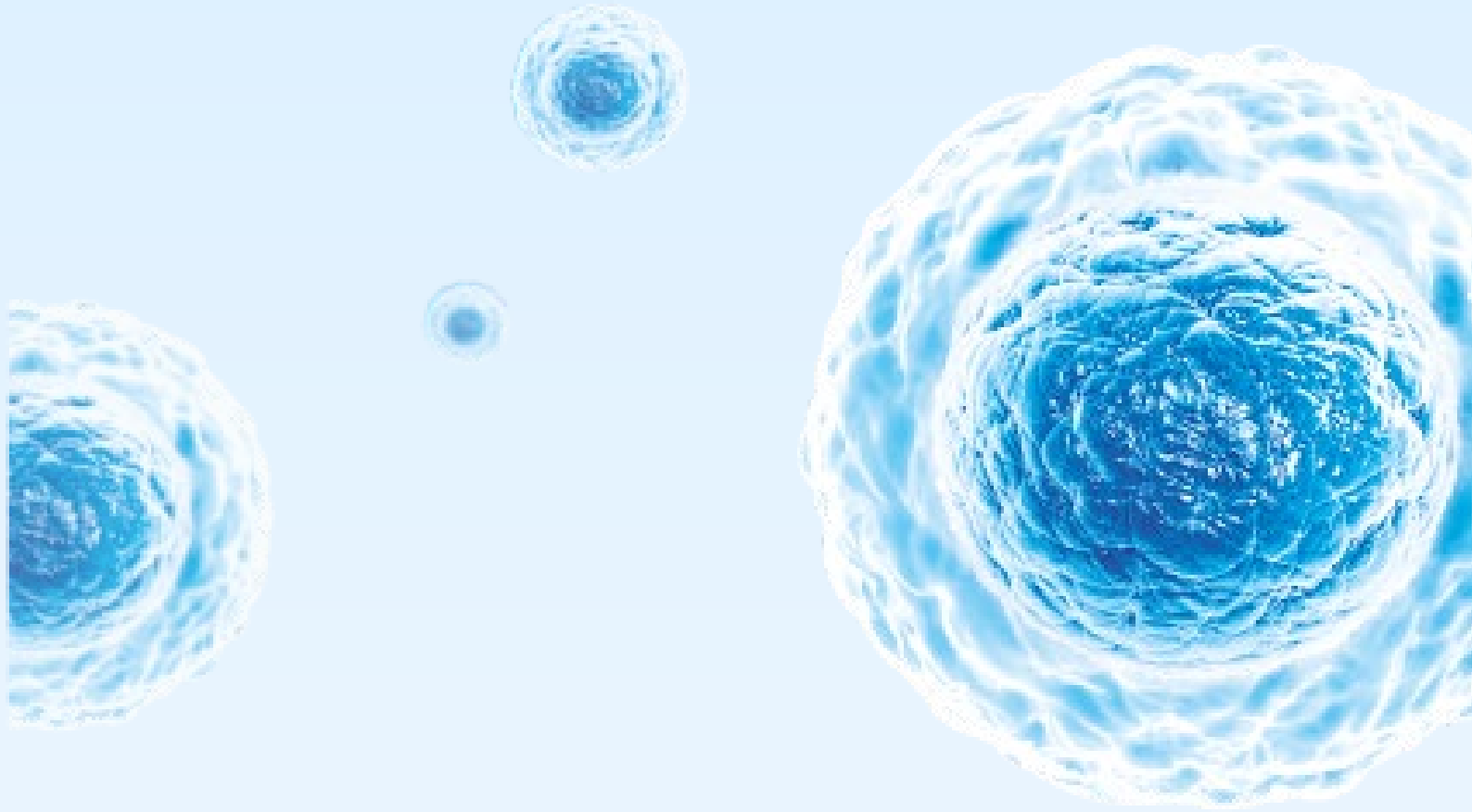
Singleron



GEXSCOPE® Single Cell RNA Library Kit

Handbook (SCOPE-chip SD/HD - Manual - 2 RXNs/ 16 RXNs, V2)

RUO Research Use Only

2025.02

This handbook applies to the following products:

Cat. No.	Product name	Reaction
4180011	GEXSCOPE® Single Cell RNA Library Kit Cell V2	2 RXNs
4180012	GEXSCOPE® Single Cell RNA Library Kit Cell V2	16 RXNs
4180031	GEXSCOPE® Single Cell RNA Library Kit Cell HD V2	2 RXNs
4180032	GEXSCOPE® Single Cell RNA Library Kit Cell HD V2	16 RXNs

Version information and revision history

Version no.		Updates
2021/11	1.	First edition
2022/07	2.	Appendix on Sequencing added
2023/04	3.	Additional notes added
2024/03	4.	UDI Added
2024/08	5.	Fragmentation time updated
2025/02	6.	RT reaction updated

Disclaimer

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1. General Information

1.1 Product Overview

The GEXSCOPE® Single Cell RNA Library Kits provide a comprehensive solution for converting the mRNA contained in the single cells into NGS libraries.

The GEXSCOPE Single Cell RNA Library Kit uses the SCOPE-chip®, a portable, microfluidic chip with microwells. The SCOPE-chip integrates multiple steps of the processing workflow such as single cell partitioning, cell lysis, and capture of cellular polyadenylated RNA, including mRNA. The SCOPE-chip is easy to use and can be operated without a special instrument. It is suitable for cells from a wide variety of tissue types.

To generate high-quality single cell suspensions from tissue, sCelLiVE® Tissue Dissociation Kits and PythoN instruments can be used. See Singleron cat. # 1190062, 11300602 and 11300603 for more information.

1.2 List of Components

GEXSCOPE® Single Cell RNA Library Kit Cell V2 (2 RXNs) and GEXSCOPE® Single Cell RNA Library Kit Cell HD V2 (2 RXNs)

Box 1: SCOPE-chip SD & Barcoding Beads V2 or SCOPE-chip HD & Barcoding Beads V2

Store between 2°C and 8°C upon receipt.

Component	SD		HD		Cap color
	Quantity	Volume	Quantity	Volume	
SCOPE-chip SD	2	-	-	-	-
SCOPE-chip HD	-	-	2	-	-
Singleron Magnetic Rack	1	-	1	-	-
Barcode Beads SD	1	1.8 mL	-	-	Black
Barcode Beads HD	-	-	1	1.8 mL	Black
Wash Buffer A	1	7 mL	1	7 mL	White
Wash Buffer B	1	1.8 mL	1	1.8 mL	White

Box 2: Single Cell Amplification Reagents SD & Library Reagents V2 or Single Cell Amplification Reagents HD & Library Reagents V2

Store between -25°C and -15°C upon receipt.

Component	SD		HD		Cap color
	Quantity	Volume	Quantity	Volume	
Lysis Buffer, Stock	1	1500 μL	1	1500 μL	Green
RNase Inhibitor	1	50 μL	1	50 μL	Green
100 mM DTT	1	880 μL	1	880 μL	Green
RT Master Mix	1	400 μL	2	400 μL	Purple
Reverse Transcriptase	1	25 μL	1	50 μL	Purple
TS Primer	1	50 μL	1	50 μL	Purple
Amplification Master Mix	1	400 μL	2	400 μL	Clear
A Primer Mix	1	200 μL	1	200 μL	Clear
Amplification Enzyme	1	20 μL	2	20 μL	Clear
Fragmentation Buffer	1	17 μL	1	17 μL	Orange
Fragmentation Enzyme Mix	1	6 μL	1	6 μL	Orange
1×TE	1	800 μL	1	800 μL	Orange
Ligation Mix	1	72 μL	1	72 μL	Blue
Ligation booster	1	4 μL	1	4 μL	Blue
Adaptor	1	50 μL	1	50 μL	Blue
Library Amp Mix v2	1	60 μL	1	60 μL	White

Box 3: UDI Adapters Set A

Store between -25°C and -15°C upon receipt.

Component	Quantity	Volume	Cap color
Unique Dual Indexes 1 to 16	16	12 µL each	White

GEXSCOPE® Single Cell RNA Library Kit Cell V2 (16 RXNs) and GEXSCOPE® Single Cell RNA Library Kit Cell HD V2 (16 RXNs)

Box 1: SCOPE-chip SD or SCOPE-chip HD

Store between 2°C and 35°C upon receipt.

