# DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set

## Instructions for use

Version: 1.0

Research Use Only

Qingdao MGI Tech Co., Ltd.

# About the instructions for use

This instructions for use is applicable to DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (hereinafter referred to as preparation set). The instructions for use version is 1.0, and the preparation set version is V1.0.

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Figures in this manual are for illustrative purpose only. The content may be slightly different from the device. For the most up-to-date details, refer to the preparation set purchased.

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# Revision history

| | Date | Version |
|---|---|---|
| **Initial release** | July 3, 2023 | 1.0 |

**Tips**

- Please download the latest instructions for use, and use it with the corresponding version of the preparation set.
- Download the instructions for use through search according to the catalog number or product name from the website:

  *www.mgi-tech.com/download/files*.

# Contents

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# Chapter 1 Product overview

This chapter describes basic information of the product, including the intended use, compatible sequencing platforms, component information, and so on.

## 1.1 About the product

Based on the proprietary DNBelab C series single-cell library preparation and DNBSEQ™ sequencing technologies, combined with self-developed single-cell analysis software, the DNBelab C Series Cell Omics Solution can realize a portable, instant, and one-stop single-cell omics research workflow.

Based on droplet microfluidics, the DNBelab C Series High-throughput single-cell ATAC library preparation set, to quickly process the single-cell or single-cell nucleus suspension and prepare the dedicated libraries applicable to DNBSEQ sequencing platforms of MGI by catching beads efficiently. All reagents, chips, and consumables included in the preparation set have undergone strict quality control and function verification, to ensure single-cell ATAC library preparation stability and reproducibility.

## 1.2 Intended use

This preparation set is applicable to preparation of high-throughput single-cell ATAC libraries from human or mammal animal samples. Before use, it is necessary to prepare the samples into single-cell nuclei suspensions.

**WARNING** This preparation set is for scientific research use only, and cannot be used for clinical diagnosis.

## 1.3 Compatible sequencing platform

| | |
|---|---|
| **Sequencing platform** | DNBSEQ-G400RS |
| | DNBSEQ-T7RS |
| **Sequencing recipe** | 115 (Read 1) + 69 (Read 2) + 10 |

## 1.4 Component information

The preparation set includes the following 3 reagent kits and 1 chip kit.

**Table 1 DNBelab C Series High-throughput Single-cell ATAC Library Preparation set (16 RXN) (Cat. No.: 940-000793-00)**

| Name | Component | Cap color | Specification |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 1 Droplet Formation Kit) (Cat. No.: 940-000792-00) | scATAC Cell Beads | Black | 1.6 mL/tube × 1 |
| | scATAC Wash Buffer | Brown | 1.6 mL/tube × 2 |
| | scATAC Lysis buffer | Black | 768 μL/tube× 1 |
| | scATAC Breakage Reagent | Brown | 1.6 mL/tube × 1 |
| | P100 Oil | Natural | 11.2 mL/bottle × 1 |
| | Mineral Oil | Natural | 6.4 mL/bottle × 1 |
| | DNA Clean Beads | Natural | 5.933 mL/bottle × 1 |
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 2 Droplet Formation Kit) (Cat. No.: 940-000794-00) | Tn5 Transposase | Green | 64 μL/tube × 1 |
| | 5 × TAG Buffer | Green | 80 μL/tube × 1 |
| | scATAC Bead Buffer | Black | 1.114 mL/tube × 1 |
| | PCR Primer | Black | 39 μL/tube × 1 |
| | PCR Enzyme I | White | 128 μL/tube × 1 |
| | scATAC Cell Buffer | White | 748 μL/tube × 1 |
| | scATAC DIR Reagent | White | 32 μL/tube × 1 |
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 3 Library Preparation Kit) (Cat. No.: 940-000910-00) | PCR Enzyme II | Blue | 800 μL/tube × 1 |
| | scATAC Splint Buffer | Yellow | 80 μL/tube × 1 |
| | scATAC Cyclization Buffer | Yellow | 157 μL/tube × 1 |
| | scATAC DNA Ligase | Yellow | 8 μL/tube × 1 |
| | scATAC Exo Enzyme | Orange | 42 μL/tube × 1 |
| | scATAC Exo Buffer | Orange | 23 μL/tube × 1 |
| | scATAC Stop Buffer | Orange | 48 μL/tube × 1 |
| | scATAC Barcode Primer 1 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 2 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 3 | Red | 16 μL/tube × 1 |

| Name | Component | Cap color | Specification |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 3 Library Preparation Kit) (Cat. No.: 940-000910-00) | scATAC Barcode Primer 4 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 5 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 6 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 7 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 8 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 9 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 10 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 11 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 12 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 13 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 14 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 15 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 16 | Red | 16 μL/tube × 1 |

**Table 2 DNBelab C Series High-throughput Single-cell ATAC Chip Kit (16 RXN)**

| Name | Component | Specification | Note |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Chip (Cat. No.: 940-000791-00) | scATAC Chip | 16 EA/box × 1 | Used together with Single Cell Droplet Generator (Cat. No.: 900-000637-00) |
| | Sealing Gasket | 5 pcs/bag × 1 | |

## 1.5 Storage and transportation condition

**Table 3 Transportation and storage condition**

| Name | Storage temperature | Transportation temperature | Validity period |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 1 Droplet Formation Kit)<br>(Cat. No.: 940-000792-00) | 2 ℃ to 8 ℃<br>(36 ℉ to 46 ℉ ) | 2 ℃ to 8 ℃<br>(36 ℉ to 46 ℉ ) | Refer to the label on the box. |
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 2 Droplet Formation Kit)<br>(Cat. No.: 940-000794-00) | -25 ℃ to -15 ℃<br>(-13 ℉ to 5 ℉ ) | -80 ℃ to -15 ℃<br>(-112 ℉ to 5 ℉ ) | |
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 3 Library Preparation Kit)<br>(Cat. No.: 940-000910-00) | -25 ℃ to -15 ℃<br>(-13 ℉ to 5 ℉ ) | -80 ℃ to -15 ℃<br>(-112 ℉ to 5 ℉ ) | |
| DNBelab C Series High-throughput Single-cell ATAC Chip<br>(Cat. No.: 940-000791-00) | 10 ℃ to 30 ℃<br>(50 ℉ to 86 ℉ ) | 0 ℃ to 30 ℃<br>(32 ℉ to 86 ℉ ) | |

Tips

- The kits are transported by using dry ice. Please check whether dry ice remains upon receipt.
- When the product is transported, stored, and used appropriately, all of the components retain full activity within the validity period.

## 1.6 Self-provided materials

**Table 4 Self-provided materials**

| Type | Name | Recommended brand | Cat. No. |
|---|---|---|---|
| Equipment | Single Cell Droplet Generator | MGI | 900-000637-00 |
| | Clean bench | / | / |
| | Fluorescence microscope<br>Tips A fluorescence microscope is required for counting the number of nuclei. | | |
| | Electronic balance | / | / |
| | Vortex mixer | / | / |
| | Mini centrifuge | / | / |
| | Manual single-channel pipette, with a measurement range as follows:<br>• 0.1 µL to 2.5 µL<br>• 0.5 µL to 10 µL<br>• 2 µL to 20 µL<br>• 10 µL to 100 µL<br>• 20 µL to 200 µL<br>• 100 µL to 1000 µL | / | / |
| | Manual 8-channel pipette, with a measurement range as follows:<br>• 1 µL to 10 µL<br>• 2 µL to 10 µL<br>• 5 µL to 50 µL<br>• 20 µL to 200 µL | / | / |
| | Deep-well PCR device (100 µL solution, with heated lid) | / | / |
| | Centrifuge or equivalents | Eppendorf | 5810R |
| | Magnetic separation rack for 1.5 mL tubes | Thermo Fisher | 12321D |
| | Magnetic separation rack for 0.2 mL tubes | New England Biolabs | S1515S |
| | Qubit 3.0 fluorometer or equivalents | Thermo Fisher | Q33216 |

| Type | Name | Recommended brand | Cat. No. |
|---|---|---|---|
| Equipment | Agilent 2100 Bioanalyze or equivalents | Agilent Technologies | G2939AA |
| Reagents | DNA-OFF SOLUTION | TAKARA | 9036 |
| | RNase Zap | AMBION | AM9782 |
| | 75% ethanol | / | / |
| | PBS, pH 7.4 | Gibco | 10010031 |
| | BSA (Bovine Serum Albumin) | Sangon Biotech | A600903-0010 |
| | DAPI or equivalents (Used to dye nuclei) | Sigma-Aldrich | D9542 |
| | Trypan Blue Solution, 0.4% or equivalents | Gibco | 15250061 |
| | Nuclease-free water (NF Water) | Ambion | AM9937 |
| | TE buffer, pH 8.0 | Ambion | AM9858 |
| | Absolute ethanol (analytical grade) | / | / |
| | Qubit ssDNA Assay Kit | Invitrogen | Q10212 |
| | Qubit dsDNA HS Assay Kit | Invitrogen | Q32854 |
| | Agilent High Sensitivity DNA Kit or equivalents | Agilent Technologies | 5067-4626 |
| Consumables | 1 mL syringe | / | / |
| | 0.22 μm filter membrane | PALL | 4612 |
| | C-Chip disposable hemocytometer or universal hemocytometer | INCYTO | DHC-N01 |
| | Low-binding sterile filter tips, boxed, with a capacity of 10 μL, 20 μL, 100 μL, 200 μL, or 1000 μL | Axygen | / |
| | Universal low-binding tips with a capacity of 10 μL, 20 μL, 100 μL, 200 μL, or 1000 μL | Axygen | / |
| | 200 μL wide-bore tips | Axygen | T-205-WB-C |
| | 0.2 mL low-binding PCR tube | Axygen | PCR-02-L-C |
| | 1.5 mL low-binding centrifuge tube | Eppendorf | 0030108051 |
| | 0.2 mL PCR tube | Axygen | PCR-02-C |
| | 1.5 mL centrifuge tube | Axygen | MCT-150-C |

| Type | Name | Recommended brand | Cat. No. |
|---|---|---|---|
| Consumables | 15 mL centrifuge tube | CORNING | 430791 |
| | 50 mL centrifuge tube | CORNING | 430291 |
| | Qubit Assay Tubes or 0.5mL transparent thin wall PCR tube | Invitrogen/ Axygen | Q32856 or PCR-05-C |

