	Components	CN	8 tests
SeekOne™ DD Single Cell BCR Amplification Kit (Mouse) V1.3, K01201-0801, store at **-20 ± 5℃**	🔴	2×PCR Master Mix	R0002102	240 µL x 2 tubes
	🔵	Mouse BCR Primers 1	R0007802	30 µL
	🔵	Mouse BCR Primers 2	R0007902	30 µL
SeekOne™ DD Library Construction Kit V1.0, K00202-0804, store at -20 ± 5℃. **Same as in Table 2.**				

Table 10 SeekOne™ DD Single Cell BCR Enrichment Kit (Mouse) , REF: K01201-08, 8 tests

1.9.4 Storage Conditions

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 5' Barcoded Beads Kit	K00501-0202/ K00501-0802	Dry ice	-80℃
SeekOne™ DD Single Cell 5' Reverse Transcription Kit	K00501-0203/ K00501-0803	Dry ice	-20 ± 5℃
SeekOne™ DD Single Cell 5' cDNA Amplification Kit	K00501-0206/ K00501-0806	Dry ice	-20 ± 5℃
SeekOne™ DD Library Construction Kit	K00202-0204/ K00202-0804	Dry ice	-20 ± 5℃
SeekOne™ DD Single-cell TCR Amplification Kit (Human)	K00601-0201/ K00601-0801	Dry ice	-20 ± 5℃
SeekOne™ DD Single-cell BCR Amplification Kit (Human)	K00701-0201/ K00701-0801	Dry ice	-20 ± 5℃
SeekOne™ DD Single-cell TCR Amplification Kit (Mouse)	K01101-0201/ K01101-0801	Dry ice	-20 ± 5℃
SeekOne™ DD Single-cell BCR Amplification Kit (Mouse)	K01201-0201/ K01201-0801	Dry ice	-20 ± 5℃

Table 11 Storage Conditions

1.9.5 Index sequence

Index Number	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	

Table 12 Index sequence

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.*

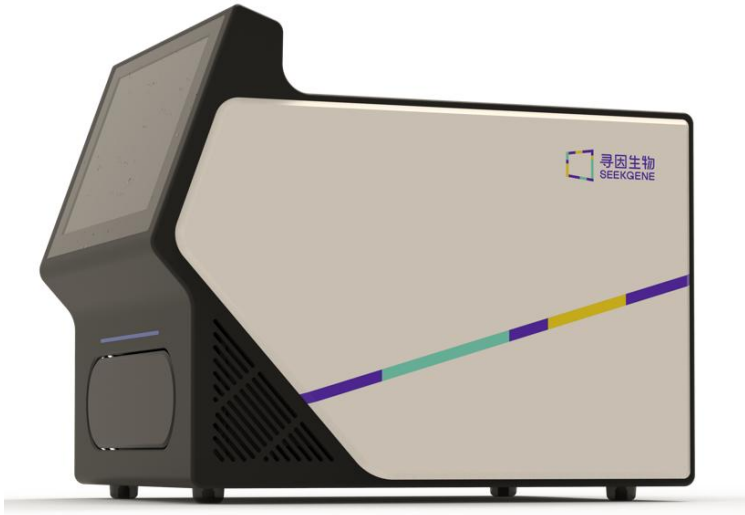
Note 2: *The Index sequence provided by this kit can label up to 16 samples simultaneously.*

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

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

1.9.6 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

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		
~2.2 h	2-1 Water-in-Oil Demulsification	**45 min**
	2-2 cDNA Amplification	30 min
	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
After step 2, for **5' Transcriptome Library Construction proceed directly to **step 3.***		
*For **V(D)J** Amplification and V(D)J Library Construction proceed to **step 4 and step 5**.*		
Step 3 5' Transcriptome 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 4 Full-Length V(D)J Fragment Enrichment		
~3 h	4-1 First Enrichment of Full-length V (D) J Fragments	30-40 min
	4-2 V (D) J Enriched Product Fragment Sorting	40 min
	○ ***STOPPING POINT: 4℃ ≤72 h or −20℃ ≤1 month***	
	4-3 Second Enrichment of Full-length V (D) J Fragments	30-40 min
	4-4 Enriched Product Fragment Sorting	40 min
	○ ***STOPPING POINT: 4℃ ≤72 h or −20℃ ≤1 month***	
	4-5: Full-length V(D)J Enrichment Product Quality Control	30 min
Step 5 V(D)J Library Construction		
~3 h	5-1 DNA Fragmentation and End Repair	35 min
	5-2 Adaptor Ligation	20 min
	5-3 Purification of Ligation Product	20 min
	5-4 Library Amplification	30 min
	5-5 Fragment Sorting	30 min
	○ ***STOPPING POINT: -20℃ ≤6 months***	
	5-6 Library Quality Control	30 min

Step1 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 5' Reverse Transcription Kit**:

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

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

☐ **SeekOne™ DD Single Cell 5' Barcoded Beads Kit**:

■ Remove **Barcoded Beads** from the -80℃ freezer in advance and equilibrate at room temperature for 30 minutes. Put back to -80℃ freezer immediately after use. It should not stay at room temperature for more than 2 hours.

■ Take out the **Enhancer** from the -80℃ freezer in advance for thawing, after thawing vortex them thoroughly, centrifuge briefly and place them on ice until use.

☐ 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.

☐ Set up the program for the deep-well thermal cycler.