Component	SD	HD
	Quantity	Quantity
SCOPE-chip SD	16	-
SCOPE-chip HD	-	16
Singleron Magnetic Rack	2	2

Box 2: Barcoding Beads SD V2 or Barcoding Beads HD V2

Store between 2°C and 8°C upon receipt.

Component	SD		HD		Cap color
	Quantity	Volume	Quantity	Volume	
Barcode Beads SD	8	1.8 mL	-	-	Black
Barcode Beads HD	-	-	8	1.8 mL	Black
Wash Buffer A	8	7 mL	8	7 mL	White
Wash Buffer B	6	1.8 mL	6	1.8 mL	White

Box 3: Single Cell Amplification Reagents SD V2 or Single Cell Amplification Reagents HD V2

Store between -25°C and -15°C upon receipt.

Component	SD		HD		Cap color
	Quantity	Volume	Quantity	Volume	
Lysis Buffer, Stock	2	1500 µL	2	1500 µL	Green
RNase Inhibitor	1	300 µL	1	300 µL	Green
100 mM DTT	1	880 µL	1	880 µL	Green
RT Master Mix	2	1500 µL	4	1500 µL	Purple
Reverse Transcriptase	1	200 µL	2	200 µL	Purple
TS Primer	1	250 µL	2	250 µL	Purple
Amplification Master Mix	2	1600 µL	4	1600 µL	Clear
A Primer Mix	1	750 µL	2	750 µL	Clear
Amplification Enzyme	1	160 µL	2	160 µL	Clear

Box 4: Library Prep Reagents V2

Store between -25°C and -15°C upon receipt.

Component	Quantity	Volume	Cap color
Fragmentation Buffer	1	135 µL	Orange
Fragmentation Enzyme Mix	1	40 µL	Orange
1×TE	1	800 µL	Orange
Ligation Mix	1	576 µL	Blue
Ligation booster	1	20 µL	Blue
Adaptor	1	50 µL	Blue
Library Amp Mix v2	1	480 µL	White

Box 5: UDI Adapters Set A

Store between -25°C and -15°C upon receipt.

Component	Quantity	Volume	Cap color
Unique Dual Indexes 1 to 16	16	12 µL each	White

Important:

- Store components according to instructions.
- Please do not store Barcoding Beads below 0°C. (Do not freeze)

2. Instruments, Reagents and Consumables Supplied by Users

For both workstations (pre-PCR and post-PCR):

- Absolute ethanol
- Nuclease-free water
- 1.5- or 2-mL nucleic acid low-binding tubes
- 15- and 50-mL conical tubes
- Serological pipets
- Single-channel pipettes (p10, p200 and p1000)
- Sterile RNase/DNase-free certified filter tips
- Mini centrifuge
- Vortex
- Janus Magnetic rack (Singleron cat # 1120020202) or equivalent

Single Cell Preparation and SCOPE-chip Loading (Pre-PCR)

- RNase Away or equivalent
- Sterile RNase/DNase-free p200 filter tips for SCOPE-chip loading (Eppendorf cat # 0030078551, Jet Biofil PMT231200, VWR cat # 732-3701, Rainin cat# 30389242)
- Refrigerated centrifuge with swing buckets rotor, compatible with 15- and 50-mL conical tubes
- Inverted microscope
- Hemocytometer
- ThermoMixer
- PBS pH 7.4 (without Ca^{2+}, without Mg^{2+})
- 0.4% Trypan Blue solution (optional, for cell counting)
- 10% Tween-20

cDNA Amplification and Library Preparation (Post-PCR)

- Thermocycler
- Agilent Fragment Analyzer 5200 (Agilent cat # M5310AA) or equivalent
- Qubit 4 Fluorometer (Thermo Fisher cat # Q33238), Assay Tubes and 1x dsDNA HS assay kit (Thermo Fisher cat # Q32856, Q33230)
- AMPure XP (Beckman Coulter cat # A63880, A63881, A63882)
- 10mM Tris-HCl pH 8.5 or Elution Buffer (EB, Qiagen cat # 19086)
- 0.2 mL 8-tube PCR strip or PCR tubes

Important: Consumables must be sterile and certified RNase/DNase-free.

3. Workflow and Timeline

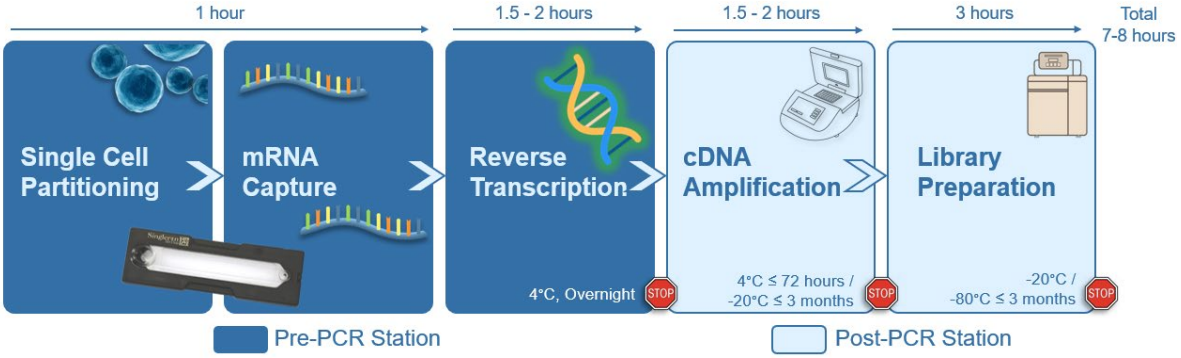


Figure 1: Workflow and timeline overview

4. Preparation

We recommend users to establish a “clean workstation” for all pre-PCR steps that require cleanroom conditions. These steps include tissue dissociation, loading the SCOPE-chip and reverse transcription. For RNA-related work, clean all working surfaces and pipettes with RNase Away (or equivalent). Wear appropriate masks and laboratory gloves to avoid contamination and RNA degradation.

Designate a second workstation (post-PCR) where cDNA amplification, purification and QC is performed, as well as library preparation and QC.

Important: Mix all buffers thoroughly before use. If precipitate or sediment is present in buffers, gently pipette to mix the solution. Ensure that the solution is clear before use.

4.1 Priming of the SCOPE-chip

Materials:

- SCOPE-chip SD or HD

User supplied:

- PBS
- 10% Tween-20
- Absolute ethanol
- Suitable p200 tips for SCOPE-chip loading*

> * The width of the tip is important for correct chip loading. Pipette tips validated for successful chip loading are: Eppendorf cat # 0030078551, Jet Biofil PMT231200, VWR cat # 732-3701 or Rainin cat# 30389242.
> Pipette tips to avoid: Rainin cat # 30389186, USA Scientific 1071-1811

1. If stored at 4°C, bring the SCOPE-chip to room temperature.
2. Prepare PBST (PBS with 0.02% v/v Tween-20) as described in the table below. Vortex and centrifuge briefly.

PBST:

Component	1 RXN（μL）	2 RXNs（μL）
PBS	998	1996
10% Tween-20	2	4
Total	1000	2000

3. Using a 200 μL pipette, inject **200 μL** of **absolute ethanol** into the SCOPE-chip via the inlet port. Dispense slowly over a period of 10 seconds to avoid the introduction of air bubbles.

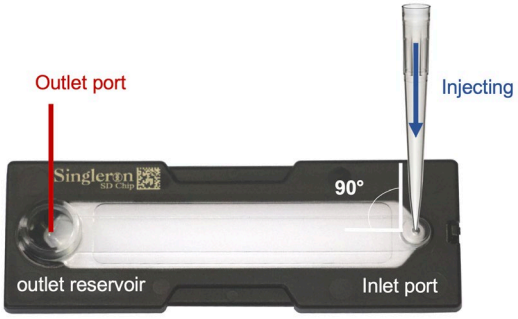


Figure 2. The position of inlet port, outlet reservoir and outlet port on the SCOPE-chip

4. Remove the ethanol from the outlet reservoir without making contact with the outlet port. (Suction applied at the outlet port may lead to the introduction of air in the SCOPE-chip).
5. Repeat the priming with absolute ethanol two additional times.
6. Inject **200 μL** of **PBST** into the SCOPE-chip via the inlet port. Dispense slowly over a period of 10 seconds to avoid the introduction of air bubbles.