## 1.7 Precautions

- This product is for research use only, and cannot be used for clinical diagnosis. Please read this instructions for use carefully before use.
- Before the experiment, make sure to be familiar with and master the operation methods and precautions of various to-be-used devices.
- Adjust and optimize library preparation procedures according to specific experiment design, sample characteristics, sequencing applications, and devices. The experiment procedures provided in this instructions for use are universal, and the reaction parameters can be adjusted as needed to achieve high performance and efficiency.
- Take out all components of the preparation set in advance, tap to mix the enzymes, briefly centrifuge them, and keep them on ice for further use. Thaw other components on ice, invert them up and down several times after thawing to thoroughly mix them, briefly centrifuge, and place them on ice for further use.
- To avoid an experiment failure caused by cross contamination, it is recommended to perform experiment operations such as sample processing, nuclei transposition, droplet formation, PCR in droplets, demulsification, library preparation, and circularization in a clean laboratory, use low-binding filter tips, and change tips for aspirating different samples.
- It is recommended to proceed with reaction steps in a PCR device with a heated lid mode. The PCR device should be preheated to a required reaction temperature before use.
- Avoid aerosol contamination caused by improper operations on PCR products, which may reduce the accuracy of experimental results. It is recommended to physically separate the PCR reaction solution preparation area from the PCR product purification and detection area. Use special pipettes or other devices, and regularly clean experimental areas (wipe by using 0.5% sodium hypochlorite or 10% bleach) to ensure the cleanliness of the experimental environment.

- Direct contact with skin and eyes should be avoided for all samples and reagents. Do not swallow. Once this happens, immediately rinse with a large amount of water and go to the hospital in time.
- All samples and wastes should be disposed of in accordance with relevant regulations.
- Any other questions, contact the technical support at *MGI-service@mgi-tech.com*.

# Chapter 2 Sample requirements and processing

This chapter describes sample processing and requirements, including precautions and preparation before experiment, sample requirements, experiment requirements, and preparation of reagents and nuclei suspension.

## 2.1 Precautions before experiment

- It is recommended to operate single-cell experiments in a class 100,000 or 300,000 clean laboratory, or in a clean bench.
- Avoid exogenous nucleic acid contamination when performing single-cell DNA experiment in the clean bench.
- Wear protective equipment such as mask and disposable powder-free latex gloves when performing experiments. During the operation, do not expose the skin of wrist. If the gloves touch the area outside the clean bench, carefully wipe the surface of the gloves with RNase-Zap before continuing the experiment.
- Place all samples on ice in the experiment.
- Consumables such as pipette tips, centrifuge tubes, and sterile water should be sterile, nucleic acid-free, and nuclease-free, and cannot be used for other purposes. Tips should be low-binding nuclease-free filter tips.

## 2.2 Pre-experiment preparation

### 2.2.1 Sample requirements

**Table 5 Sample requirements**

| | |
|---|---|
| **Cell type** | Organism/cell samples of human or mammals |

| | |
|---|---|
| **Nuclei size** | Recommended diameter: < 40 μm |
| **Recommended total nuclei input** | • Recommended total input for transposition : 50,000 to 100,000 nuclei<br>• Recommended total input after transposition on chip: 5,000 to 20,000 nuclei |
| **Requirements of nuclei extracted from cell** | • Before nuclei extraction:<br>▪ Cytoactivity > 80%<br>▪ Clumping rate < 5%<br>▪ Impurity rate < 5%<br>• After nuclei extraction:<br>▪ Cytoactivity < 5%<br>▪ Clumping rate < 5%<br>▪ Impurity rate < 5% |
| **Requirements of nuclei extracted from organism** | • Cytoactivity < 5%<br>• Clumping rate < 5%<br>• Impurity rate < 5% |

## 2.2.2 Experiment requirements

- Before experiment, carefully wipe the gloves, pipettes, bench, and devices with RNase-Zap.
- If a clean bench is used, turn on the light of the clean bench in advance and perform the following steps:

  1) Wipe the device and operating deck of the clean bench with DNA-OFF, especially the metal and plastic surfaces.
  2) Wait for 10 minutes for degrading the DNA.
  3) Turn off the light, and turn on the UV lamp for sterilization for at least 15 minutes.
  4) Turn on the light and ventilator after sterilization.

## 2.2.3 Preparing reagents

Prepare the following reagents:

- PBS (containing 10% BSA)

**Table 6 PBS (containing 10% BSA) preparation**

| Component | Volume |
|---|---|
| BSA powder | 1 g |
| PBS | Fixed volume to 10 mL |

After the BSA powder fully dissolves, use a syringe and 0.22 μm filter membrane to filter the solution.

Tips The PBS (containing 10% BSA) can be stored at -25 ℃ to -15 ℃ (-13 ℉ to 5 ℉) for up to 6 months.

- PBS (containing 1% BSA)

**Table 7 PBS (containing 1% BSA) preparation**

| Component | Volume |
|---|---|
| PBS | 45 mL |
| PBS (containing 10% BSA) | 5 mL |

Add the components with the required volumes in proportion and mix them thoroughly.

Tips The PBS (containing 1% BSA) can be stored at 2 ℃ to 8 ℃ (36 ℉ to 46 ℉) for up to 1 month.

## 2.3 Preparing nuclei suspension

For different samples such as a cell-line or live tissue sample, prepare single nuclei suspension in an appropriate way, and wash the nuclei once or twice with the PBS (containing 1% BSA). Resuspend nuclei with an appropriate volume of the PBS (containing 1% BSA) to obtain nuclei suspension. Detect and record the concentration of the nuclei suspension.

Tips
- It is recommended to mix the nuclei suspension thoroughly by pipetting with a wide-bore tip.
- When nuclei are counted by using the C-Chip disposable hemocytometer or the universal hemocytometer, a counting result must be correct, or it may affect a final yield. It is recommended to repeat the counting step at least 3 times.
- After preparation of nuclei suspension is completed, perform the nuclei transposition in 30 minutes.

# Chapter 3 Nuclei transposition

This chapter describes how to transpose nuclei by using DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set. The whole procedure takes about 40 minutes.

## 3.1 Pre-experiment preparation

**Table 8 Required reagents**

| Name | Component | Cap color | Specification |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 2 Droplet Formation Kit)<br>(Cat. No.: 940-000794-00) | Tn5 Transposase | Green | 64 μL/tube × 1 |
| | 5× TAG Buffer | Green | 80 μL/tube × 1 |

## 3.2 Nuclei transposition

Perform the following steps:

1. Gently pipette the nuclei suspension prepared in *Preparing nuclei suspension on Page 10* by using a pipette to mix it thoroughly. Take out a 1.5 mL low-binding centrifuge tube, and prepare the transposition reaction solution according to the following table on ice.

**Table 9 Transposition reaction solution preparation**

| Component | Volume (μL) required for each tube |
|---|---|
| 5× TAG Buffer | 5 |
| Tn5 Transposase | 4 |
| PBS (containing 1% BSA) | 16-X |
| Nuclei suspension | X |
| **Total** | 25 |

Tips
- The recommended total number of input nuclei ranges from 50,000 to 100,000, and the recommended concentration of the nuclei is 6,250 nuclei/μL < N < 10, 000 nuclei/μL. The biggest volume of the nuclei should be 16 μL and may vary according to the nuclei concentration. If the volume is less than 16 μL, the PBS (containing 1% BSA) is added as a complement.
- Tn5 Transposase should be tapped gently and centrifuged to the bottom of the tube briefly before use. If not mixed thoroughly, it may cause in failure of the experiment.
- Ensure that all reagents are added to the bottom of the centrifuge tube. Failure to do so may cause the reagents to remain on the tube wall and cause in loss of reaction solution.

2. After the reaction solution is prepared, adjust the pipette range to 20 μL, Gently pipette to mix the reaction solution thoroughly.

   Tips Gently pipette and tap the tube, ensure that no bubbles exist in the tube.

3. Put the centrifuge tube in the mixer at 37 ℃ (99 ℉ ), and transpose at 500 rpm for 30 minutes. After transposing for 15 minutes, take out the tube from the mixer, pipette to mix it thoroughly, and then place the tube in the mixer to continue transposing.
4. After the transposition is completed, place the 1.5 mL centrifuge tube on ice immediately and gently pipette to mix it thoroughly. Count and records the number of the nuclei.

   Tips After the transposition is completed, perform the droplet formation in 30 minutes.

# Chapter 4 Droplet formation

This chapter describes how to form droplets from the prepared nuclei suspension after transposition by using DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set. The whole procedure takes about 20 minutes.

# 4.1 Pre-experiment preparation

## 4.1.1 Preparing reagents and consumables

**Table 10 Required reagents and consumables**

| Name | Component | Cap color | Specification |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 1 Droplet Formation Kit) (Cat. No.: 940-000792-00) | scATAC Cell Beads | Black | 1.6 mL/tube × 1 |
| | scATAC Wash Buffer | Brown | 1.6 mL/tube × 2 |
| | scATAC Lysis Buffer | Black | 768 μL/tube × 1 |
| | P100 Oil | Natural | 11.2 mL/bottle × 1 |
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 2 Droplet Formation Kit) (Cat. No.: 940-000794-00) | scATAC Bead Buffer | Black | 1.114 mL/tube × 1 |
| | PCR Primer | Black | 39 μL/tube × 1 |
| | PCR Enzyme I | White | 128 μL/tube × 1 |
| | scATAC Cell Buffer | White | 748 μL/tube × 1 |
| | scATAC DIR Reagent | White | 32 μL/tube × 1 |
| DNBelab C Series High-throughput Single-cell ATAC Chip (Cat. No.: 940-000791-00) | scATAC Chip | / | 16 EA/box* 1 |
| | Sealing Gasket | / | 5 pcs/bag* 1 |

Tips
- Take out the P100 Oil for at least 30 minutes in advance to equilibrate to room temperature.
- Take out the scATAC Lysis Buffer in advance to equilibrate to room temperature until all the crystals in the solution are dissolved.
- DNBelab C Series High-throughput Single-cell ATAC Chip is used together with Single Cell Droplet Generator (Cat. No.: 900-000637-00).