***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.

Components	Volume/Sample
3x RT Buffer	26.6 μL
RT Enzyme	5.2 μL
RT Primer	2.0 μL
Enhancer	1.0 μL
Reducing Buffer	1.6 μL
Total	**36.4 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 has high viscosity. When pipetting, avoid inserting the pipette tip too deep into the liquid, and aspirate slowly to prevent insufficient reagent due to wall adhesion.*

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

a. 36.4 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. The Master Mix is always 36.4 μL. The remaining volume (43.6 μL) is made up of nuclease-free water and cells.

b. Subtract the calculated volume of cells from 43.6 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 cells × 2) / 900 cells/μL = 11.1 μL of cell suspension.

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

a. Pipette 36.4 μL of the Master Mix into a small tube (e.g. 500μL) on ice.

b. Subtract the calculated volume of cells from 43.6 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 36.4 μL of the Master Mix.

Then pipette 32.5 μL nuclease-free water (43.6 μL-11.1 μL=32.5 μL).

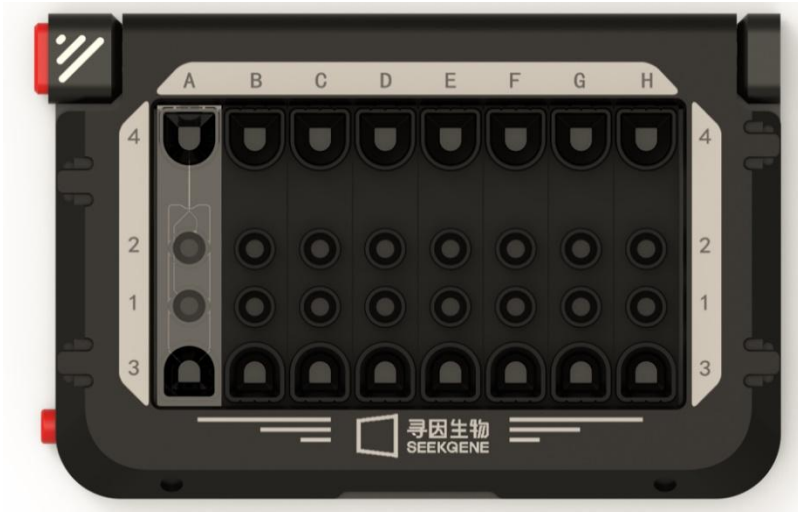
Finally, pipette 11.1 μL of cells into the mixture.

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 allow loading of the Chip S3. For unused positions, insert the 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 mix from above (total of 80 μL) by pipetting it up and down 15 times with a pipette. Take **78 μL** of the single-cell mix and insert the tip of the pipette tip 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**: 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 the **Well 2**, keeping the tip slightly above the bottom, and pipette slowly to avoid bubbles.

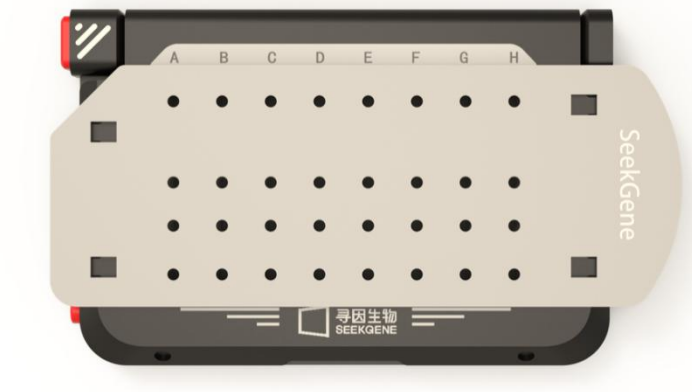
***Note 1:** When adding reagents, keep the pipette tip moving with the liquid level and always ensure that only the tip of the pipette is below the liquid surface by 3 mm to avoid generating bubbles.*

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

4. **Add Carrier Oil**: Pipette 120 μL of Carrier Oil with a 200 μL pipette into the **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.



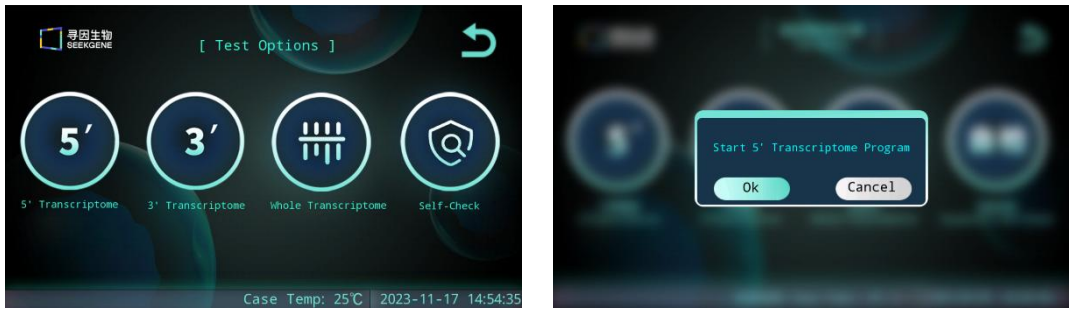
Note: *Do not touch the smooth surface of Gasket.*

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 holder tray.

3. **Start the Program**: Click the "5' 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.