7. Remove the liquid from the outlet reservoir without making contact with the outlet port.
8. Repeat the priming with PBST two additional times.
9. Leave 50-100 µL of PBST in the outlet reservoir to avoid the appearance of air in the chip.
10. Cover the SCOPE-chip to protect it from contaminants and let it sit at room temperature.

4.2 Barcode Beads Preparation

Materials:

- Barcode Beads SD or HD (Black)

User supplied:

- Janus Magnetic rack (Singleron cat # 1120020202) or equivalent
- PBS

1. Vortex the Barcode Beads thoroughly for 30 seconds until fully resuspended.
2. For each reaction, pipet **900 µL** of the **Barcode Beads** into a new, labeled 1.5 mL tube.
3. Centrifuge briefly, place in a magnetic rack until the liquid is clear.
4. Discard the supernatant without disturbing the beads.
5. Remove the tube from the magnetic rack and resuspend the beads in **1 mL** of **PBS**.
6. Centrifuge briefly, place the tube back to the magnetic rack place until the liquid is clear.
7. Discard the supernatant without disturbing the beads.
8. Wash two additional times with **1 mL** of **PBS**.
9. After the last wash, resuspend the beads in **60 µL** of **PBS**.
10. Store the Barcode Beads at room temperature for later use.

Important: If not used within 1 hour, store barcode beads at 4°C.

5. Single-Cell Partitioning and mRNA Capture

5.1 Lysis Mix Preparation

Materials:

- Lysis Buffer, Stock (Green)
- 100 mM DTT (Green)
- RNase Inhibitor (Green)

1. Thaw the Lysis Buffer, Stock and 100 mM DTT on ice. Vortex, centrifuge briefly, keep on ice. Ensure that the reagents are fully thawed.

2. Prepare the Lysis Mix on ice according to the tables below. Vortex, centrifuge briefly, and store on ice.

Lysis Mix (SCOPE-chip SD):

Component	1 RXN（μL） ×1	2 RXNs（μL） ×2.2
Lysis Buffer, Stock	92.5	203.5
100 mM DTT	5	11
RNase Inhibitor	2.5	5.5
Total	100	220

Lysis Mix (SCOPE-chip HD):

Component	1 RXN（μL） ×1	2 RXNs（μL） ×2.2
Lysis Buffer, Stock	138.7	305
100mM DTT	7.5	16.5
RNase Inhibitor	3.8	8.5
Total	150	330

5.2 Cell Suspension Preparation

User supplied:

- PBS
- Single cell suspension

1. Dilute the cell suspension using pre-chilled PBS to a concentration between 1.5 × 10^5 to 5.0 × 10^5 cells /mL according to the tables below.

Important: Note that only a subset of the cells loaded on the chip will be captured into the microwells. For example, to capture 10 000 cells on the SCOPE-chip SD (first table, left column), 100 μL of a 3.5 x 10^5 cells/mL suspension (right column) (35000 cells) is needed to load the SCOPE-chip.

2. Proceed immediately to the loading of the cells into the SCOPE-chip.

SCOPE-chip SD:

Targeted number of cells captured	Recommended cell concentration
3 000 – 5 000	（1.5 - 2.0）×10^5 cells /mL
5 000 – 7 000	（2.0 - 2.5）×10^5 cells /mL
7 000 – 9 000	（2.5 - 3.0）×10^5 cells /mL
9 000 - 10 000	（3.0 - 3.5）×10^5 cells /mL

SCOPE-chip HD:

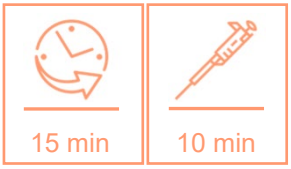
Targeted number of cells captured	Recommended cell concentration
9 000 - 12 000	（1.5 - 2.0）×10^5 cells /mL
12 000 - 15 000	（2.0 - 2.5）×10^5 cells /mL
15 000 - 18 000	（2.5 - 3.0）×10^5 cells /mL
18 000 - 24 000	（3.0 - 4.0）×10^5 cells /mL
24 000 - 30 000	（4.0 - 5.0）×10^5 cells /mL

5.3 Loading of the Cells on the SCOPE-chip

(See Appendix B for link to video manual)

Materials:

- Primed SCOPE-chip SD or HD (from Section 4.1)
- Diluted cell suspension (from Section 5.2)



User supplied:

- PBS
- p200 tips for SCOPE-chip loading (See Recommendation in Section 4.1)

1. Retrieve the previously primed SCOPE-chip.
2. Remove the residual liquid from the outlet reservoir without making contact with the port.
3. Inject **200 µL** of **pre-chilled PBS** into the SCOPE-chip via the inlet port. Dispense slowly over a period of 10 seconds to avoid the introduction of air bubbles.
4. Remove the liquid from the inlet port and outlet reservoir without making contact with the port.
5. Repeat the PBS wash one additional time.
6. Resuspend the diluted cells by pipetting gently up and down.
7. Inject **100 µL** (SCOPE-chip SD) or **150 µL** (SCOPE-chip HD) of the homogenous **cell suspension** into the SCOPE-chip via the inlet port. Dispense slowly over a period of 30 seconds to avoid the introduction of air bubbles.

Important:

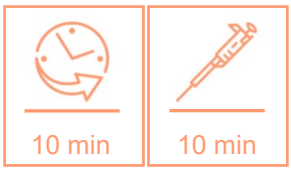
- When loading cells into the SCOPE-chip, a drop of liquid can be kept at the inlet port to avoid the introduction of bubbles.
- The pipette should be kept in a vertical position when loading cells into the SCOPE-chip.
- Injecting cells in less than 30 seconds could cause the capture of fewer cells into the chip.
- Injecting cells for over 30 seconds could increase the cell doublet rate by biasing the distribution of the cell at the entrance of the chip.

8. Remove excess liquid from the outlet reservoir without making contact with the port. (Suction applied at the outlet port may lead to the removal of the cells from the SCOPE-chip and introduction of air in the SCOPE-chip).
9. Let the SCOPE-chip sit for 5 minutes at room temperature to allow the cells to fall into the microwells.
10. Under the microscope, look at the distribution of the cells in the microwells. Ideally, 5 to 15% of the wells should be populated with cells.
11. Inject **200 µL** of **pre-chilled PBS** into the SCOPE-chip via the inlet port. Dispense slowly over a period of 30 seconds to avoid the introduction of air bubbles.
12. Remove the excess of liquid from the outlet reservoir without making contact with the port.
13. Repeat the PBS wash and proceed to the loading of Barcode Beads.

Important: Under the microscope, look for the presence of cells floating above the microwells. If detected, repeat the PBS wash.

5.4 Loading of the Barcode Beads

(See Appendix C for link to video manual)



Materials:

- SCOPE-chip SD or HD containing cells (from the previous step)
- Washed Barcode Beads (from Section 4.2)

User supplied:

- PBS
- p200 tips for SCOPE-chip loading (See Recommendation in Section 4.1)

1. Resuspend the previously prepared Barcode Beads by pipetting up and down.
2. Immediately, inject **60 µL** of the **Barcode Beads** resuspension into the SCOPE-chip via the inlet port. Dispense slowly over a period of 30 seconds. Incubate for **1 minute**.
3. Inject **100 µL** of **PBS** to the inlet port. Dispense slowly over a period of 30 seconds. The beads will spread inside the SCOPE-chip.
4. Inject **200 µL** of **PBS** into the SCOPE-chip via the inlet port. Dispense slowly over a period of 30 seconds.
5. In a new labeled, 1.5 mL tube, collect the excess of beads from the output reservoir without making contact with the outlet port.
6. Repeat the **200 µL PBS** wash two additional times to get rid of the excess of Barcode Beads.

7. Under the microscope, look at the distribution of the Barcode Beads. The beads should occupy at least 95% of the total number of microwells.