## 4.1.2 Preparing sample phase suspension

Perform the following steps:

1. Gently pipette the nuclei suspension prepared in *Nuclei transposition on Page 11* by using a pipette to mix it thoroughly, and prepare the sample phase suspension according to the following table.

**Table 11 Sample phase suspension preparation**

| Component | Volume (μL) required for each tube |
|---|---|
| scATAC Cell Buffer | 46.7 |
| PCR Enzyme I | 8 |
| scATAC DIR Reagent | 2 |
| NF Water | 43.3-X |
| Nuclei suspension | X |
| **Total** | 100 |

Tips
- 'X' indicates the volume of the nuclei suspension.
- The recommended total number of input nuclei suspension ranges from 5,000 to 20,000, and the biggest volume of the nuclei suspension should be 43.3 μL and may vary according to the nuclei concentration. If the volume is less than 43.3 μL, the NF Water is added as a complement.
- If there are identical samples that need to use multiple chips, these samples can be combined to make the sample phase suspension.
- You can add the lost reagents in the process when making the sample phase suspension.
- For the resuscitated or fragile nuclei, it is recommended to use a wide-bore tip to pipette and mix the suspension thoroughly.

2. After the suspension is prepared, adjust the pipette range to 80 μL, gently pipette the mixture to mix it thoroughly, and place it on ice for future use.

### 4.1.3 Preparing beads phase suspension

Perform the following steps:

1. Take out the scATAC Cell Beads, and gently invert or pipette it to mix it thoroughly.
2. Aspirate 100 μL of the scATAC Cell Beads (for each sample), and transfer it to a 0.2 mL low-binding centrifuge tube (the volume varies according to the number of samples).
3. Place the PCR tube on the magnetic separation rack for 3 to 5 minutes. Gently remove and dispose of the supernatant to avoid loss of the beads.
4. Add 200 μL of the scATAC Wash Buffer (for each sample) and clean the beads by rotating the centrifuge tube 180 degrees on the magnetic separation rack. After the supernatant becomes clear, use a pipette to remove and dispose of the supernatant.

5. Remove the PCR tube from the magnetic separation rack, and add 69.6 μL of the scATAC Bead Buffer, 48 μL of the scATAC Lysis buffer, 2.4 μL of the PCR Primer accordingly to prepare the beads phase suspension.
6. Adjust the pipette range to 100 μL, gently pipette the mixture to mix it thoroughly, and place it on ice for future use.

**Tips** If there are multiple samples that need to use multiple chips, it is recommended to prepare the beads phase suspension required for all the samples in a 1.5 mL low-binding centrifuge tube.

## 4.2 Performing droplet formation

Perform the following steps:

1. Take out the scATAC Chip and the single cell droplet generator.

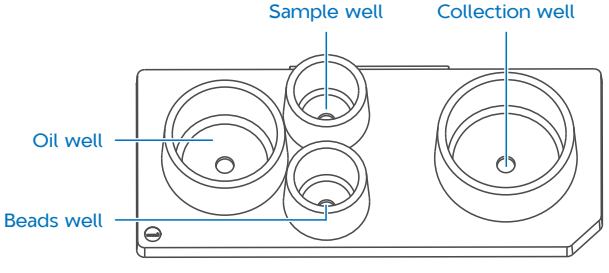


**Figure 1 scATAC chip**

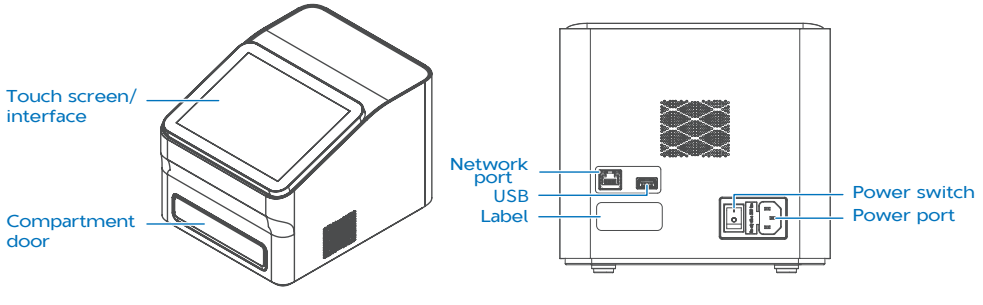


**Figure 2 Single cell droplet generator (left: front view, right: back view)**

2. Switch the power switch of the single cell droplet generator to | position. Tap **Open**, the compartment door opens automatically. Take out the chip stage.

3. Unlock the buckle of the chip stage, install the chip by pushing it into the chip stage from the side (the number of the loaded chip depends on the number of the sample), and lock the buckle at the oil well position.

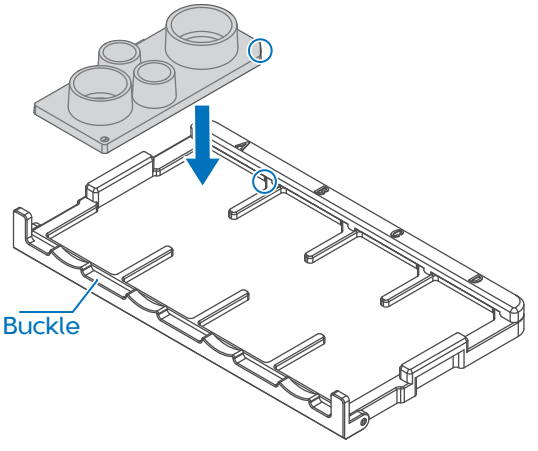


**Figure 3 Loading the chip**

4. Snap the sealing gasket into the collection well (tailor the gasket according to the number of the chip).

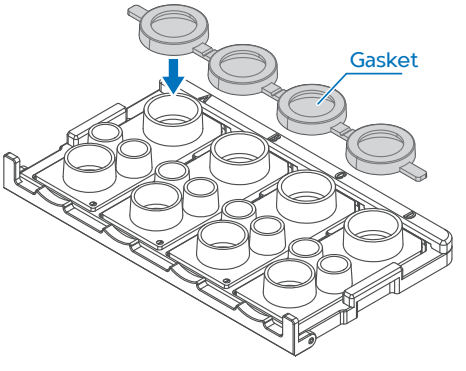


**Figure 4 Loading the sealing gasket**

5. Place the loaded chip stage into the compartment,

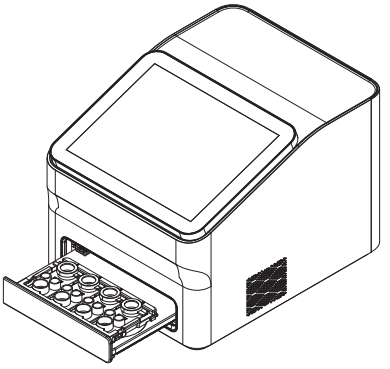

**Figure 5 Loading the loaded chip stage**

6. Add 100 μL of the sample phase suspension, 700 μL of the P100 oil, 120 μL of the beads phase suspension in order accordingly to the sample well, oil well and beads well.

Tips
- Before adding the sample phase suspension and beads phase suspension, separately pipettes them to mix thoroughly. Ensure that no bubbles exist during pipetting. When adding the solutions, do not suspend the tip. Gently add the sample near the edge of the well to avoid forming bubbles at the bottom.
- The total time for adding the three solutions should be within 1 minute.
- Ensure that you add the sample phase suspension first, then add the P100 Oil, and add the beads phase suspension at last. Strictly follow this order. Otherwise, it may cause in failure of droplet formation.

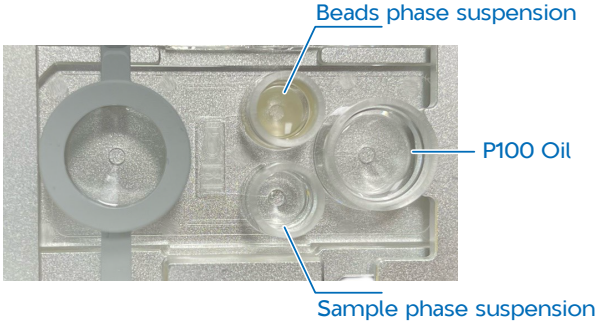


**Figure 6 Adding solutions to the chip**

7. Tap **Close** to close the compartment door.
8. Select **ATAC** as the reaction type, and select the corresponding channel.

Tips The channel **A**/**B**/**C**/**D** on the screen corresponds to the channel "A"/"B"/"C"/"D" on the stage.

9. Tap **Start** to start the reaction.

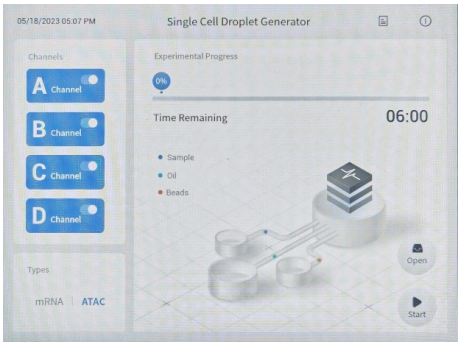


**Figure 7 Droplet formation starts**

10. After the reaction is completed, tap **Confirm**.

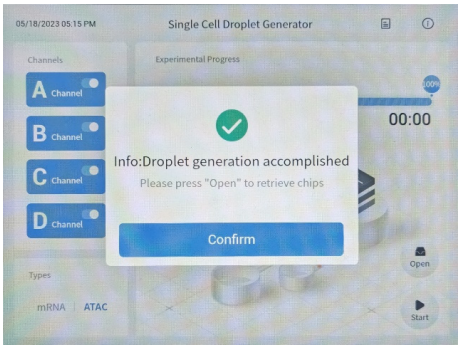


**Figure 8 Droplet formation completed**

11. Tap **Open** to open the compartment door and take out the chip stage. Remove the sealing gasket, unlock the buckle, take out the chip, and recover the droplets.