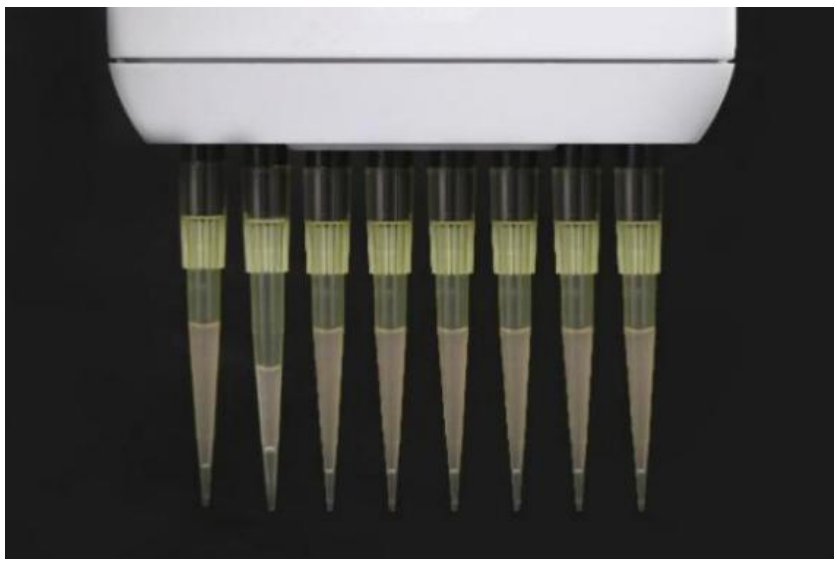
***Note**: Well 1 should have ≤ 10 μL, and Well 2 should have ≤ 15 μL. Larger volumes indicate a blocked chip.*

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 sec) 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℃	100 µL	~95 min
Step	**Temperature**	**Time**
1	42℃	90 min
2	85℃	5 min
3	4℃	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.

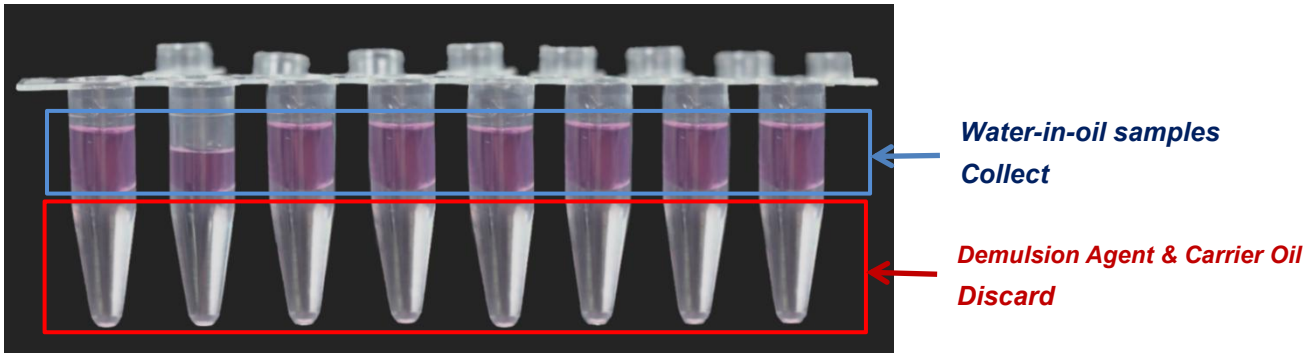
☐ **Thaw Reagents**: Remove **2× PCR Master Mix, cDNA Primers, Reducing Buffer** and **Human Additive** from -20℃, put on ice in advance to thaw. When thawed, vortex thoroughly, centrifuge briefly, and place on ice until use.

☐ **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 sure to prepare it just before use and use it within 24 hours.

☐ **Warm Cleanup Beads**: Equilibrate the Cleanup Beads (from 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:

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 slowly pipetting from the **bottom** of the PCR tube, leaving 2-5 μL clear mixture at the bottom of the tube to avoid taking 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 the tubes at room temperature for a total of 10 minutes with the lid open (the tube is too full to close, liquid would stick to the lid). 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 adsorption, 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 23 µL of nuclease-free water to fully resuspend 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 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 22 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**:

A. For **human** tissue or cell samples, prepare the mix using the table below.

Component	Volume/Sample
2×PCR Master Mix	25 µL
cDNA Primers	2 µL
Human Additive	1 µL
Total	**28 µL**

B. For samples from **non-human** species, prepare the mix using the table below.

Component	Volume/Sample
2×PCR Master Mix	25 µL
cDNA Primers	2 µL
Nuclease-free Water	1 µL
Total	**28 µL**

2. **Mix and Start PCR**: Add the prepared 28 μL cDNA amplification mix to the 22 μL cDNA sample purified in Step 2-1, pipetting and mixing 10 times, briefly centrifuge, and then proceed with PCR. Set up the PCR program as follows:

Lid Temperature	Reaction Volume	Run Time
105℃	50 μL	~28-38 min
Total Cycles	**Temperature**	**Time**
	98℃	3 min
11-16 (See table below)	98℃	20 sec
	63℃	30 sec
	72℃	1 min
	72℃	5 min
	4℃	Hold

Cell diameter	Number of loaded cells	Recommended cycles
≤10 μm	500-5,000	16
	5,000-15,000	14
	15,000-24,000	13
＞10 μm	500-5,000	14
	5,000-15,000	12
	15,000-24,000	11

Centrifuge the samples after the PCR run is completed.

Step 2-3 cDNA Enrichment Product Purification

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

***Note**: 0.6x refers to the volume ratio of added DNA sorting magnetic beads to PCR products, which is 30μL / 50μL=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. The beads will stick to the magnet and the solution will appear clear after 1-2 minutes. **Then 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.