Important: If less than 95% of the microwells are occupied with beads, use the beads collected in step 5 to load the SCOPE-chip a second time. Place the recovered beads on a magnetic rack. Remove the supernatant. Resuspend the beads in 30 µL of PBS. Inject the beads in the SCOPE-chip following steps 2 to 7 above.

If many empty wells are observed at proximity of the outlet reservoir, the recovered Barcode Beads can be injected via the outlet reservoir port.

5.5 Cell Lysis and mRNA Capture

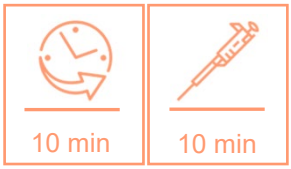
Materials:
- Loaded SCOPE-chip SD or HD (from the previous step)
- Lysis Mix (from Section 5.1)

User supplied:
- p200 tips for SCOPE-chip loading (See Recommendation in Section 4.1)

1. Inject **100 µL** (SCOPE-chip SD) or **150 µL** (SCOPE-chip HD) of previously prepared **Lysis Mix** into the SCOPE-chip via the inlet port. Dispense slowly over a period of 30 seconds to avoid the introduction of air bubbles.
2. Remove the excess liquid from the outlet reservoir without making contact with the port.
3. Incubate the SCOPE-chip at room temperature for **20 minutes**. During this time, the cells will be lysed, and the released mRNA molecules will bind to the Barcode Beads.
4. Proceed immediately to the Barcode Beads retrieval.

5.6 Barcode Beads Retrieval

(See Appendix D for detailed pictured workflow and link to video manual)



Materials:
- Loaded SCOPE-chip SD or HD (from the previous step)
- Singleron Magnetic Rack
- Wash Buffer A

User supplied:
- p200 tips for SCOPE-chip loading (See Recommendation in Section 4.1)

1. Place the Singleron Magnetic Rack under the SCOPE-chip and let sit for 1 minute at room temperature.
2. Add **200 µL** of pre-chilled **Wash Buffer A** into the **outlet reservoir** to remove impurities. Avoid making contact with the port. (***Do not inject the chip).

Figure 3. Addition of Wash Buffer A to the outlet reservoir

3. Remove liquid from the outlet reservoir without making contact with the outlet port.
4. Clean the reservoir two additional times, by repeating steps 2 and 3.
5. Place the Singleron Magnetic Rack on top of the SCOPE-chip and incubate for 1 minute at room temperature.
6. While keeping the magnet on top of the SCOPE-chip, add **200 µL** of pre-chilled **Wash Buffer A** to the SCOPE-chip **outlet reservoir** without making contact with the port. (***Do not inject the chip).
7. Aspirate **200 µL** of liquid containing the **Barcode Beads** with bound mRNA via the **inlet port**.

Figure 4. Collection of the Barcode Beads via inlet port

8. Transfer the collected Barcode Beads to a pre-cooled labeled, 1.5 mL tube.
9. Repeat steps 6 to 8 two additional times to collect most of the Barcode Beads.
10. Proceed to the reverse transcription.

6. Reverse Transcription and cDNA Amplification

6.1 Reverse Transcription

Materials:

- Wash Buffer A
- Wash Buffer B
- RT Master Mix (Purple)
- TS Primer (Purple)
- 100 mM DTT (Green)
- RNase Inhibitor (Green)
- Reverse Transcriptase (Purple)
- mRNA-containing Barcode Beads (from the previous step)

User supplied:

- Nuclease-free water
- Janus Magnetic rack (Singleron cat # 1120020202) or equivalent

1. Thaw RT Master Mix and TS Primer reagents at room temperature. Vortex, centrifuge briefly, keep on ice. Ensure that the reagents are fully thawed.

Important: If a precipitate is observed in the RT Master Mix, mix by vortexing or pipetting up and down until the solution becomes clear.

2. Prepare the RT Mix on ice according to the tables below. Add the reagents in the order listed from top to bottom. Vortex, centrifuge briefly and keep on ice.

RT Mix (SCOPE-chip SD):

Component	1 RXN（μL） × 1	2 RXNs（μL） × 2.2
RT Master Mix	120	264
Cold nuclease-free water	35	77
TS Primer	10	22
100 mM DTT	20	44
RNase Inhibitor	5	11
Reverse Transcriptase	10	22
Total	200	440

RT Mix (SCOPE-chip HD):

Component	1 RXN（μL） × 1	2 RXNs（μL） × 2.2
RT Master Mix	240	528
Cold nuclease-free water	70	154
TS Primer	20	44
100 mM DTT	40	88
RNase Inhibitor	10	22
Reverse Transcriptase	20	44
Total	400	880

3. Briefly centrifuge the tube containing the beads previously collected from the SCOPE-chip.
4. Place the tube on a magnetic rack. Incubate until the supernatant becomes clear.
5. Carefully discard the supernatant without disturbing the beads.
6. Remove the tube from the magnetic rack. Add **1 mL** of pre-chilled **Wash Buffer A.** Mix well by pipetting up and down.
7. Centrifuge briefly and return the tube to the magnetic rack. Let sit until the supernatant becomes clear.
8. Carefully discard the supernatant without disturbing the beads. Remove the tube from the magnetic rack.
9. Add **500 μL** of **Wash Buffer B** and mix well by pipetting up and down.
10. Centrifuge briefly and return the tube to the magnetic rack. Let sit until the supernatant becomes clear.
11. Carefully discard the supernatant without disturbing the beads.
12. Centrifuge briefly and return the tube to the magnetic rack. Remove all residual buffer using a fine pipet tip.
13. Remove the tube from the magnetic rack and add:
    - **200 μL of the pre-chilled RT Mix for SCOPE-chip SD**
    - **400 μL of the pre-chilled RT Mix for SCOPE-chip HD**
14. Mix by pipetting up and down gently to homogenize the beads.
15. Place the tube in a preheated ThermoMixer at **42°C** and shake at **1300 rpm** for **90 minutes**.
16. Procced to the cDNA Amplification.

Important: If the cDNA amplification reaction cannot be carried out immediately, incubate the product of the reverse transcription reaction at 70°C for 15 minutes. Store at 4°C overnight.

6.2 cDNA Amplification

Materials:

- Amplification Master Mix (Clear)
- A Primer Mix (Clear)
- Amplification Enzyme (Clear)
- Product of the Reverse transcription reaction (from the previous step)

User supplied:

- 0.2 mL 8-tube PCR strips or PCR tubes
- Nuclease-free water
- Janus Magnetic rack (Singleron cat # 1120020202) or equivalent

1. On a thermocycler, set up the **cDNA Amplification program** according to the following table. The lid temperature of the thermocycler should be set at **105°C**.

Lid Temperature 105°C		Reaction Volume 50μL
Step	Temperature	Time
1	95°C	3 min
	98°C	20 sec
2	65°C	45 sec
cycles = 4	72°C	3 min
	98°C	20 sec
3	67°C	20 sec
cycles = 9	72°C	3 min
4	72°C	5 min
5	4°C	Hold

2. Thaw the Amplification Master Mix and A Primer Mix at room temperature. Vortex, centrifuge briefly, keep on ice. (Ensure that the reagents are fully thawed)

3. Prepare the PCR Mix on ice according to the table below. Add the reagents in the order listed, from top to bottom. Vortex, centrifuge briefly, and keep on ice.

PCR Mix (SCOPE-chip SD):

Component	1 RXN（μL） ×1	2 RXNs（μL） ×2.2
Amplification Master Mix	172	378.4
A Primer Mix	32	70.4
Cold nuclease-free water	188	413.6
Amplification Enzyme	8	17.6
Total	400	880

PCR Mix (SCOPE-chip HD):

Component	1 RXN（μL） ×1	2 RXNs（μL） ×2.2
Amplification Master Mix	344	756.8
A Primer Mix	64	140.8
Cold nuclease-free water	376	827.2
Amplification Enzyme	16	35.2
Total	800	1760

4. Briefly centrifuge the reverse transcription product and place it in the magnetic rack.
5. Let sit undisturbed until the supernatant becomes clear. Then, carefully discard the supernatant without disturbing the beads.
6. Centrifuge the tube briefly.
7. Place the tube back in the magnetic rack. Using a fine pipet tip to remove all residual liquid.
8. Remove the tube from the magnetic rack and add:
   - **400 μL of the PCR Mix for SCOPE-chip SD**
   - **800 μL of the PCR Mix for SCOPE-chip HD**
9. Mix well by pipetting up and down.
10. Distribute the mixture evenly into a labeled 8-tube PCR strip by pipetting **50 μL** of the PCR Mix + beads into each tube. For SCOPE-chip HD, use two 8-tube PCR strips.
11. Close the caps of the 8-tube strip and place it in the preheated thermocycler.