**Tips** After the droplet formation is completed, recover the droplets immediately to avoid droplets evaporation due to exposure to the air or loss of droplets due to attachment on the well.

# Chapter 5 Performing PCR in droplets

This chapter describes how to recover droplets after the droplet formation is completed and how to perform PCR in droplets. The whole procedure takes about 1 hour and 20 minutes.

## 5.1 Pre-experiment preparation

**Table 12 Required reagents**

| Name | Component | Cap color | Specification |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 1 Droplet Formation Kit) (Cat. No.: 940-000792-00) | Mineral Oil | Brown | 6.4 mL/bottle × 1 |

Tips Take out the Mineral Oil at least 30 minutes in advance and equilibrate to room temperature.

## 5.2 Recovering droplets

Perform the following steps:

1. Take out a clean PCR 8-strip tube and mark it with a marker.

   Tips Use 1 PCR 8-strip tube for 1 sample.

2. Gently aspirate all the droplets in the collection well of the chip by using a 200 μL low-binding tip (you can slightly lean the chip to collect the droplets). Suspend the pipette vertically for a few seconds until the droplets float to the upper level of the oil. Gently transfer the oil at the bottom to the last 4 tubes of the PCR 8-strip tube and the droplets to the first 4 tubes of the PCR 8-strip tube. Repeat this step until all the droplets have been transferred.

   Tips
   - The first 4 tubes of the PCR 8-strip tube are loaded with all the droplets of 1 sample, and the last 4 tubes are loaded with P100 Oil. Transfer the dropets gently. Pipetting or tapping the droplets intensively will break the droplets.
   - Divide the droplets of 1 sample into the first 4 tubes of the PCR 8-strip tube on average. The volume of the droplets in each tube should be about 50 μL to 100 μL.

3. Use the oil collected in the last 4 tubes of the PCR 8-strip tube to rinse the remained droplets in the collection well (do not pipette). Transfer the droplets to the first 4 tubes of the PCR 8-strip tube. Try to recover all the droplets.

   Tips Do not place the droplets after formation for over 40 minutes. Otherwise, the data quality may be influenced.

4. Add 100 µL of the Mineral Oil on the top level of the first 4 tubes of the PCR 8-strip PCR tube.

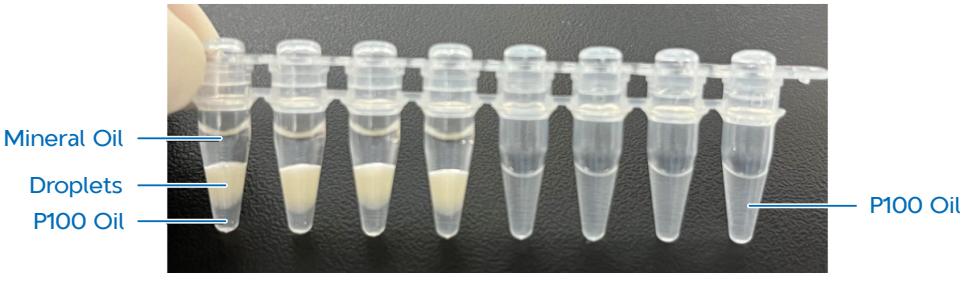


**Figure 9 Liquid in the 8-strip tube**

**Tips** The liquid in the PCR 8-strip tube is divided into 3 levels: the upper level is the mineral oil, the middle level is the droplets, and the bottom level is P100 Oil (little or none).

5. Perform PCR in droplets in the PCR device according to the following table.

**Table 13 PCR in droplets condition (100 µL reaction solution)**

| Temperature | Time | Cycles |
|---|---|---|
| 105 °C (221 °F) (heated lid) | On | / |
| 72 °C (162 °F) | 5 min | 1 |
| 98 °C (208 °F) | 30 s | 1 |
| 98 °C (208 °F) | 10 s | 10 |
| 63 °C (145 °F) | 30 s | |
| 72 °C (162 °F) | 1 min | |
| 72 °C (162 °F) | 5 min | 1 |
| 4 °C (39 °F) | Hold | / |

**Stop point** After the PCR reaction is completed, the solution can store at 2 °C to 8 °C (36 °F to 46 °F ) for 72 hours.

# Chapter 6 Demulsification and beads selection

This chapter describes the procedure of recovering DNA by demulsification. The whole procedure takes about 1 hour.

## 6.1 Pre-experiment preparation

**Table 14 Required reagents**

| Name | Component | Cap color | Specification |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 1 Droplet Formation Kit) (Cat. No.: 940-000792-00) | scATAC Breakage Reagent | Brown | 1.6 mL/tube × 1 |
| | DNA Clean Beads | Natural | 5.933 mL/bottle × 1 |

Tips
- Take out the DNA Clean Beads at least 30 minutes in advance to equilibrate to room temperature. Before use, mix it thoroughly.
- Use the scATAC Breakage Reagent in the ventilation cabinet.

## 6.2 Performing demulsification

Perform the following steps:

1. Transfer the droplets in the middle level of the first 4 tubes of the PCR 8-strip tube prepared in *Recovering droplets on Page 19* to a clean 1.5 mL low-binding centrifuge tube.

   Tips Avoid pipetting the upper level of the Mineral Oil. Pipetting the P100 Oil in the bottom level will not make an affect.

2. Add 100 μL of the scATAC Breakage Reagent in the centrifuge tube, invert the centrifuge tube 15 to 20 times, and place it at room temperature for 3 minutes.

   Tips Do not shake or vortex the tube strongly.

3. Centrifuge the centrifuge tube for 1 minute at room temperature with a centrifugal force of 1000 ×g.
4. Place the centrifuge tube on the magnetic separation rack for 3 to 5 minutes.
5. Slowly pipette 100 μL of the aqueous phase to a new 1.5 mL low-binding centrifuge tube.

   Tips Slowly pipette the aqueous phase. Avoid pipetting the oil phase in the bottom level, the interface layer between the aqueous phase and the oil phase in the middle and the remained mineral oil in the upper level.

## 6.3 Beads selection

Tips
- Take out the DNA Clean Beads at least 30 minutes in advance to equilibrate to room temperature. Before use, mix it thoroughly.
- Before operation, carefully read through *Appendix 1 About the DNA Clean Beads and purification on Page 36*.

Perform the following steps:

1. Aspirate 50 µL of the DNA Clean Beads into the low-binding centrifuge tube with the aqueous phase prepared in *Performing demulsification on Page 21* and pipette to mix it thoroughly. In the last pipetting, ensure that all the liquids and beads in the pipette are transferred into the centrifuge tube.
2. Incubate the centrifuge tube at room temperature for 5 minutes.
3. After centrifuge briefly, place and keep the centrifuge tube on the magnetic separation rack for 2 to 5 minutes until the liquid becomes clear. Gently transfer the supernatant to a new 1.5 mL low-binding centrifuge tube by using a pipette.

   Tips
   - Do not dispose of but reserve the supernatant in this step.
   - Do not pipette the beads.
4. Aspirate 50 µL of the DNA Clean Beads to the centrifuge tube containing the supernatant and gently pipette and mix it thoroughly. In the last pipetting, ensure that all the liquid and beads in the pipette are transferred into the PCR tube.
5. Incubate the PCR tube at room temperature for 5 minutes.
6. After centrifuge briefly, place and keep the centrifuge tube on the magnetic separation rack for 2 to 5 minutes until the liquid becomes clear. Gently remove and dispose of the supernatant.
7. Keep the centrifuge tube on the magnetic separation rack, add 500 µL of the 80% ethanol freshly prepared, rinse the beads and the tube wall, and keep the tube for 30 seconds. Gently remove and dispose of the supernatant.
8. Repeat the last step, try to remove all the liquid from the tube. If some residuals remain on the tube wall, briefly centrifuge the tube, place it on the magnetic separation rack for separation, and remove all liquid at the bottom of the tube by using a pipette with a small measurement range.
9. Keep the tube on the magnetic rack, and open the cap of the tube to dry the beads at room temperature until no wetness or glossiness is visible on the beads surface. Do not let the beads overdried to avoid beads from cracking.
10. Remove the centrifuge tube from the magnetic separation rack, add 22 µL of the NF water to elute DNA, and pipette to mix it thoroughly.
11. Incubate the centrifuge tube at room temperature for 5 minutes.

12. Centrifuge the tube briefly, place the tube on the magnetic separation rack, and wait for 2 to 5 minutes until the liquid becomes clear. Transfer 20 μL of the supernatant to a new 1.5 mL centrifuge tube and mark it as 'Product'.
13. Take out 1 μL of the 'Product' and detect its concentration of product by using the Qubit dsDNA HS Assay Kit.

**Stop point** The 'Product' can be stored at -25 ℃ to -15 ℃ (-13 ℉ to 5 ℉ ) for 6 months.

# Chapter 7 Library preparation

This chapter describes the library preparation. The whole procedure takes about 1 hour and 20 minutes.

## 7.1 Pre-experiment preparation

**Table 15 Required reagents**

| Name | Component | Cap color | Specification |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 1 Droplet Formation Kit) (Cat. No.: 940-000792-00) | DNA Clean Beads | Natural | 5.933 mL/bottle × 1 |
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 3 Library Preparation Kit) ) (Cat. No.: 940-000910-00) | PCR Enzyme II | Blue | 800 μL/tube × 1 |
| | scATAC Barcode Primer 1 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 2 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 3 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 4 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 5 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 6 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 7 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 8 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 9 | Red | 16 μL/tube × 1 |

| Name | Component | Cap color | Specification |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 3 Library Preparation Kit) )<br>(Cat. No.: 940-000910-00) | scATAC Barcode Primer 10 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 11 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 12 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 13 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 14 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 15 | Red | 16 μL/tube × 1 |
| | scATAC Barcode Primer 16 | Red | 16 μL/tube × 1 |

Tips Take out the DNA Clean Beads at least 30 minutes in advance to equilibrate to room temperature.