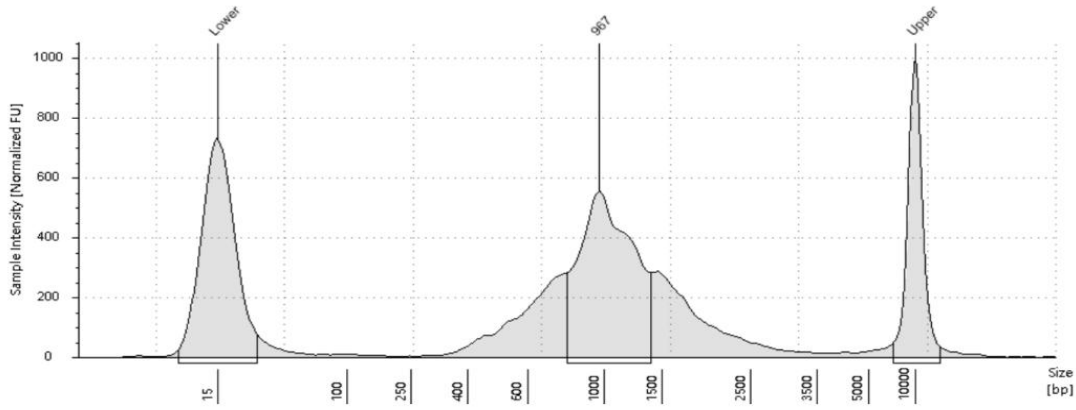
5. **Dry and Resuspend Beads**: Keep the tubes open and allow the ethanol to evaporate for about 3-5 minutes. Monitor the beads closely—they should turn dull and matte but not dry out completely (avoid cracks on the bead surface). Adjust drying time based on your beads status and environment. Add 41 µL of nuclease-free water to fully suspend the beads, vortex for ~20 seconds. 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.

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 250-5000 bp with major peaks in the 750-2,000bp range without small fragments, and there is no small fragment. 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 **500-5,000 cells**: cDNA concentration (Qubit) **≥ 1 ng/µL**, fragment size range: 250-5,000 bp, main peak within 750-2,500 bp range, indicating a pass.

(2) For capturing **6,000-10,000 cells**: cDNA concentration (Qubit) **≥ 3 ng/μL**, fragment size range: 250-5,000 bp, main peak within 750-2,500 bp range, indicating a pass.

(3) If the concentration (Qubit) is between 0.5 ng/μL and <1 ng/μL: fragment size range: 250-5,000 bp, main peak within 750-2,500 bp range. Alternatively, if the concentration (Qubit) is ≥ 1 ng/μL: detected peak range between 250-5,000 bp, but main peak falls outside the 750-2,500 bp range, indicating 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.

***Note**: After step 2, for 5' Transcriptome Library Construction proceed directly to step 3. For V(D)J Amplification and V(D)J Library Construction proceed to step 4 and step 5.*

Step 3 5' Transcriptome Library Construction

Step 3-0 Prepare Before the Experiment

☐ **Prepare Ice**: Prepare a box with ice in advance.

☐ **Thaw Reagents**: Take out in advance the **Fragmentation Buffer, Ligation Buffer, Adaptor** and **2×PCR Mix** from -20℃ 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℃ directly before use, immediately briefly centrifuge, then pipetted and put back at -20℃, 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℃	50 μL	~35 min
Step	**Temperature**	**Time**
1	4℃	Hold
2	32℃	5 min
3	65℃	30 min
4	4℃	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℃ for up to 72 h or at −20℃ 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 ng ÷ 5 ng/μL = 10 μL).*

Components	Volume/Sample
CDNA enriched product	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, and 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, then centrifuge briefly. Place the tube on the magnetic rack until the solution appears 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 another PCR tube containing 10 μL of DNA selection beads (0.8×). Mix by pipetting up and down 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 volume ratio of added DNA selection beads to PCR products, i.e., (first 30 μL + second 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), wait for about 30 seconds. Carefully **remove the supernatant ethanol and discard it. 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 let the tubes stand for 2 minutes at room temperature on a normal rack (not a magnetic rack), then centrifuge briefly.

7. **Collect Supernatant:** Place the tube on the magnetic rack until the solution becomes clear. **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 the reaction system according to the following table, fully vortex and centrifuge briefly.

Components	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
Step	**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 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 (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 the reaction system according to the following table, fully vortex and centrifuge briefly.

Components	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 program according to the table below and run the thermal cycler.

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 is completed, centrifuge briefly to ensure proper settling. 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, centrifuge briefly. Place the tube on the 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 another PCR tube containing 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, then close the lid and centrifuge briefly. Place the tube on the magnetic rack until the solution appears 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).

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. **Collect Supernatant:** Place the tube on the magnetic rack to allow the beads to attach and the solution to become clear. **Transfer 30 μL of the supernatant (containing the final library) to a new tube.** Take 1 μL for Quibit measurement.

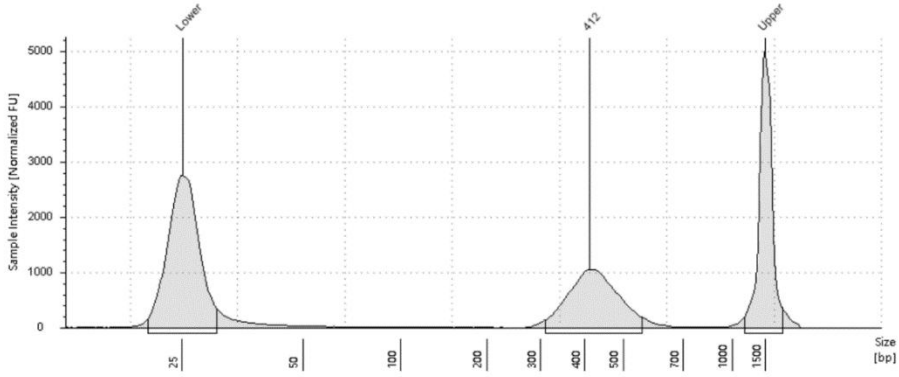
Note 1*: During adsorption, gently pipette up and down 5 times to enhance bead adhesion.*

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 measure library concentration.

Library Quality Control Criteria:

1. Library concentration ≥ 5 ng/μL, with fragment sizes ranging from 250 bp to 1500 bp, no small fragment contamination, and the main peak falling within the 350-750 bp range. Considered qualified.