Important: The following steps should be carried out at a post-PCR workstation.

12. Perform PCR amplification using the cDNA Amplification program.
13. Once the thermocycler reaches 4°C, the cDNA can be stored at 4°C for 48h or at -20°C for up to a week.

6.3 cDNA Purification

Materials:

- Amplified cDNA (from the previous step)

User supplied:

- 15 mL conical tubes
- Absolute ethanol
- Nuclease-free water
- 10 mM Tris-HCl pH 8.5 or Elution Buffer
- AMPure XP
- Janus Magnetic rack (Singleron cat # 1120020202) or equivalent

Important:

- Vortex the AMPure beads thoroughly before use.
- Bring the AMPure beads to room temperature by allowing a ~1 mL aliquot to sit for 30 minutes or until they reach room temperature.
- Pipet the AMPure beads slowly to ensure the accuracy of the retrieved volume.

1. Prepare **2 mL** (SCOPE-chip SD) or **4 mL** (SCOPE-chip HD) of **80% ethanol** per reaction.
2. For SCOPE-chip SD, combine the contents of the 8-tube PCR strip containing the amplified cDNA into a labeled 1.5 mL tube. For SCOPE-chip HD, combine the contents of each 8-tube PCR strip into separate labeled 1.5 mL tubes.
3. Centrifuge briefly and measure the volume with a pipette.
4. Calculate the volume of AMPure magnetic beads equivalent to **0.6×** the total volume of the amplified cDNA. (For example, if the volume of the measured product is 400 µL, then use 0.6 × 400 µL = **240 µL of AMPure beads**.)
5. Vortex the AMPure beads until homogenized, then add the calculated volume to the amplified cDNA tube(s).
6. Mix well by vortexing for 15 seconds. Incubate at room temperature for 5 minutes.
7. Centrifuge the tube briefly and place in the magnetic rack for 5 minutes or until the liquid is clear.

Figure 5. AMPure beads in Janus magnetic rack

8. Carefully discard the supernatant without disturbing the beads.
9. Keep the tube(s) in the magnetic rack and add **800 µL** of freshly prepared **80% ethanol** to wash the magnetic beads. Incubate at room temperature for 30 seconds.
10. Carefully discard the supernatant without disturbing the beads.
11. Repeat the 80% ethanol wash one more time.
12. Centrifuge briefly and return the tube in the magnetic rack.
13. Remove all residual ethanol using a fine pipet tip.
14. Keep the lid open to dry the beads for about 2 minutes or until they are no longer shiny (no more than 5 minutes).
15. Remove the tube from the magnetic rack and add **20 µL Elution Buffer** (10 mM Tris-HCl pH 8.5). Vortex to mix.
16. Incubate at room temperature for 5 minutes. Centrifuge briefly, and place back in the magnetic rack until the liquid is clear.
17. Transfer the supernatant containing the purified cDNA to a new labeled, 1.5 mL tube. For SCOPE-chip HD, combine the supernatants from the two 1.5 mL tubes into a single new tube.
18. The cDNA can be stored at 4°C for 72 hours or at -20°C for up to three months.

6.4 cDNA QC

Materials:

- Purified cDNA (from the previous step)

User supplied:

- Qubit Assay Tubes and
- Qubit 1x dsDNA HS assay kit

1. Take an aliquot (1 µL) of the cDNA and measure its concentration using a Qubit 4 Fluorometer.
2. Take an aliquot of the cDNA (2-5 ng/µL) to measure the fragment size profile using Agilent Fragment Analyzer 5200 or equivalent.
3. The ideal cDNA should meet the following criteria:
   a) Average peak from region between 200 - 5,000 bp: > 1,200 bp;
   b) The fragments between 1,000 – 5,000 bp should represent more than 30% of the total molecules.

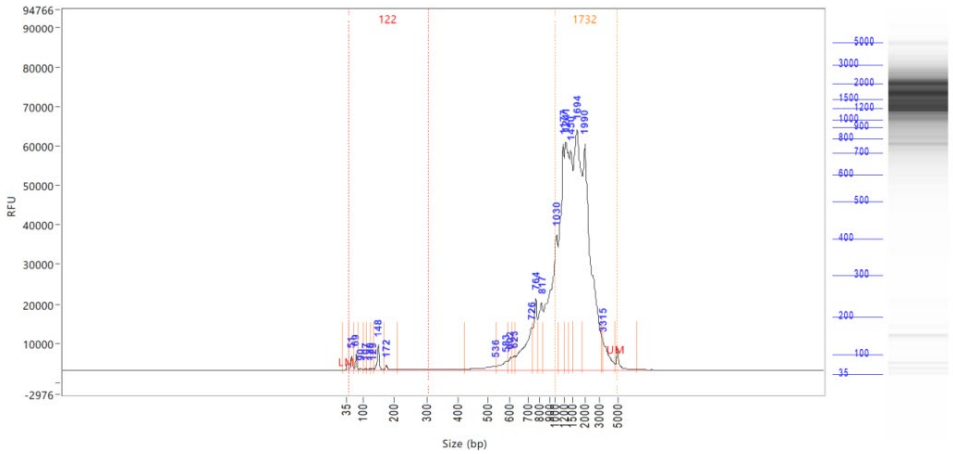


Figure 6: QC of cDNA on Agilent Fragment Analyzer 5200

c) If the fragments below 300 bp represent more than 10% of the total molecules, perform a second AMPure purification according to the table below.

Percentage of fragment between 40-300 bp	Ratio of AMPure beads added
10% - 20%	0.8 x
21% - 35%	0.7 x
> 35%	0.6 x

It is recommended to dilute the cDNA with nuclease-free water to a volume of 100 µl to perform the second round of AMPure purification.

7. Library Preparation

7.1 Fragmentation

Materials:

- Fragmentation Buffer (Orange)
- Fragmentation Enzyme Mix (Orange)
- 1xTE (Orange)
- Purified cDNA (from the previous step)

User supplied:

- PCR tubes

1. On a thermocycler, set up the **Fragmentation program** according to the following table. The lid temperature of the thermocycler should be set at **75°C**.

Lid Temperature 75°C		Reaction Volume 35μL
Step	Temperature	Time
1	37°C	10 min
2	65°C	30 min
3	4°C	Hold

2. Thaw the Fragmentation Buffer at room temperature. Vortex 5 to 8 seconds, centrifuge briefly, and keep on ice. If a precipitate is visible, mix by vortexing until the solution is clear.
3. Vortex the Fragmentation Enzyme Mix 5 to 8 seconds before use.
4. Dilute the cDNA with 1xTE according to the following recommendations:
   a) If the total amount of cDNA is less than 10 ng, use all the cDNA.
   b) If the total amount of cDNA is between 10 and 50 ng, use 10 ng as input.
   c) If the total amount of cDNA is above 50 ng, use 50 ng as the input.
5. Prepare the Fragmentation Reaction on ice according to the table below. Add the reagents in the order listed from top to bottom. Vortex for 10 seconds, centrifuge briefly, and keep on ice.

   Fragmentation Reaction:

Component	Volume（μL）
Diluted cDNA (10 ng or 50 ng）	26
Fragmentation Buffer	7
Fragmentation Enzyme Mix	2
Total	35

6. Immediately place the PCR tube in the pre-heated thermocycler and run the Fragmentation program.
7. Once the thermocycler reaches 4°C, proceed immediately with the adapter ligation.