## 7.2 Performing library preparation

Tips Please read through *Appendix 2 Using scATAC Barcode Primer (1 to 16) on Page 37*.

Perform the following steps:

1. According to the 'Product' concentration detected in *Demulsification and beads selection on Page 20*, add 3 ng (if less than 3 ng, put them all in) of the 'Product' in a new 0.2 mL PCR tube, add NF Water as a complement to a total volume of 46 μL, and place the PCR tube on ice.
2. Add 4 μL of the scATAC Barcode Primer in the PCR tube.

   Tips Record the number of scATAC Barcode Primer added in each sample.

3. Add 50 μL of the PCR Enzyme II, vortex to mix it thoroughly, and centrifuge the tube briefly to collect the reaction solution to the bottom of the tube.
4. Place the PCR tube on the PCR device and perform the reaction according to the following table.

**Table 16 PCR reaction condition (100 µL reaction solution )**

| Temperature | Time | Cycles |
|---|---|---|
| 105 °C (221 °F) (heated lid) | On | / |
| 98 °C (208 °F) | 30 s | 1 |
| 98 °C (208 °F) | 10 s | 12 |
| 63 °C (145 °F) | 30 s | |
| 72 °C (162 °F) | 1 min | |
| 72 °C (162 °F) | 5 min | 1 |
| 4 °C (39 °F) | Hold | / |

## 7.3 Beads selection

Tips Before operation, carefully read through *Appendix 1 About the DNA Clean Beads and purification on Page 36*.

Perform the following steps:

1. Aspirate 40 µL of the DNA Clean Beads into the centrifuge tube with the PCR products prepared in *Performing library preparation on Page 24* and gently pipette and mix it thoroughly. In the last pipetting, ensure that all the liquids and beads in the pipette are transferred into the PCR tube.
2. Incubate the PCR tube at room temperature for 5 minutes.
3. After centrifuge briefly, place and keep the PCR tube on the magnetic separation rack for 2 to 5 minutes until the liquid becomes clear. Gently transfer the supernatant to a new 1.5 mL low-binding centrifuge tube by using a pipette.

   Tips
   - Do not dispose of but reserve the supernatant in this step.
   - Do not pipette the beads.

4. Aspirate 70 µL of the DNA Clean Beads to the centrifuge tube containing the supernatant prepared in the last step and gently pipette and mix it thoroughly. In the last pipetting, ensure that all the liquid and beads in the pipette are transferred into the centrifuge tube.
5. Incubate the centrifuge tube at room temperature for 5 minutes.
6. After centrifuge briefly, place and keep the centrifuge tube on the magnetic separation rack for 2 to 5 minutes until the liquid becomes clear. Gently remove and dispose of the supernatant.

7. Place and keep the centrifuge tube on the magnetic separation rack, add 500 µL of the 80 % ethanol freshly prepared to rinse the beads and the tube wall, keep the tube for 30 seconds, and dispose of the supernatant.
8. Repeat the last step, try to remove all liquid from the tube. If some residuals remain on the tube wall, briefly centrifuge the tube, place it on the magnetic separation rack for separation, and remove all liquid at the bottom of the tube by using a pipette with a small measurement range.
9. Keep the tube on the magnetic rack, and uncap the tube to dry the beads at room temperature until no wetness or glossiness is visible on the beads surface. Do not let the beads overdried to avoid the beads from cracking.
10. Remove the centrifuge tube from the magnetic separation rack, add into 42 µL of the TE Buffer, and pipette to mix it thoroughly.
11. Incubate the centrifuge tube at room temperature for 5 minutes.
12. Centrifuge the tube briefly, place the tube on the magnetic separation rack, and wait for 2 to 5 minutes until the liquid becomes clear. Transfer 40 µL of the supernatant to a new 1.5 mL centrifuge tube and mark it as 'Library'.
13. Take out 1 µL of the 'Library' and detect the concentration by using the Qubit dsDNA HS Assay Kit. Take out 1 µL of the 'Library' and detect the segment distribution by using the Agilent High Sensitivity DNA Kit. The following figure is an example of the library segment distribution:

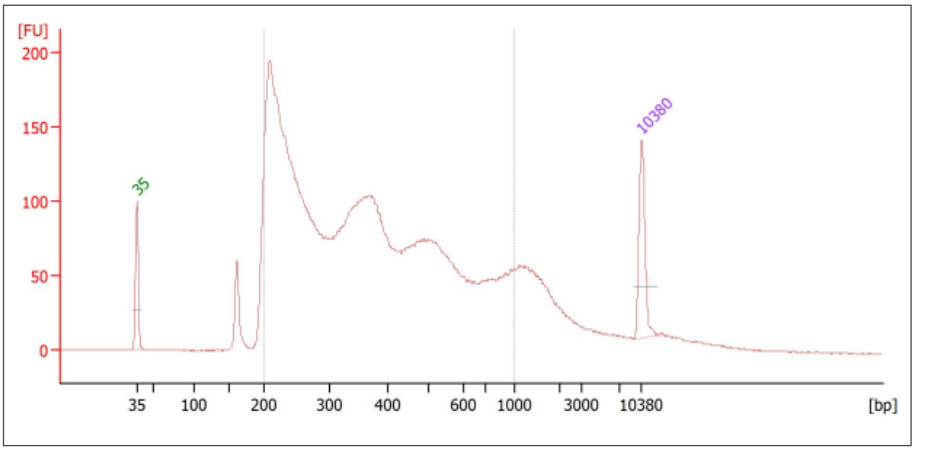


**Figure 10 Library segment distribution (Agilent 2100)**

**Tips** Reference value: When the 'Product' input is 3 ng, library concentration should be greater than 50 ng/µL.

**Stop point** The 'Library' can be stored at -25 ℃ to -15 ℃ (-13 ℉ to 5 ℉ ) for 6 months.

# Chapter 8 Circularization

This chapter describes the whole procedure of single-stranded circularization and products purification, which takes about 1 hour and 30 minutes.

## 8.1 Pre-experiment preparation

**Table 17 Required reagents**

| Name | Component | Cap color | Specification |
|---|---|---|---|
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 1 Droplet Formation Kit) (Cat. No.: 940-000792-00) | DNA Clean Beads | Natural | 5.933 mL/bottle × 1 |
| DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 3 Library Preparation Kit) (Cat. No.: 940-000910-00) | scATAC Splint Buffer | Yellow | 80 μL/tube × 1 |
| | scATAC Cyclization Buffer | Yellow | 157 μL/tube × 1 |
| | scATAC DNA Ligase | Yellow | 8 μL/tube × 1 |
| | scATAC Exo Enzyme | Orange | 42 μL/tube × 1 |
| | scATAC Exo Buffer | Orange | 23 μL/tube × 1 |
| | scATAC Stop Buffer | Orange | 48 μL/tube × 1 |

Tips Take out the DNA Clean Beads at least 30 minutes in advance to equilibrate to room temperature. Vortex to mix it thoroughly before use.

## 8.2 Denaturation

Perform the following steps:

1. Add 400 ng of the 'Library' into a new 0.2 mL PCR tube and add TE Buffer as a complement to a total volume of 45 μL.
2. Add 5 μL of the scATAC Splint Buffer into the PCR tube, vortex to mix it thoroughly, and centrifuge briefly to collect the reaction solution to the bottom of the tube.
3. Put the PCR tube on the PCR device and perform the reaction according to the following table.

**Table 18 Denaturation condition (50 μL reaction solution)**

| Temperature | Time |
|---|---|
| 105 °C (221 °F) (heated lid) | On |
| 95 °C (203 °F) | 3 min |

4. After the reaction is completed, place and keep the PCR tube on ice for 5 minutes, and then add single-stranded circularization reaction solution into the tube.

Tips
- After the reaction at 95 °C (203 °F) is completed, place the PCR tube on ice immediately. Otherwise, circularization efficiency may be affected by the DNA renaturation.
- You can prepare the single-stranded circularization reaction solution during the waiting period of 5 minutes.

## 8.3 Single-stranded circularization

Perform the following steps:

1. Prepare the reaction solution for single-stranded circularization on ice according to the following table.

**Table 19 Single-stranded circularization reaction solution**

| Component | Volume (μL) required for each tube |
|---|---|
| scATAC Cyclization Buffer | 9.5 |
| scATAC DNA Ligase | 0.5 |
| **Total** | 10 |

2. Aspirate 10 μL of the single-stranded circularization reaction solution into the PCR tube, vortex to mix it thoroughly, and centrifuge briefly to collect the reaction solution to the bottom of the tube.
3. Put the PCR tube on the PCR device and perform the reaction according to following table.

**Table 20 Single-stranded circularization condition (60 μL reaction solution)**

| Temperature | Time |
|---|---|
| 50 °C (122 °F) (heated lid) | On |
| 37 °C (99 °F) | 30 min |
| 4 °C (39 °F) | Hold |

4. After the reaction is completed, centrifuge the PCR tube briefly and place the tube on ice. Perform the enzyme digestion immediately.

## 8.4 Enzyme digestion

Perform the following steps:

1. Prepare the enzyme digestion reaction solution on ice according to the following table.

**Table 21 Enzyme digestion reaction solution**

| Component | Volume (μL) required for each tube |
|---|---|
| scATAC Exo Enzyme | 2.6 |
| scATAC Exo Buffer | 1.4 |
| Total | 4.0 |

2. Aspirate the 4 μL enzyme digestion reaction solution into the PCR tube prepared in *Single-stranded circularization on Page 28* by using the pipette, vortex to mix it thoroughly, and centrifuge briefly to collect the reaction solution to the bottom of the tube.
3. Place the PCR tube on the PCR device and perform the reaction according to the following table.

**Table 22 Digestion condition (64 μL reaction solution)**

| Temperature | Time |
|---|---|
| 50 °C (122 °F) (heated lid) | On |
| 37 °C (99 °F) | 30 min |
| 4 °C (39 °F) | Hold |

4. After the reaction is completed, add 3 μL of the scATAC Stop Buffer into the PCR tube, vortex to mix it thoroughly, and centrifuge it briefly to collect the reaction solution to the bottom of the tube.