2. Library concentration between 1 ng/μL and < 5 ng/μL, with fragment sizes ranging from 250 bp to 1500 bp, no small fragment contamination, and the main peak falling within the 350-750 bp range. Can proceed with some risk.

3. Library concentration ≥ 5 ng/μL, with fragment sizes ranging from 250 bp to 1500 bp, and the main peak falling within the 350-750 bp range, but with small fragment contamination. The height of the small fragments is lower than that of the target fragments. Can proceed with some risk.

4. Library concentration < 1 ng/μL, or no detectable target fragments in the 250-1500 bp range, no clear main peak, or the height of the small fragments is higher than that of the target fragments. Considered not qualified.

Step 4: Full-Length V(D)J Fragment Enrichment

Step 4-0 Preparation Before the Experiment

1. **Prepare Ice**: Prepare a box with ice in advance.

2. **Thaw Reagents**: Take out in advance the **TCR or BCR enrichment reagents** of the corresponding species (human or mouse) from -20℃ put onto ice to thaw, vortex thoroughly, centrifuge briefly, and place on ice until use.

3. **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.

4. **Warm DNA Selection Beads**: Let the DNA selection beads reach room temperature for at least 30 minutes before use.

Step 4-1 First Enrichment of Full-length V (D) J Fragments

1. **Prepare the Reaction Mix:** Prepare the reaction system according to the following table. Vortex it well, centrifuge briefly, and put on ice until use.

Components	Volume/Sample	
2×PCR Master Mix	25 μL	
Human BCR Primers 1	3 μL	*Choose one suitable reagent for your experiment
Mouse BCR Primers 1	3 μL	
Human TCR Primers 1	3 μL	
Mouse TCR Primers 1	3 μL	
Nuclease-free Water	19 μL	
Total	**47μL**	

2. **Add cDNA Samples:** Add 3 μL of cDNA sample generated from the 5' transcriptome (Step 2) to the prepared 47 μL reaction system, pipette up and down 15 times on ice, centrifuge briefly and put back onto ice.

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

Lid Temperature	Reaction Volume	Run Time
105℃	50 μL	~26-30 min
Total cycles	**Temperature**	**Time**
	98℃	3 min
TCR:12 BCR:10	98℃	20 sec
	60℃	30 sec
	72℃	1 min
	72℃	5 min
	4℃	Hold

Step 4-2 V (D) J Enriched Product Fragment Sorting

1. **First Selection:** Immediately after the reaction, centrifuge briefly to ensure proper settling. Add 25 μL DNA selection beads (0.5×) 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, then close the lid and centrifuge briefly. Place the tube on the magnetic rack until the solution appears clear.

***Note 1**: 0.5× means 25 μL of beads for a 50 μL 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 it)** to another PCR tube containing 15 μL of DNA selection beads (0.8×) . Mix by pipetting up and down 10 times. Incubate the mixture at room temperature for 5 minutes, then close the lid and centrifuge briefly. Place the tube on the magnetic rack until the solution appears clear. **Remove the supernatant and discard it.**

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 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).

6. **Resuspend Beads:** Add 23 μL of nuclease-free water to fully suspend 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. **Transfer 22 μL of the supernatant to a new 0.2 mL PCR tube.**

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

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

Step 4-3 Second Enrichment of Full-length V (D) J Fragments

1. **Prepare the Reaction Mix**: Prepare the reaction system according to the following table. Vortex it well, centrifuge briefly, and put on ice until use.

Components	Volume/Sample	
2×PCR Master Mix	25 μL	
Human BCR Primers 2	3 μL	*Choose one suitable reagent for your experiment
Mouse BCR Primers 2	3 μL	
Human TCR Primers 2	3 μL	
Mouse TCR Primers 2	3 μL	
Total	**28μL**	

2. **Add Mix to Samples**: Add the prepared 28 μL of the mixture to the purified 22 μL enrichment product from the previous step. Pipette up and down 10 times to mix thoroughly. Centrifuge briefly, and then proceed with PCR.

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

Lid Temperature	Reaction Volume	Run Time
105℃	50 μL	~26-30 min
Total cycles	**Temperature**	**Time**
	98℃	3 min
TCR:10 BCR:10	98℃	20 sec
	60℃	30 sec
	72℃	1 min
	72℃	5 min
	4℃	Hold

Step 4-4 Enriched Product Fragment Sorting

1. **First Selection:** After the end of the PCR reaction, centrifuge briefly and add 25 μL of well-mixed DNA selection beads (0.5×), mix 10 times with a pipette or mix by vortexing and centrifuge briefly. Incubate the mixture at room temperature for 5 minutes, then close the lid and centrifuge briefly. Place the tube on the magnetic rack until the solution appears clear.

***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 it)** to another PCR tube containing 15 μL of DNA selection beads (0.8×). Mix by pipetting up and down 10 times. Incubate the mixture at room temperature for 5 minutes, then close the lid and centrifuge briefly. Place the tube on the magnetic rack until the solution appears clear. **Remove the supernatant and discard it.**

***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 **remove the supernatant ethanol and discard it. Repeat this step once.**

4. **Remove Ethanol:** Briefly centrifuge the tube, place it back on the magnetic rack, and use a 10 μL pipette to **remove any remaining ethanol**. During centrifugation, ensure that the beads side of the tubes is facing away from the centrifuge's center.

5. **Dry Beads:** Let the beads dry on the magnetic rack for 3-5 minutes until they appear dull but not cracked. 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 let the tubes stand for 2 minutes at room temperature on a normal rack (not a magnetic rack), then centrifuge briefly.