7.2 Adapter Ligation

Materials:

- Adaptor (Blue)
- Ligation booster (Blue)
- Ligation Mix (Blue)
- Fragmentation product (from the previous step)

1. On a thermocycler, set up the **Ligation program** according to the table below. The lid temperature of the thermocycler should be **off** and allowed to cool down to room temperature.

Lid Temperature OFF		Reaction Volume 70μL
Step	Temperature	Time
1	20°C	15 min
2	4°C	Hold

2. Thaw the Adaptor at room temperature, mix well, keep on ice.
3. Gently mix the Ligation Mix and the Ligation booster by pipetting up and down. Centrifuge briefly and keep on ice. **Do not vortex.**
4. On ice, add the Adapter Ligation reagents directly to the fragmented cDNA according to the table below.

   Adapter Ligation*:

Component	Volume（μL）
Fragmented cDNA	35
Ligation Mix**	30
Ligation booster	1
Adaptor	2.5
Total	68.5

** It is not recommended to prepare a master mix*

***Ligation Mix is viscous. Pipet carefully to ensure an accurate volume*

5. Mix gently by pipetting up and down and centrifuge briefly. Place the reaction tube into the pre-cooled (20°C) thermocycler and proceed with the Ligation PCR program. Do not vortex.
6. Once the thermocycler reaches 4°C, immediately proceed to the purification of the adapter-ligated cDNA.

7.3 Purification of the Adapter-Ligated cDNA

Materials:

- 1xTE (Orange)
- Adapter-ligated cDNA (from the previous step)

User supplied:

- PCR tubes
- AMPure XP
- Nuclease-free water
- Absolute ethanol
- Janus Magnetic rack (Singleron cat # 1120020202) or equivalent

Important:

- Vortex the AMPure beads thoroughly before use.
- Bring the AMPure beads to room temperature by allowing a ~1 mL aliquot to sit for 30 minutes or until they reach room temperature.
- Pipet the AMPure beads slowly to ensure the accuracy of the retrieved volume.

1. Prepare **0.5 mL** of **80% ethanol** and **25 µl 0.1x TE** per reaction (1:10 dilution of 1×TE with nuclease-free water).
2. Centrifuge the adapter-ligated cDNA from the previous step briefly and measure the volume with a pipette.
3. Calculate the volume of AMPure beads equivalent to **0.5×** the total volume of the ligated product. (For example, if the volume of the measured product is 68.5 µL, then 0.5 × 68.5 µL = 34.3 µL of AMPure magnetic beads should be used).
4. Vortex the AMPure beads until homogenized and add the calculated volume to the ligated product.
5. Mix well by vortexing and incubate at room temperature for 5 minutes.
6. Centrifuge the tube briefly and place in the magnetic rack for 5 minutes or until the liquid is clear.
7. Carefully discard the supernatant without disturbing the beads.
8. Keep the tube in the magnetic rack and add **200 µL** of freshly prepared **80% ethanol** to wash the magnetic beads. Let sit at room temperature for 30 seconds.
9. Carefully discard the supernatant without disturbing the beads.
10. Repeat the 80% ethanol wash one more time.
11. Centrifuge briefly and return the tube to the magnetic rack.
12. Remove all residual ethanol using a fine pipet tip.

13. Keep the lid open to dry the beads for about 2 minutes or until the beads are no longer shiny (no more than 5 minutes).
14. Remove the tube from the magnetic rack and add **17 µL** of **0.1x TE**. (1:10 dilution of 1×TE with nuclease-free water). Vortex to mix.
15. Incubate at room temperature for 5 minutes. Centrifuge briefly, and place back in the magnetic rack until the liquid is clear.
16. Transfer **15 µL** the **supernatant** containing the purified adapter-ligated product to a new, labeled PCR tube.
17. The adapter-ligated product can be stored at 4°C for 72 hours or at -20°C for one week.

7.4 Library Amplification

Materials:

- Library Amp Mix v2 (White)
- Unique Dual Indexes (White)
- Purified adapter-ligated cDNA (from the previous step)

1. On a thermocycler, set up the **Library Amplification program** according to the following table. The lid of the thermocycler should be set at **105°C**.

Lid Temperature 105°C		Reaction Volume 50 µL
Step	Temperature	Time
1	98°C	30 sec
2（cycle number varies*）	98°C	10 sec
	65°C	75 sec
3	65°C	5 min
4	4°C	Hold

** The number of cycles should be selected based on the amount of cDNA input as depicted in the table below.*

cDNA input	Number of cycles
50ng	10
10ng	13

2. Thaw the Library Amp Mix v2 and Unique Dual Indexes on ice. Vortex, centrifuge briefly, keep on ice. Ensure that the reagents are fully thawed.
3. On ice, add the following reagents directly to the purified adapter-ligated product:

Component	Volume（µL）
Purified adapter-ligated product	15
Library Amp Mix v2	25
Unique Dual Indexes*	10
Total	50

** Choose a different UDI Adapter for each sample and use Illumina pooling recommended strategies. See Appendix for NovaSeq X.*

4. Mix by pipetting up and down and centrifuge briefly.
5. Place the reaction in the thermocycler and run the Library Amplification program.
6. Once the thermocycler reaches 4°C, immediately proceed to the purification and size selection of the amplified library.

7.5 Purification and Dual Size Selection of the Amplified Library

Materials:

- Amplified library (from the previous step)

User supplied:

- 1.5 mL tubes
- Absolute ethanol
- Nuclease-free water
- AMPure XP
- Janus Magnetic rack (Singleron cat # 1120020202) or equivalent
- 10mM Tris-HCl pH 8.5 or Elution Buffer

Important:

- Vortex the AMPure beads thoroughly before use.
- Bring the AMPure beads to room temperature by allowing a ~1 mL aliquot to sit for 30 minutes or until they reach room temperature.
- Pipet the AMPure beads slowly to ensure the accuracy of the retrieved volume.

1. Prepare **0.5 mL** of **80% ethanol** per reaction.
2. Briefly centrifuge the amplified library and measure the volume with a pipette.
3. If needed, fill up to 50 µL with nuclease free water.
4. Vortex the AMPure beads until homogenized and add **25 µL** to the amplified libraries.
5. Mix thoroughly by vortexing 30 seconds and incubate at room temperature for 5 minutes.
6. Centrifuge the tube briefly and place in the magnetic rack for 5 minutes or until the liquid is clear.
7. Transfer **70 µL** of the **supernatant** containing the amplified library to a new tube.
8. Vortex the **AMPure beads stock tube** until homogenized and add the **7.5 µL** to the amplified libraries.
9. Mix well by vortexing and incubate at room temperature for 5 minutes.

10. Centrifuge the tube briefly and place in the magnetic rack for 5 minutes or until the liquid is clear.
11. Carefully remove and discard the supernatant without disturbing the beads.
12. Keep the tube in the magnetic rack and add 200 µL of freshly prepared 80% ethanol to wash the magnetic beads. Incubate at room temperature for 30 seconds.
13. Carefully discard the supernatant without the beads.
14. Repeat the 80% ethanol wash one more time.
15. Centrifuge briefly and return the tube to the magnetic rack.
16. Remove all residual ethanol using a fine pipet tip.
17. Keep the lid open to dry the beads for about 2 minutes or until the beads are no longer shiny (no more than 5 minutes).
18. Remove the tube from the magnetic rack and add **20 µL** of **Elution Buffer** or 10mM Tris-HCl pH 8.5. Vortex to mix.
19. Incubate at room temperature for 5 minutes. Centrifuge briefly, and place back in the magnetic rack until the liquid is clear.
20. Transfer **18 µL** the supernatant containing the purified library to a new, labeled tube.
21. The libraries can be stored at -20°C or -80°C for up to three months.

7.6 QC of the Library

Materials:

- Purified library (from the previous step)

User supplied:

- Qubit Assay Tubes
- Qubit 1x dsDNA HS assay kit

1. Take an aliquot (1 μL) of the library to measure the concentration using Qubit 4 Fluorometer.
2. Take an aliquot of the library (2 - 5 ng/μL) to measure the fragment size profile using Agilent Fragment Analyzer 5200 or equivalent.
3. The ideal library should meet the following criteria:
    a. The main peak should average between 400 – 700 bp when the region analyzed is set between 300 bp and 2000 bp.
    b. The fragments between 900 bp and 1500 bp should account for less than 10% of the molecules.
    c. The fragments below 300 bp should account for less than 5% of the molecules.
4. If a peak is observed around 200 bp or if the fragments below 300 bp account for more than 5%, an extra AMPure purification using a bead ratio of 0.7x the volume of your sample should be performed.