## 8.5 Beads purification

Tips

- Take out the DNA Clean Beads at least 30 minutes in advance to equilibrate to room temperature. Vortex to mix it thoroughly before use.
- Before operation, carefully read through *Appendix 1 About the DNA Clean Beads and purification on Page 36*.

Perform the following steps:

1. Transfer the digestion products prepared in *Enzyme digestion on Page 29* into a new 1.5 mL low-binding centrifuge tube. Add 160.8 µL of the DNA Clean Beads into the centrifuge tube and gently pipette and mix it thoroughly. In the last pipetting, ensure that all the liquids and beads in the pipette are transferred into the centrifuge tube.
2. Incubate the centrifuge tube at room temperature for 10 minutes.
3. Centrifuge briefly, place and keep the centrifuge tube on the magnetic separation rack for 2 to 5 minutes until the liquid becomes clear. Gently remove and dispose of the supernatant.
4. Place and keep the centrifuge tube on the magnetic separation rack. Add 500 µL of the 80 % ethanol freshly prepared to rinse the beads and the tube wall, wait for 30 seconds, and dispose of the supernatant.
5. Repeat the last step, try to remove all liquids from the tube. If some residuals remain on the tube wall, briefly centrifuge the tube, place it on the magnetic separation rack for separation, and remove all liquid at the bottom of the tube by using a pipette with a small measurement range.
6. Keep the tube on the magnetic rack, and uncap the tube to dry the beads at room temperature until no wetness or glossiness is visible on the beads surface. Do not let the beads overdried to avoid the beads from cracking.
7. Remove the centrifuge tube from the magnetic separation rack, add into 32 µL of the TE Buffer, and pipette to mix it thoroughly.
8. Incubate the centrifuge tube at room temperature for 10 minutes.
9. After centrifuge briefly, place and keep the centrifuge tube on the magnetic separation rack for 2 to 5 minutes until the liquid becomes clear. Transfer 30 µL of the supernatant to a new 1.5 mL centrifuge tube gently and mark the tube as 'ssDNA'.

   **Tips** Do not pipette the beads in this step. Failure to do so will take a great effect on the quality of the library sequencing.

10. Take out 1 µL of the 'ssDNA' and detect the concentration by using the Qubit ssDNA Assay Kit.

    **Tips** Reference value: The 'ssDNA' concentration should be greater than 0.5 ng/µL.

    **Stop point** The 'ssDNA' can be stored at -25 °C to -15 °C (-13 °F to 5 °F ) for 1 month.

# Chapter 9 Sequencing

This chapter describes the sequencer, sequencing reagents and read length that are compatible to the library. The library structure is as shown below:

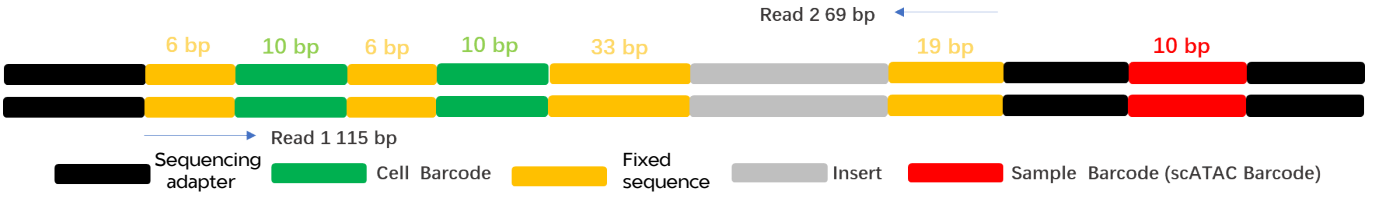


**Figure 11 Library structure**

Tips
- Read 1 = 115 bp ( Read 1 fixed sequence 6 + 6 + 33 = 45 bp dark reaction )
- Read 2 = 69 bp ( Read 2 fixed sequence 19 bp dark reaction )
- Sample Barcode = 10 bp

## 9.1 Sequencing requirements of DNBSEQ-G400RS

### 9.1.1 Pre-experiment preparation

Tips According to the sequencing set, carefully read *DNBSEQ-G400RS HotMPS High-throughput Sequencing Set User Manual* or *DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set User Manual* before starting sequencing, and strictly perform all operations according to the operations in the user manual.

**Table 23 Preparation list**

| Type | Model No. | Cat. No. |
|---|---|---|
| Genetic sequencer | DNBSEQ-G400RS | / |
| Sequencing set | HotMPS High-throughput Sequencing Set (G400 HM FCL PE100) | 940-000489-00 |
| | HotMPS High-throughput Sequencing Set (G400 HM FCL PE150) | 940-000244-00 |
| | DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100) | 1000016950 |
| | DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150) | 1000016952 |

### 9.1.2 Making DNB

Make DNB according to the following table.

**Table 24 DNBSEQ-G400RS making DNB requirements**

| Model No. | DNBSEQ-G400RS (HotMPS) | | DNBSEQ-G400RS (StandardMPS) | |
|---|---|---|---|---|
| Sequencing read | PE100 | PE150 | PE100 | PE150 |
| Make DNB input | 10 ng | 10 ng | 10 ng | 10 ng |
| RCA time | 20 min | 20 min | 20 min | 20 min |

### 9.1.3 Pooling libraries

For details about protocols for pooling different libraries based on the scATAC Barcode Primer (1 to 16), refer to *Appendix 2 Using scATAC Barcode Primer (1 to 16) on Page 37*.

### 9.1.4 Sequencing parameters

**Table 25 DNBSEQ-G400RS (HotMPS) sequencing software version and read length (mixed sample, sequencing barcode)**

| | |
|---|---|
| Software version | ECR 1.0 |
| Control software version | 1.7.1.1799 |
| Basecall version | 1.5.0.323 |
| Sequencing script | Z_scATAC_BC_HM |
| Read 1 | 115 cycles (1 to 6 bp, 17 to 22 bp, 33 to 65 bp are set for dark reaction) |
| Read 2 | 69 cycles (1 to 19 bp are set for dark reaction) |
| Sample barcode | 10 cycles |

**Table 26 DNBSEQ-G400RS (HotMPS) sequencing software version and read length (unmixed sample, not sequencing barcode)**

| | |
|---|---|
| Software version | ECR 1.0 |
| Control software version | 1.7.1.1799 |
| Basecall version | 1.5.0.323 |
| Sequencing script | Z_scATAC_noBC_HM |

| | |
|---|---|
| **Read 1** | 115 cycles (1 to 6 bp, 17 to 22 bp, 33 to 65 bp are set for dark reaction) |
| **Read 2** | 69 cycles (1 to 19 bp are set for dark reaction) |
| **Sample barcode** | / |

**Table 27 DNBSEQ-G400RS (StandardMPS) sequencing software version and read length (mixed sample, sequencing barcode)**

| | |
|---|---|
| **Software version** | ECR 3.0 or later version |
| **Control software version** | DNBSEQ-G400_1.0.0.34 or later version |
| **Basecall version** | Basecall_1.0.8.208 or later version |
| **Sequencing script** | scATAC_BC |
| **Read 1** | 115 cycles (1 to 6 bp, 17 to 22 bp, 33 to 65 bp are set for dark reaction) |
| **Read 2** | 69 cycles (1 to 19 bp are set for dark reaction) |
| **Sample barcode** | 10 cycles |

**Table 28 DNBSEQ-G400RS (StandardMPS) sequencing software version and read length (unmixed sample, not sequencing barcode)**

| | |
|---|---|
| **Software version** | ECR 3.0 or later version |
| **Control software version** | DNBSEQ-G400_1.0.0.34 or later version |
| **Basecall version** | Basecall_1.0.8.208 or later version |
| **Sequencing script** | scATAC_noBC |
| **Read 1** | 115 cycles (1 to 6 bp, 17 to 22 bp, 33 to 65 bp are set for dark reaction) |
| **Read 2** | 69 cycles (1 to 19 bp are set for dark reaction) |
| **Sample barcode** | / |

**Tips** If the number of nuclei input is 10,000, the recommended sequencing data is 400 M Reads. The average sequencing depth for each nucleus is 50 K and can be modified according to requirements.

## 9.2 Sequencing requirements of DNBSEQ-T7RS

### 9.2.1 Pre-experiment preparation

Tips According to the sequencing set, carefully read *DNBSEQ-T7RS HotMPS High-throughput Sequencing Set User Manual* or *DNBSEQ-T7RS High-throughput Sequencing Set User Manual* before starting sequencing, and strictly perform all operations according to the operations in the user manual.

**Table 29 Preparation list**

| Type | Model No. | Cat. No. |
|---|---|---|
| Genetic sequencer | DNBSEQ-T7RS | / |
| Sequencing set | DNBSEQ-T7RS HotMPS High-throughput Sequencing Set (FCL PE100) | 940-000247-00 |
| | DNBSEQ-T7RS HotMPS High-throughput Sequencing Set (FCL PE150) | 940-000248-00 |
| | DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE100) | 1000028455 |
| | DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE150) | 1000028454 |

### 9.2.2 Making DNB

Make DNB according to the following table.

**Table 30 DNBSEQ-T7RS making DNB requirements**

| Model No. | DNBSEQ-T7RS (HotMPS) | | DNBSEQ-T7RS (StandardMPS) | |
|---|---|---|---|---|
| Sequencing read | PE100 | PE150 | PE100 | PE150 |
| Make DNB input | 15 ng | 15 ng | 10 ng | 10 ng |
| RCA time | 10 min | 10 min | 30 min | 15 min |

### 9.2.3 Pooling libraries

For details about protocols for pooling different libraries based on the scATAC Barcode Primer (1 to 16), refer to *Appendix 2 Using scATAC Barcode Primer (1 to 16) on Page 37*.