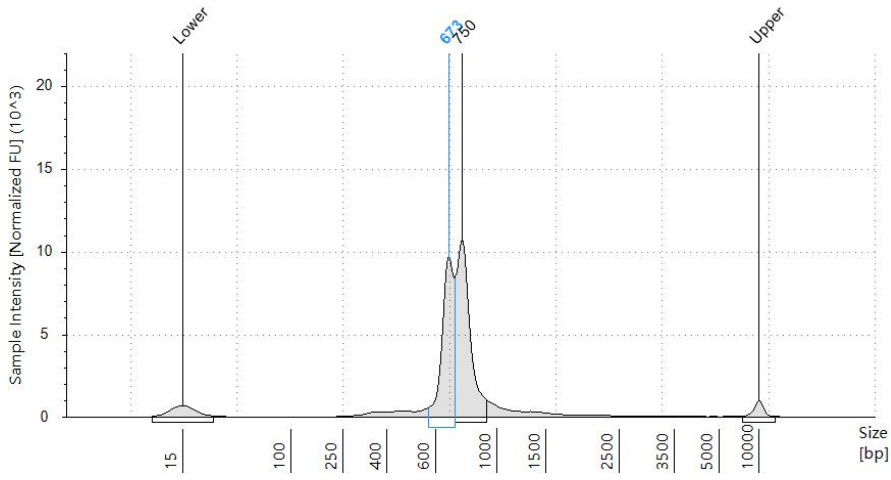
7. Collect Supernatant: Place the tube on the magnetic rack until the solution becomes clear. **Transfer 50 μL of the supernatant to a new 0.2 mL PCR tube.**

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

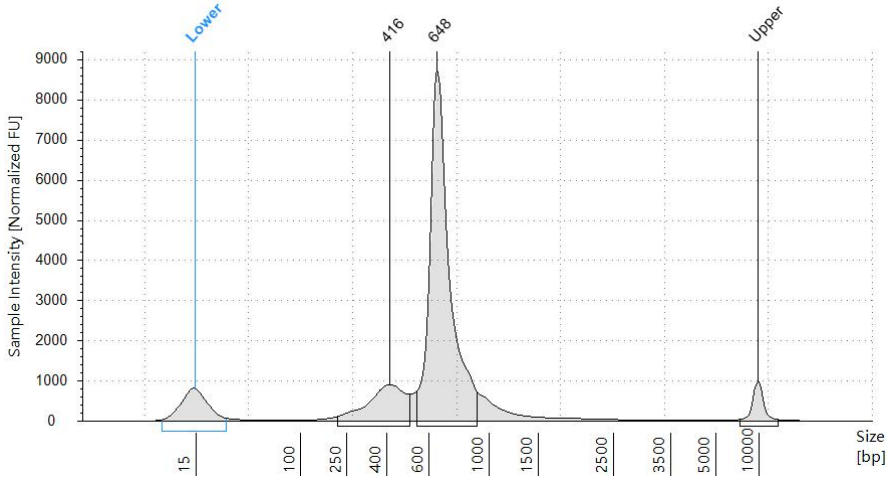
***STOPPING POINT**: The products generated in step 4-4 can be stored at 4℃ for up to 72 hours or at -20℃ for up to 1 month.*

Step 4-5: Full-length V(D)J Enrichment Product Quality Control

1. For BCR/TCR Enrichment products, if there are small fragments below 200bp present, perform an additional 0.75X purification step until no small fragments are detected (using Agilent 4200 TapeStation/ Qsep).



BCR Enrichment products



TCR Enrichment product

2. Measure the concentration of the full-length V(D)J enriched products using Qubit 4.0.

cDNA Quality Control Criteria:

(1) For capturing 500-5,000 cells: cDNA concentration (Qubit) should be ⩾5 ng/μL, fragment size range: 250-5,000 bp, main peak within 500-1,000 bp range, indicates a qualified sample.

(2) For capturing 6,000-10,000 cells: cDNA concentration (Qubit) should be ⩾10 ng/μL, fragment size range: 250-5,000 bp, main peak within 500-1,000 bp range, indicates a qualified sample.

(3) If the cDNA concentration (Qubit) is between 0.5 ng/μL and <1 ng/μL, the fragment size range: 250-5,000 bp, the main peak within 750-2,500 bp range, indicates a potential risk.

(4) If the cDNA concentration (Qubit) is ⩾1 ng/μL, the fragment size range: 250-5,000 bp, the main peak not in the 750-2,500 bp range, indicates a potential risk.

(5) If the cDNA concentration (Qubit) is <0.5 ng/μL or if no target fragments are detected in the 250-5,000 bp range and no clear main peak is observed, the sample is deemed unqualified.

Step 5: V(D)J Library Construction

Step 5-0 Preparation Before the Experiment

1. **Prepare Ice**: Prepare a box with ice in advance.

2. **Thaw Reagents**: Take out in advance the **Fragmentation Buffer, Ligation Buffer, Adaptor,** and **2×PCR Master Mix** from -20℃ put onto ice to thaw, vortex thoroughly, and centrifuge briefly, and place on ice until use.

3. **Handle Enzymes**: **Fragmentation Enzyme** and **DNA Ligase** should only be taken out from -20℃ directly before use, immediately briefly centrifuge, then pipetted and put back at

-20˚C, because enzymes are temperature sensitive.

4. **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.

5. **Warm DNA Selection Beads**: Let the DNA selection beads reach room temperature for at least 30 minutes before use.

Step 5-1 DNA Fragmentation and End Repair

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

Lid Temperature	Reaction Volume	Run Time
70˚C	50 µL	~32 min
Step	**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 nuclease-free water to complete the reaction.

If the total cDNA amount is less than 50 ng, take 15 µL of cDNA enrichment products 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 for up to 72 h or at −20˚C for up to 1 month for generating additional libraries.