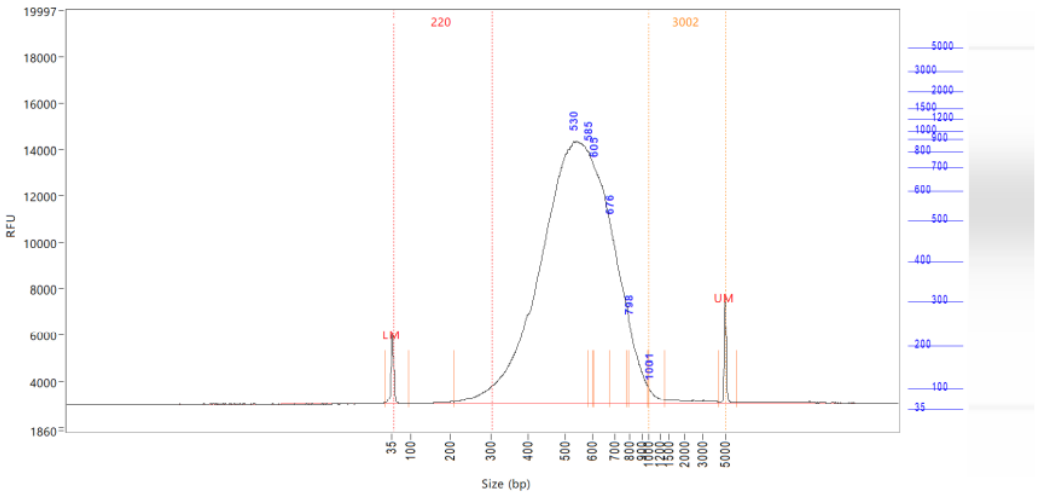


Figure 7. QC of the NGS library on Agilent Fragment Analyzer 5200

Appendix A: Principle of Technology

Single Cell Partitioning and mRNA Capture

The SCOPE-chip® isolates single cells into thousands of microwells. Subsequently, Barcode Beads are added to the microwells on the SCOPE-chip, ensuring that only one Barcode Bead falls into each microwell.

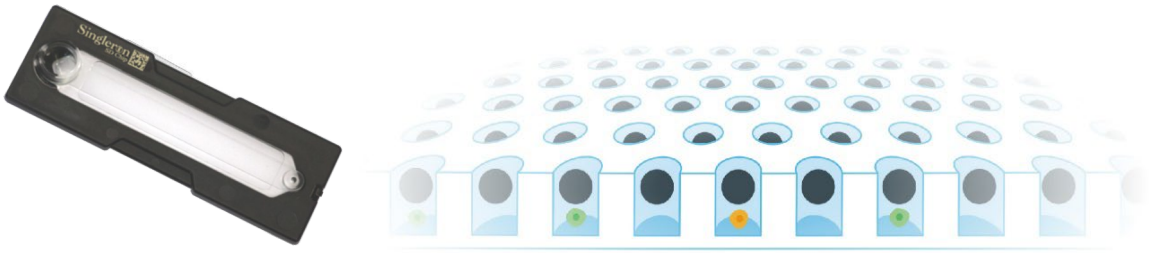


Figure 8. SCOPE-chip and microwells containing cells and Barcode Beads

Barcode Beads contain the Illumina Read 1 sequencing primer sequence, a unique cell label (**Cell Barcode**) for identifying the cell origin of RNA, a unique molecular identifier (**UMI**) for cDNA quantification, and poly (dT) sequence to capture total mRNA (see below). After cell lysis, mRNA is captured onto the Barcode Beads by the poly (dT) sequence. Barcode Beads with captured mRNA molecules are subsequently collected from the SCOPE-chip and subjected to a reverse transcription reaction. Synthesized cDNA molecules will each contain a unique Cell Barcode and a UMI sequence.

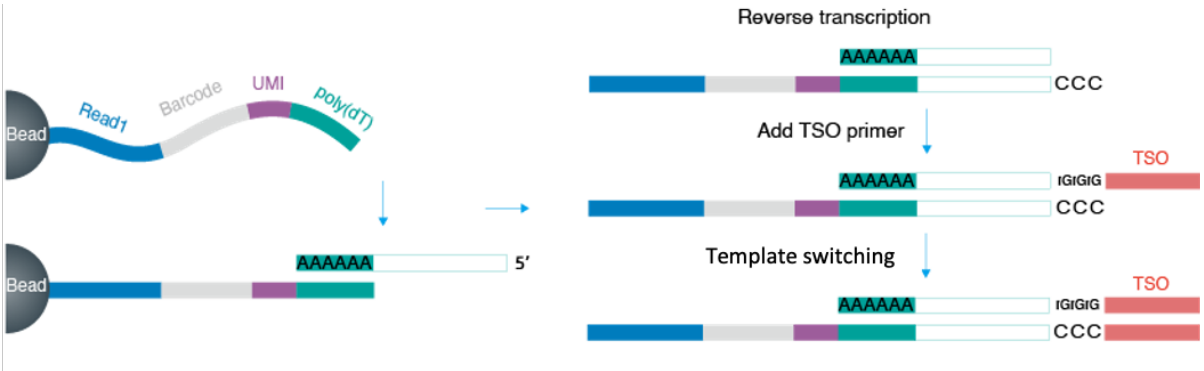


Figure 9. mRNA capture on Barcode Beads and reverse transcription

cDNA Amplification

The PCR handle sequence at the 5' end of the single cell Barcode Beads and the TSO sequence added during the reverse transcription process are both used for the amplification of the full-length cDNA (sequencing primers are adapted to the Illumina second-generation sequencing platform).

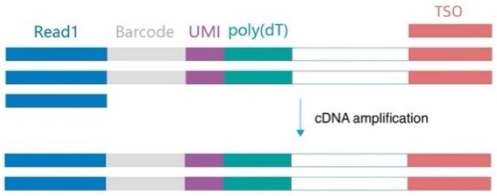


Figure 10. cDNA amplification with PCR

Library preparation

The cDNA obtained by reverse transcription amplification is first fragmented. The fragmented product is repaired and ligated to truncated adapters. The ligated product is then indexed by PCR, resulting in an NGS library compatible with Illumina sequencing instruments.

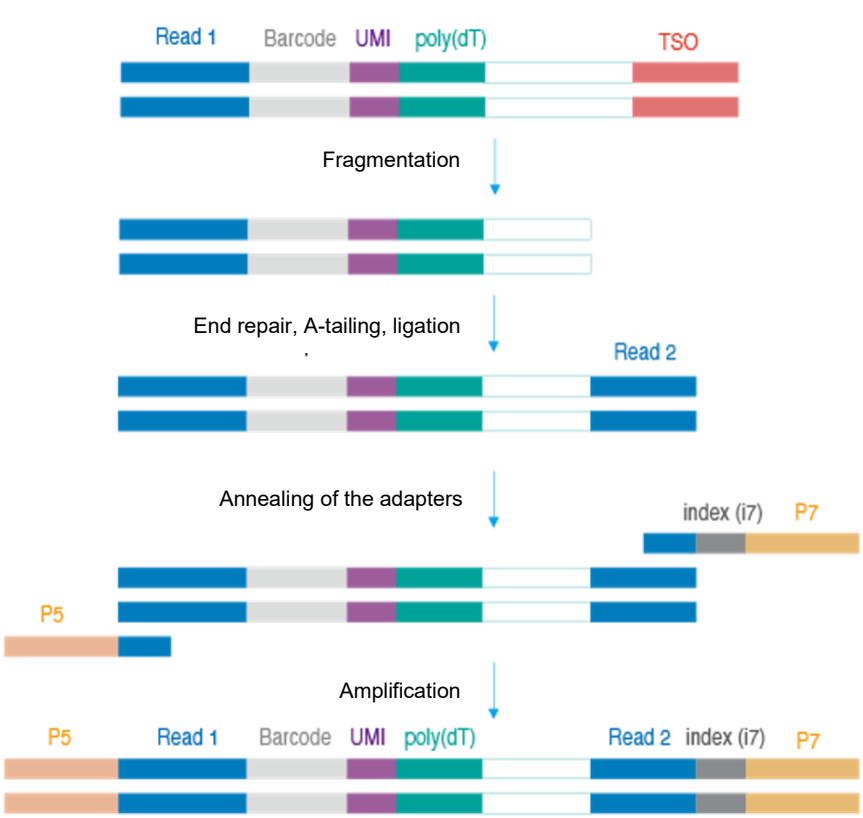


Figure 11. Library preparation step

Appendix B: Loading of the SCOPE-chip

The video manual depicting the loading of the SCOPE-chip with cells can be accessed here:

https://youtu.be/xrvxoe19iZk

The video manual depicting the loading of the SCOPE-chip with Barcode Beads can be accessed here:

https://youtu.be/6Lg6aySKA3o

The video manual depicting the retrieval of Barcode Beads can be accessed here:

https://youtu.be/UuNip_AJ2Qs

Appendix C: Sequencing

Sequencing Libraries

GEXSCOPE® Single Cell RNA Library Kits generate standard Illumina paired-end constructs, starting with the P5 adapter and ending with the P7 adapter. These adapters are standard Illumina sequences used in bridge amplification. A 60-base barcode, consisting of 27 bases of unique sequences and linkers, is located next to the "Read1" sequencing primer site. The library also includes 8-base i5 and i7 sample indexes (see table below: Indexing sequences for sample sheet). Paired-end sequencing is performed, with Read 1 resolving the 60-base barcode and 12-base UMI, while Read 2 is used to capture gene-specific sequences. The sequencing produces standard Illumina FASTQ files.

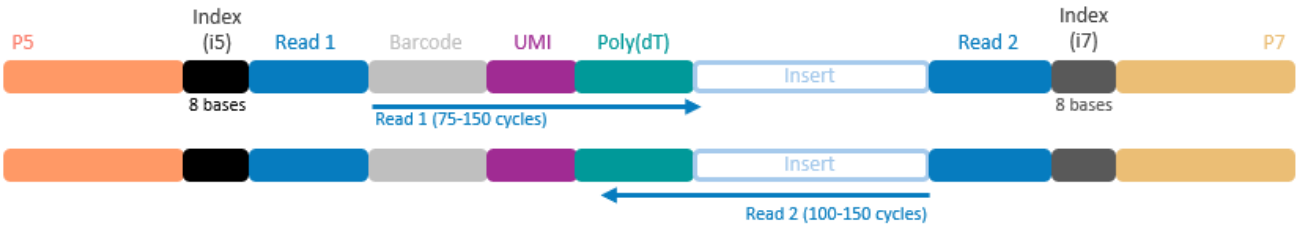


Figure 12: Sequencing Library.

Illumina Sequencer Compatibility

Singleron Biotechnologies verified the compatibility of the sequencers listed below. Sequencer choice can affect assay performance. For more information about performance variation, visit the Singleron Biotechnologies Support website.

- NovaSeq 6000
- NovaSeq X (see pooling strategies below)

Sequencing Depth & Run Parameters

Sequencing Depth:

- Peripheral blood mononuclear cells (PBMC) or lymphocytes: 30 000 read pairs per cell.
- Other sample types: 50 000 read pairs per cell.

Sequencing Format:

Sequencing read	Recommended number of cycles
Read 1*	75-150
i7 Index	8
i5 Index	8
Read 2#	150

**For Read 1, a minimum of 75 cycles is required to cover cell barcodes and UMI.*

#For Read 2, Mapping quality can be compromised when using a reduced number of cycles. We strongly suggest performing a minimum of 100 cycles.

Library Denaturation and Loading

After quantification and normalization, the libraries should be denatured and diluted as recommended for Illumina sequencing platforms. For information on denaturing and diluting libraries refer to the Illumina latest documentation.

Indexing sequences for sample sheet

Index Name	i7 for sample sheet	i5 for sample sheet in Forward orientation	i5 for sample sheet in Reverse orientation
Unique Dual Index 1	CCGCGGTT	AGCGCTAG	CTAGCGCT
Unique Dual Index 2	TTATAACC	GATATCGA	TCGATATC
Unique Dual Index 3	GGACTTGG	CGCAGACG	CGTCTGCG
Unique Dual Index 4	AAGTCCAA	TATGAGTA	TACTCATA
Unique Dual Index 5	ATCCACTG	AGGTGCGT	ACGCACCT
Unique Dual Index 6	GCTTGTCA	GAACATAC	GTATGTTC
Unique Dual Index 7	CAAGCTAG	ACATAGCG	CGCTATGT
Unique Dual Index 8	TGGATCGA	GTGCGATA	TATCGCAC
Unique Dual Index 9	AGTTCAGG	CCAACAGA	TCTGTTGG
Unique Dual Index10	GACCTGAA	TTGGTGAG	CTCACCAA
Unique Dual Index 11	TCTCTACT	CGCGGTTC	GAACCGCG
Unique Dual Index 12	CTCTCGTC	TATAACCT	AGGTTATA
Unique Dual Index 13	CCAAGTCT	AAGGATGA	TCATCCTT
Unique Dual Index 14	TTGGACTC	GGAAGCAG	CTGCTTCC
Unique Dual Index 15v2	CAGTAGGC	TGACGAAT	ATTCGTCA
Unique Dual Index 16v2	TGACGAAT	CAGTAGGC	GCCTACTG

Pooling strategies for NovaSeq X / X Plus instruments

Illumina NovaSeq X/X Plus uses two-color XLEAP-SBS Chemistry:

- T: Green
- **A**: Blue
- C: Green + Blue
- **G**: Dark

Illumina recommends avoiding index arrangements that result in dark (**G**) or blue (A) signal bases during any cycle. See examples below.

Examples of good indexing strategies:

Strategy 1:

Index Name	i7 for sample sheet	i5 in reverse orientation
Unique Dual Index 1	CCGCGGTT	CTAGCGCT
Unique Dual Index 4	AAGTCCAA	TACTCATA
Unique Dual Index 5	ATCCACTG	ACGCACCT
Unique Dual Index 8	TGGATCGA	TATCGCAC
	YYYYYYYY	YYYYYYYY

Strategy 2:

Index Name	i7 for sample sheet	i5 in reverse orientation
Unique Dual Index 2	TTATAACC	TCGATATC
Unique Dual Index 3	GGACTTGG	CGTCTGCG
Unique Dual Index 5	ATCCACTG	ACGCACCT
Unique Dual Index 7	CAAGCTAG	CGCTATGT
	YYYYYYYY	YYYYYYYY

Strategy 3:

Index Name	i7 for sample sheet	i5 in reverse orientation
Unique Dual Index 9	AGTTCAGG	TCTGTTGG
Unique Dual Index11	TCTCTACT	GAACCGCG
Unique Dual Index 13	CCAAGTCT	TCATCCTT
Unique Dual Index 15v2	CAGTAGGC	ATTCGTCA
	YYYYYYYY	YYYYYYYY

Strategy 4:

Index Name	i7 for sample sheet	i5 in reverse orientation
Unique Dual Index 10	GACCTGAA	CTCACCAA
Unique Dual Index 12	CTCTCGTC	AGGTTATA
Unique Dual Index 14	TTGGACTC	CTGCTTCC
Unique Dual Index 16v2	TGACGAAT	GCCTACTG
	YYYYYYYY	YYYYYYYY

An example of an incompatible strategy:

Index Name	i7 for sample sheet	i5 in reverse orientation
Unique Dual Index 1	CCGCGGTT	CTAGCGCT
Unique Dual Index 2	TTATAACC	TCGATATC
Unique Dual Index 15v2	CAGTAGGC	ATTCGTCA
Unique Dual Index 16v2	TGACGAAT	GCCTACTG
	YYXYXXYY	YYYYYYYY

Positions 3, 5 and 6 of i7 only present blue or dark bases.

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