### 9.2.4 Sequencing parameters

**Table 31 DNBSEQ-T7RS (HotMPS) sequencing software version and read length**

| | |
|---|---|
| Software version | ECR 4.0 or later version |
| Control software version | 1.4.1.812 or later version |
| Basecall version | BCS_1.0.10.142 or later version |
| Sequencing script | Customize |
| Custom primers | No |
| Read 1 | 115 cycles (1 to 6 bp, 17 to 22 bp, 33 to 65 bp are set for dark reaction) |
| Read 2 | 69 cycles (1 to 19 bp are set for dark reaction) |
| Sample barcode | 10 cycles |

**Table 32 DNBSEQ-T7RS (StandardMPS) sequencing software version and read length**

| | |
|---|---|
| Software version | ECR 3.0 or later version |
| Control software version | 1.3.3.553 or later version |
| Basecall version | 1.4.2.47_Ubuntu or later version |

| | |
|---|---|
| Sequencing script | Customize |
| Custom primers | No |
| Read 1 | 115 cycles (1 to 6 bp, 17 to 22 bp, 33 to 65 bp are set for dark reaction) |
| Read 2 | 69 cycles (1 to 19 bp are set for dark reaction) |
| Sample barcode | 10 cycles |

Tips If the number of nuclei input is 10,000, the recommended sequencing data is 400 M Reads. The average sequencing depth for each nucleus is 50 K and can be modified according to requirements.

# Appendix 1 About the DNA Clean Beads and purification

## Precautions before use of the DNA Clean Beads

- To ensure recovery efficiency of the DNA Clean Beads (hereinafter called the beads), take it out from the 4 ℃ (39 ℉) refrigerator 30 minutes in advance, vortex it to mix thoroughly and equilibrate to room temperature.
- Vortex or invert the beads before use each time, to ensure that the beads are mixed thoroughly.
- The volume of the beads directly affects the lower limit length of the purified DNA fragments. The higher volume of the beads leads to the smaller lower limit length of the purified DNA fragments.

## Precautions during use of the DNA Clean Beads

- If the volume of to be purified DNA segments decreases due to evaporation caused by incubation, the TE Buffer should be added to complement the liquid to the required volume, and then the recommended volume of the beads is added to perform purification.
- After mixing the DNA segments and the beads thoroughly and placing the centrifuge tube on the magnetic separation rack for separation, remove the supernatant after the solution becomes completely clear. This process usually

takes 2 to 5 minutes. Because magnetism of magnetic separation racks might be different, the separation time may be longer, depending on the time in which the solution becomes completely clear.

- When separating the beads from the solution, avoid contact between the pipette tip and the beads. Reserve 2 or 3 μL of the solution, to avoid aspirating the beads. If the beads are aspirated accidentally, pipette down all the beads and the liquid into the centrifuge tube, and re-aspirate the supernatant after separation.
- Rinse the beads by using 80% ethanol that is freshly prepared and equilibrated to room temperature. During rinsing, keep the centrifuge tube on the magnetic separation rack, and operate the pipette tip on the side that is away from the magnetic separation rack. Do not pipette and stir the beads.
- Try to remove all liquid from the tube in second rinsing with the ethanol. If some residuals remain on the tube wall, briefly centrifuge the tube, place it on the magnetic separation rack for separation, and remove all liquid at the bottom of the tube by using a pipette with a small measurement range.
- After rinsing the beads with the ethanol twice, completely dry the beads at room temperature. Incomplete drying of the beads (with a reflective surface) easily causes absolute ethanol residuals and affects subsequent reactions. Over-drying of the beads (beads with cracks) reduces the purification yield. Drying at room temperature usually takes 5 to 10 minutes. The drying time varies with the room temperature and humidity. Start elution after observing that the beads are not reflective.
- Avoid contact between the pipette tip and the beads when removing the supernatant after elution, as removal of the beads might affect subsequent purification reactions. Therefore, the elution volume should be 2 μL greater than the volume of the aspirated supernatant.
- Carefully open or close the lid of the 1.5 mL centrifuge tube on the magnetic separation rack, to avoid spill of the beads or liquid caused by strong shaking. It is recommended to hold the tube at the middle and lower part with your fingers when opening the lid.

# Appendix 2 Using scATAC Barcode Primer (1 to 16)

DNBelab C Series High-throughput Single-cell ATAC Library Preparation Set (Box 3 Library Preparation Kit) includes 16 tubes of scATAC Barcode Primer. The scATAC Barcode Primers in the preparation set are designed based on the base balancing principle. To ensure high performance, carefully read through this appendix.

Tips
- Avoid placing the tubes at a temperature higher than the room temperature. Otherwise, melting occurs, which affects the performance.
- Mix each tube of scATAC Barcode Primer thoroughly and centrifuge it before use, and wipe the lid of the tube with the lint-free paper. Gently open the lid during use, to prevent the liquid from splashing and avoid cross-contamination. Close the lid in time after use.

scATAC Barcode Primer use rules/protocols:

Based on the base balancing principle, the scATAC Barcode Primers are used individually or in groups.

- First group: The scATAC Barcode Primer 1 to 4 are used as a base balancing barcode group.
- Second group: The scATAC Barcode Primer 5 to 8 are used as a base balancing barcode group.
- Third group: The scATAC Barcode Primer 9 to 12 are used as a base balancing barcode group.
- Forth group: The scATAC Barcode Primer 13 to 16 are used as a base balancing barcode group.

  Totally four groups exist. When all samples have the same data amount requirement, the group of scATAC Barcode Primer varies according to the number of samples. For details, refer to the following table.

**Table 30 scATAC Barcode Primer use rules/protocols**

| Samples/ lane | Method 1 | Method 2 | Method 3 | Method 4 |
|---|---|---|---|---|
| 1 | 1 to 4 | 5 to 8 | 9 to 12 | 13 to 16 |
| 2 | • Sample 1: 1 and 2<br>• Sample 2: 3 and 4 | • Sample 1: 5 and 6<br>• Sample 2: 7 and 8 | • Sample 1: 9 and 10<br>• Sample 2: 11 and 12 | • Sample 1: 13 and 14<br>• Sample 2: 15 and 16 |
| 3 | • Sample 1: 1<br>• Sample 2: 2<br>• Sample 3: 3 and 4 | • Sample 1: 5<br>• Sample 2: 6<br>• Sample 3: 7 and 8 | • Sample 1: 9<br>• Sample 2: 10<br>• Sample 3: 11 and 12 | • Sample 1: 13<br>• Sample 2: 14<br>• Sample 3: 15 and 16 |
| 4 | • Sample 1: 1<br>• Sample 2: 2<br>• Sample 3: 3<br>• Sample 4: 4 | • Sample 1: 5<br>• Sample 2: 6<br>• Sample 3: 7<br>• Sample 4: 8 | • Sample 1: 9<br>• Sample 2: 10<br>• Sample 3: 11<br>• Sample 4: 12 | • Sample 1: 13<br>• Sample 2: 14<br>• Sample 3: 15<br>• Sample 4: 16 |

| Samples/ lane | Method 1 | Method 2 | Method 3 | Method 4 |
|---|---|---|---|---|
| 5 | • Sample 1: 1<br>• Sample 2: 2<br>• Sample 3: 3<br>• Sample 4: 4<br>• Sample 5: select any group from the remaining three groups | • Sample 1: 5<br>• Sample 2: 6<br>• Sample 3: 7<br>• Sample 4: 8<br>• Sample 5: select any group from the remaining three groups | • Sample 1: 9<br>• Sample 2: 10<br>• Sample 3: 11<br>• Sample 4: 12<br>• Sample 5: select any group from the remaining three groups | • Sample 1: 13<br>• Sample 2: 14<br>• Sample 3: 15<br>• Sample 4: 16<br>• Sample 5: select any group from the remaining three groups |
| 6 | • Sample 1: 1<br>• Sample 2: 2<br>• Sample 3: 3<br>• Sample 4: 4<br>• Samples 5 and 6: select any two groups from the remaining three groups | • Sample 1: 5<br>• Sample 2: 6<br>• Sample 3: 7<br>• Sample 4: 8<br>• Samples 5 and 6: select any two groups from the remaining three groups | • Sample 1: 9<br>• Sample 2: 10<br>• Sample 3: 11<br>• Sample 4: 12<br>• Samples 5 and 6: select any two groups from the remaining three groups | • Sample 1: 13<br>• Sample 2: 14<br>• Sample 3: 15<br>• Sample 4: 16<br>• Samples 5 and 6: select any two groups from the remaining three groups |
| 7 | • Sample 1: 1<br>• Sample 2: 2<br>• Sample 3: 3<br>• Sample 4: 4<br>• Samples 5 to 7: select groups by referencing the methods used for 3 samples/lane | • Sample 1: 5<br>• Sample 2: 6<br>• Sample 3: 7<br>• Sample 4: 8<br>• Samples 5 to 7: select groups by referencing the methods used for 3 samples/lane | • Sample 1: 9<br>• Sample 2: 10<br>• Sample 3: 11<br>• Sample 4: 12<br>• Samples 5 to 7: select groups by referencing the methods used for 3 samples/lane | • Sample 1: 13<br>• Sample 2: 14<br>• Sample 3: 15<br>• Sample 4: 16<br>• Samples 5 to 7: select groups according to the methods used for 3 samples/lane |
| 8 | Select any two groups from the four groups | | | |
| 8+x (x = 1 to 8, totally 9 to 16 samples) | Perform the following steps:<br>1. Classify samples 1 to 8 as a group, and add the scATAC Barcode Primer by referencing the methods used for 8 samples/lane.<br>2. Classify the remaining samples as a group, and correspondingly add different groups of scATAC Barcode Primer based on a value of x by referencing the methods used for 1 to 8 samples/lane. | | | |

Tips It is required to add a mixture of the scATAC Barcode Primer 1 to 16 to a sample. Specifically, take the same volume of the N types of scATAC Barcode Primer, mix them, and add the mixture to the sample.

# Appendix 3 96 sequences information and concentration of scATAC Barcode Primer (1 to 96)

This preparation set provides 16 tubes of scATAC Barcode Primer. To meet requirements of multiple samples, this appendix provides 96 sequences information and concentration of scATAC Barcode Primer (1 to 96). You can make the primer according to specific needs.