Components	Volume/Sample
V (D) J enriched product	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 mix on ice, mix by pipetting up and down 15 times, 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 5-2 Adaptor Ligation

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

Components	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 mixture to the fragmentation product generated from step 5-1. Mix 15 times with a pipette, and centrifuge briefly.

3. **Run the PCR Program:** Set the PCR program according to the following table. Perform the reaction with a thermal cycler.

Lid Temperature	Reaction Volume	Run Time
30˚C	100 μL	~15 min
Step	**Temperature**	**Time**
1	20˚C	15 min
2	4˚C	Hold

Step 5-3 Purification of Ligation Product

1. **Add DNA Selection Beads**: After the end of the PCR reaction, 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. **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 (keeping on the magnetic rack) for approximately 30 seconds, carefully **remove the supernatant ethanol and discard it. Repeat this step once.**

4. **Remove Ethanol:** Briefly centrifuge the tube, place it back on the magnetic rack, and use a 10 μL pipette to **remove any remaining ethanol**. 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 post-ligation 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 5-4 Library Amplification

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

Components	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 mixture to the post-ligation products generated from step 5-3, mix 15 times with a pipette, and centrifuge briefly.

3. **Run PCR**: Set the PCR program according to the following conditions, perform the reaction with a thermal cycler.

Lid Temperature	Reaction Volume	Run Time
105℃	50 μL	~15-20 min
Total cycles	**Temperature**	**Time**
	98℃	3 min
6-10 (See table below)	98℃	20 sec
	54℃	30 sec
	72℃	20 sec
	72℃	5 min
	4℃	**Hold**

CDNA	Recommended cycles
1-50 ng	8-10
50-100 ng	6-8

Step 5-5 Fragment Sorting

1. **Add DNA Selection Beads:** After the reaction is completed, centrifuge briefly to ensure proper settling. Add 40 μL of the well-mixed DNA selection beads(0.8×), pipette up and down 10 times or mix by vortexing, then centrifuge briefly.

Note: 0.8× refers to the volume ratio of added DNA selection beads to PCR products, i.e., 40 μL / 50μL = 0.8×.

2. Incubate and Remove Supernatant: Incubate the mixture at room temperature for 5 minutes, then close the lid and centrifuge briefly. Place the tube on the magnetic rack until the solution appears clear. **Remove the supernatant and discard it.**

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

4. **Remove Ethanol:** 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:** Leave them stand 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.

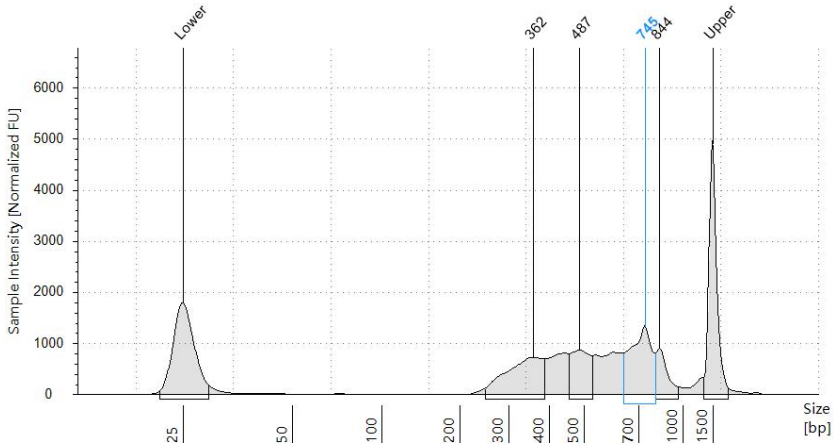
7. **Collect Supernatant:** Place the tube on the magnetic rack to allow the beads to attach and the solution to become clear. **Transfer 30 μL of the supernatant to a new tube**. Take 1 μL for Quibit measurement.

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

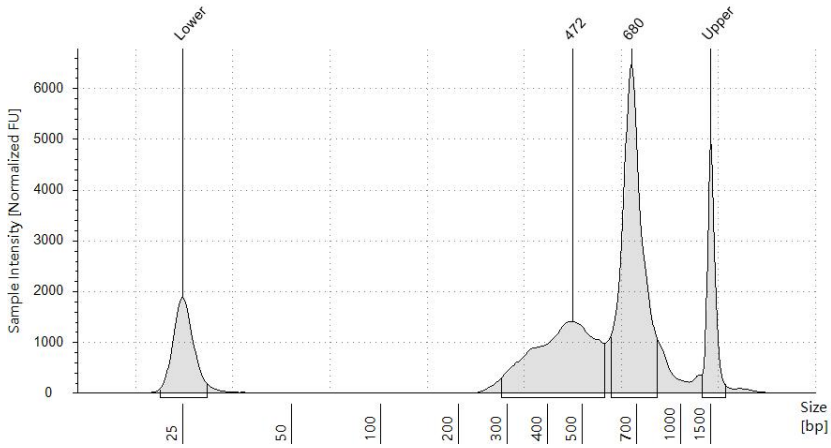
STOPPING POINT：*The products generated in step 5-5 can be sorted to store up to 6 months at -20℃.*

Step 5-6 Library Quality Control

1. For qualified BCR libraries, the main peak fragment size should be between 350-850 bp. If there are smaller fragments below 200 bp present, perform another 0.75× purification until no small fragments are remaining (Agilent 4200 TapeStation/ Qsep).



2. For qualified TCR libraries, the main peak fragment size should be between 350-750 bp. If there are smaller fragments below 200 bp present, perform another 0.75× purification until there are no small fragments remaining (Agilent 4200 TapeStation/ Qsep).



3. Measure the library concentration using Qubit 4.0.

Library Quality Control Criteria:

(1) Concentration (Qubit) ≥ 5 ng/μL, the fragment size range is 250 bp-1500 bp, no small fragment contamination, and the main peak is between 350-700 bp, indicating a qualified sample.

(2) 1 ng/μL ≤ Concentration (Qubit) < 5 ng/μL, the fragment size range is 250 bp-1500 bp, no small fragment contamination, and the main peak is between 350-700 bp, indicating a potential risk.

(3) Concentration (Qubit) ≥ 5 ng/μL, the fragment size range is 250 bp-1500 bp, the main peak is between 350-700 bp, but there is small fragment contamination with the height of small fragments lower than the target peak, indicating a potential risk.

(4) Concentration (Qubit) < 1 ng/μL, or the fragment size analysis shows no specific fragments between 250-1500 bp, no obvious main peak, or the height of small fragments is higher than the target peak, the sample is 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

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

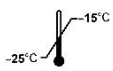
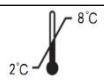
SRN Code: DE-AR-000000087

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

【Edition】

V1.3

【Revised date】

2024/12/20

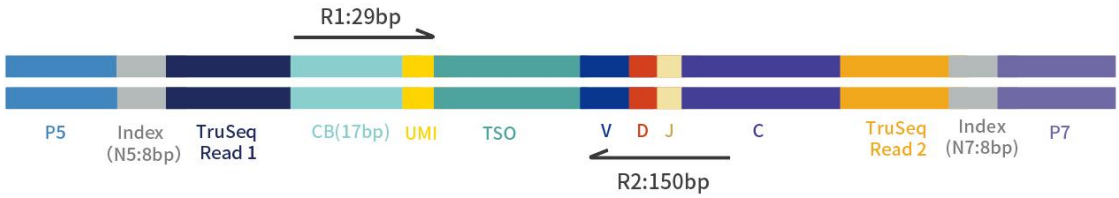
Appendix 1 High-throughput sequencing

1. Sequencing Library

SeekOne™ DD Single-Cell 5' Transcriptome and V(D)J libraries start with P5 and end with P7 sequences. The cell barcode (CB) contains 17 bp, UMI is 12 bp, and sample dual-indexes are N5 (8 bp) and N7 (8 bp). Sequencing the library will yield basic FASTQ data for standard single-cell 5' transcriptome and V(D)J analysis.

Single-Cell 5' Transcriptome Library

Single-Cell V(D)J Library



2. Sequencing Platforms

Libraries constructed with this kit are compatible with GeneMind sequencing platforms and Illumina sequencing platforms.

- GeneMind sequencing platform: SURFSeq 5000

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

3. Library Sequencing Depth and Run Parameters:

	5' Transcriptome Library	**V(D)J Library**
Sequencing Depth	Minimum 20,000 reads per cell, recommended ≥50,000 reads per cell	≥5,000 reads/cell
Sequencing Type	Paired-end, dual indexing	Paired-end, dual indexing
Sequencing Read Length	Read 1: 29 bp N7 Index: 8 bp N5 Index: 8 bp Read 2: 90 bp	Read 1: 29 bp N7 Index: 8 bp N5 Index: 8 bp Read 2: 150 bp

Note 1: *Recommended sequencing depth is ≥50,000 reads/cell for 5' transcriptome and ≥5,000 reads/cell for V(D)J library to ensure the accuracy of single-cell sequencing data analysis.*

Note 2*: Recommended read length for paired-end sequencing. Read1 should be at least 29 bp to capture complete Cell Barcode and UMI sequences. For 5' transcriptome, Read2 should be at least 90 bp for subsequent data analysis, and for V(D)J library, Read2 should be at least 150 bp for efficient full-length VDJ assembly.*

4. Library Loading Concentration:

Platform	Instrument	Loading concentration（pM）	PhiX（%）
Illumina	NextSeq 500	1.5	1
	HiSeq 2500(RR)	10	1
	HiSeq 4000	180	1
	NovaSeq	150*/300	1
	NextSeq 2000	650	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

When pooling 5' gene expression libraries or V(D)J libraries for sequencing on the same lane, ensure that no samples with the same index are used for sequencing. Samples with the same index cannot be demultiplexed in subsequent data analysis. When mixing 5' gene expression and V(D)J libraries, adjust the library mixing ratio based on the desired sequencing depth as follows:

Library	**Sequencing depth(reads/cell)**	**Library mixing ratio**
V(D)J Library	5,000	1
5' Gene Expression Library	50,000	10

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.

- Input Files: FASTQ

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

- Operating System: Linux

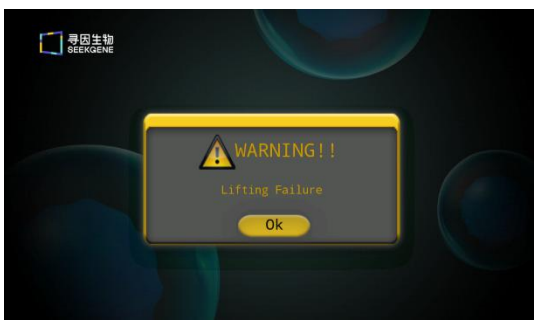
Software Access: To obtain the software package and installation instructions, please visit http://seeksoul.seekgene.com/en/index.html

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
	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	2022/08/05
2	V1.1 to V1.2	Component change, process optimization	2023/02/28
3	V1.2 to V1.3	Library reagent replacement, library amplification process optimization. New reagent components added to optimize detection sensitivity	2024/12/19
4	Details revised	The number of cycles for TCR enrichment in 4-3 has been modified.	2024/04/22
5	Revised	Updated link to SeekSoul Tools.	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