## Preparing scATAC Barcode Primer (1 to 96)

Before preparing primer, use the Nuclease-free Water to dissolve the primer powder into 100 μm solution, and then prepare the primer according to the following table,

Tips You can prepare the primer by percentage according to requirements.

**Table 31 scATAC Barcode primer preparation**

| Name | Volume (μL) |
|---|---|
| NF Water | 80 |
| scATAC Barcode Primer-F (100 μM, NF Water) | 10 |
| scATAC Barcode Primer (any one among 1 to 96) (100 μM, NF Water) | 10 |
| Total | 100 |

## scATAC Barcode Primer (1 to 96) sequences information

Based on the base balancing principle, the scATAC Barcode Primer are used individually or in groups.

- 4 scATAC Barcode Primer (1 to 16) are used as a base balancing barcode group. 1 to 4 are used as a base balancing barcode group, 5 to 8 are used as a base balancing barcode group, and so on.
- 8 scATAC Barcode Primer (17 to 96) are used as a base balancing barcode group. 17 to 24 are used as a base balancing barcode group, 25 to 32 are used as a base balancing barcode group, and so on.
- Details about the sequence information refer to the following table.

Tips The purification method is HPLC.

**Table 32 Primer sequence information**

| Primer | Sequence information (5' → 3') |
|---|---|
| scATAC Barcode Primer-F | (5'Phosphorylation)GAACGACATGGCTACGATCCGACTT |
| scATAC Barcode Primer 1 | TGTGAGCCAAGGAGTTGATCGGACCTATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 2 | TGTGAGCCAAGGAGTTGGATTCCGTCCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 3 | TGTGAGCCAAGGAGTTGCGGCAGTAAGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 4 | TGTGAGCCAAGGAGTTGTCAATTAGGTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 5 | TGTGAGCCAAGGAGTTGCGGATACGAATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 6 | TGTGAGCCAAGGAGTTGGCTCGTTACCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 7 | TGTGAGCCAAGGAGTTGTTATACGTTGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 8 | TGTGAGCCAAGGAGTTGAACGCGACGTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 9 | TGTGAGCCAAGGAGTTGGCTAGCAGAATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 10 | TGTGAGCCAAGGAGTTGCTATCTTCCTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 11 | TGTGAGCCAAGGAGTTGAAGCAAGAGCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 12 | TGTGAGCCAAGGAGTTGTGCGTGCTTGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |

| Primer | Sequence information (5' → 3') |
|---|---|
| scATAC Barcode Primer 13 | TGTGAGCCAAGGAGTTGCGGATTGCCGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 14 | TGTGAGCCAAGGAGTTGGAATCCTGATTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 15 | TGTGAGCCAAGGAGTTGTCTGGAATGATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 16 | TGTGAGCCAAGGAGTTGATCCAGCATCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 17 | TGTGAGCCAAGGAGTTGCATCACTCACTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 18 | TGTGAGCCAAGGAGTTGCAGCTGACTCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 19 | TGTGAGCCAAGGAGTTGTTCGCAGACATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 20 | TGTGAGCCAAGGAGTTGTTGTACCAATTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 21 | TGTGAGCCAAGGAGTTGACCACAATCGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 22 | TGTGAGCCAAGGAGTTGGGAAGTCTGTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 23 | TGTGAGCCAAGGAGTTGAGAGTGTGGATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 24 | TGTGAGCCAAGGAGTTGGCTTGTGGTGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 25 | TGTGAGCCAAGGAGTTGTTGTCCTCTATTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 26 | TGTGAGCCAAGGAGTTGATTCGCTAGGTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 27 | TGTGAGCCAAGGAGTTGCGATGACTACTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 28 | TGTGAGCCAAGGAGTTGACAGCTCAGCTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 29 | TGTGAGCCAAGGAGTTGTATCTAGGTTTTGTCTTCCTAAGACCGCTTGGCCTCCGACTT |

| Primer | Sequence information (5' → 3') |
|---|---|
| scATAC Barcode Primer 30 | TGTGAGCCAAGGAGTTGGAGATGGCAATTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 31 | TGTGAGCCAAGGAGTTGCGCAAGATCTTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 32 | TGTGAGCCAAGGAGTTGGCCGATAGCGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 33 | TGTGAGCCAAGGAGTTGCCATCGTTGCTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 34 | TGTGAGCCAAGGAGTTGTGAACGATTATTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 35 | TGTGAGCCAAGGAGTTGTAGAGCGAACTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 36 | TGTGAGCCAAGGAGTTGATGTGTGAGATTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 37 | TGTGAGCCAAGGAGTTGATCCTAACAGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 38 | TGTGAGCCAAGGAGTTGCGCGTCTGCGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 39 | TGTGAGCCAAGGAGTTGGATGATCCTTTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 40 | TGTGAGCCAAGGAGTTGGCTCAACGCTTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 41 | TGTGAGCCAAGGAGTTGATGCATCTAATTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 42 | TGTGAGCCAAGGAGTTGAGCTCTGGACTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 43 | TGTGAGCCAAGGAGTTGCTATCACGTGTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 44 | TGTGAGCCAAGGAGTTGGGACTAGTGGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 45 | TGTGAGCCAAGGAGTTGGCCAAGTCCATTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 46 | TGTGAGCCAAGGAGTTGCCTGTCAAGCTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |

| Primer | Sequence information (5' → 3') |
|---|---|
| scATAC Barcode Primer 47 | TGTGAGCCAAGGAGTTGTAGAGGTCTTTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 48 | TGTGAGCCAAGGAGTTGTATGGCAACTTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 49 | TGTGAGCCAAGGAGTTGCTGCGTACATTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 50 | TGTGAGCCAAGGAGTTGATCTCATTAATTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 51 | TGTGAGCCAAGGAGTTGAAGTGGCGCATTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 52 | TGTGAGCCAAGGAGTTGGGCCTTAATGTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 53 | TGTGAGCCAAGGAGTTGTCTGAGGCGGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 54 | TGTGAGCCAAGGAGTTGCGAGCCGATTTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 55 | TGTGAGCCAAGGAGTTGGATAACCGGCTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 56 | TGTGAGCCAAGGAGTTGTCAATATTCCTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 57 | TGTGAGCCAAGGAGTTGTCCGTTGAATTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 58 | TGTGAGCCAAGGAGTTGCAGTACAGTTTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 59 | TGTGAGCCAAGGAGTTGATTGAGGTACTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 60 | TGTGAGCCAAGGAGTTGATTAGAAGTCTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 61 | TGTGAGCCAAGGAGTTGCAACGCTTCATTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 62 | TGTGAGCCAAGGAGTTGGGATCGCACGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 63 | TGTGAGCCAAGGAGTTGTGCCTTCCGATTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |

| Primer | Sequence information (5' → 3') |
|---|---|
| scATAC Barcode Primer 64 | TGTGAGCCAAGGAGTTGGCGACATCGGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 65 | TGTGAGCCAAGGAGTTGCATTCTAAGTTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 66 | TGTGAGCCAAGGAGTTGCAGGCTTGGATTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 67 | TGTGAGCCAAGGAGTTGATCATCGTCTTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 68 | TGTGAGCCAAGGAGTTGGTCTTGTGAGTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 69 | TGTGAGCCAAGGAGTTGAGTAGGAACGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 70 | TGTGAGCCAAGGAGTTGTCACAACCACTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 71 | TGTGAGCCAAGGAGTTGGCAGGCCTTCTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 72 | TGTGAGCCAAGGAGTTGTGGCAAGCTATTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 73 | TGTGAGCCAAGGAGTTGGAGCATTGTCTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 74 | TGTGAGCCAAGGAGTTGTGTGATTAGCTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 75 | TGTGAGCCAAGGAGTTGCCTATGGACTTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 76 | TGTGAGCCAAGGAGTTGTAGGCGATAGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 77 | TGTGAGCCAAGGAGTTGAGACCACGATTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 78 | TGTGAGCCAAGGAGTTGGTATTAGCCATTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 79 | TGTGAGCCAAGGAGTTGCTCTGCACTGTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 80 | TGTGAGCCAAGGAGTTGACCAGCCTGATTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |

| Primer | Sequence information (5' → 3') |
|---|---|
| scATAC Barcode Primer 81 | TGTGAGCCAAGGAGTTGGCGTGAGTATTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 82 | TGTGAGCCAAGGAGTTGCGCGGAGCATTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 83 | TGTGAGCCAAGGAGTTGCAAGTTCACATTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 84 | TGTGAGCCAAGGAGTTGAGCACCTCTCTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 85 | TGTGAGCCAAGGAGTTGTTACAGTGCATTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 86 | TGTGAGCCAAGGAGTTGTTGCCTAGGCTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 87 | TGTGAGCCAAGGAGTTGGCTATGATGGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 88 | TGTGAGCCAAGGAGTTGAATTACCATGTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 89 | TGTGAGCCAAGGAGTTGAGACATGGTGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 90 | TGTGAGCCAAGGAGTTGCCAGACATATTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 91 | TGTGAGCCAAGGAGTTGACGCTTCCTTTTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 92 | TGTGAGCCAAGGAGTTGGACGTCTTGATTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 93 | TGTGAGCCAAGGAGTTGTACTGAGCGGTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 94 | TGTGAGCCAAGGAGTTGTGTACACACCTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 95 | TGTGAGCCAAGGAGTTGCTTACGTGAATTGTCTTCCTAAGA CCGCTTGGCCTCCGACTT |
| scATAC Barcode Primer 96 | TGTGAGCCAAGGAGTTGGTGTGGAACCTTGTCTTCCTAAG ACCGCTTGGCCTCCGACTT |

# Appendix 4 Manufacturer information

| | |
|---|---|
| Manufacturer | Qingdao MGI Tech Co., Ltd |
| Address | Building 4, No.2, Hengyunshan Road, Qingdao Area, Pilot Free Trade Zone, Shandong, China |
| Technical support | Qingdao MGI Tech Co., Ltd |
| Technical support E-mail | MGI-service@mgi-tech.com |

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Part No.: H-020-000746-00

## Leading Life Science Innovation

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Website: www.mgi-tech.com