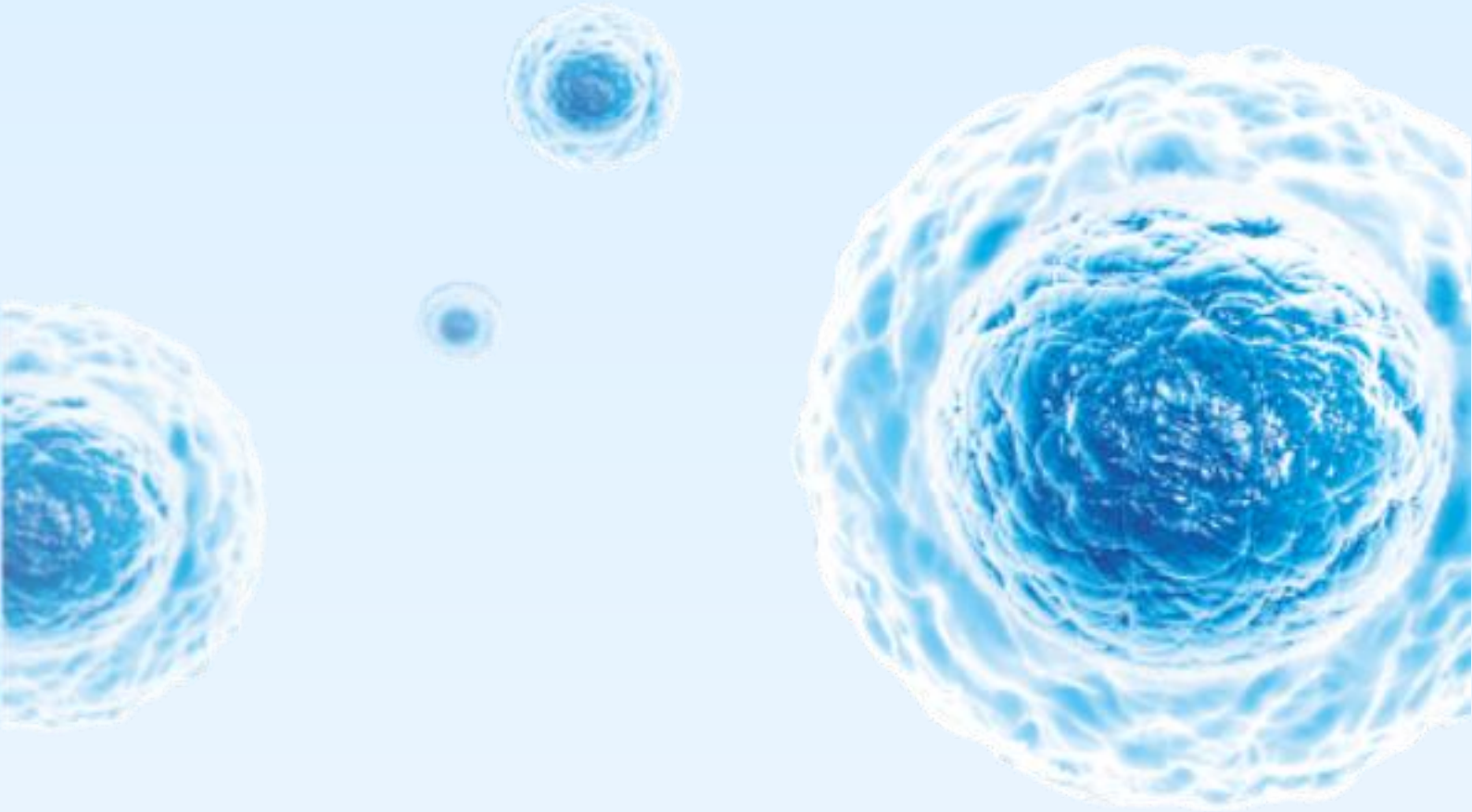
Singleron



AccuraSCOPE® Single Cell Full-Length Transcriptome Library Kit Handbook (96-Well)

RUO Research Use Only

2025.04

This handbook applies to the following products:

Cat. No.	Product name	Reaction
1403464	AccuraSCOPE® Single Cell Full-Length Transcriptome Library Kit (96-Wells)	1 RXN

Version Information

Version no.: 2025.04

Release date: 2025.04

Version no.	Updates
2023/12	• First version
2025/04	• Logo and fragmentation time updates

Disclaimer

Trademarks

Singleron Biotechnologies GmbH

Address:
Gottfried-Hagen-Strasse 60
51105 Cologne, Germany

Phone: +49 (0) 221 16824777
Email: info@singleronbio.com
Website: www.singleron.bio

Table of Contents

1. General Information

1.1 Product Overview

With the Single-cell full-length transcriptome library kit (96-Well version), RNA capture and mixed library construction can be completed for 96 samples simultaneously, enabling rapid access to full-length gene expression information for each sample.

The Single-cell full-length transcriptome library kit (96-Well version) captures mRNA in the PCR plate, performs reverse transcription and cDNA amplification, thereby constructing a mixed RNA-Seq library containing transcripts from multiple samples. After NGS sequencing, transcripts from different samples are identified based on Well Barcode information.

1.2 Modular Components

Single Cell Full-Length Transcriptome Library Kit (96-Well)		
Components	Quantity	Volume
DR barcode beads	1	5 μL/well
ASF TS Primer	1(96)	10 μL/well
ASF Lysis Buffer	1	600 μL
ASF Lysis Enhancer	1	100 μL
RNase Inhibitor	2	50 μL
100 mM DTT	1	100 μL
Wash Buffer B	2	7 mL
RT Master Mix	2	400 μL
Reverse Transcriptase	1	70 μL
Amplification Master Mix	2	400 μL
ASF Amplification Primer	1	120 μL
Amplification Enzyme	2	20 μL
Fragmentation Buffer V3	1	17 μL
Fragmentation Enzyme Mix V3	1	6 μL
1×TE	1	800 μL
Ligation Mix	1	72 μL
Ligation booster	1	4 μL
Adaptor	1	50 μL
Library Amp Mix V3	1	60 μL
Indexing Primer Mix 1	ATCACGTT	30 μL
Indexing Primer Mix 2	CGATGTTT	30 μL
Purification beads wash buffer	1	1500 μL

2. Instruments, Reagents and Consumables Supplied by Users

For both working stations (pre-PCR and post-PCR):

- Absolute ethanol
- Nuclease-free water
- 1.5 mL nucleic acid low-binding tubes
- 15-mL conical tubes
- 50-mL conical tubes
- Serological pipettes
- Single-channel pipettes (p10, p200 and p1000)
- Multi-channel pipettes (8 channel or 12 channel)
- Sterile RNAse/DNAse-free certified filter tips
- Mini centrifuge
- Vortex
- DynaMag™-2 Magnetic rack (Thermo Fisher cat # 12321D) or equivalent

Single Cell lysis and RNA capture (Pre-PCR)

- RNase Away or equivalent
- PBS pH 7.4 (without Ca2+, without Mg2+)
- 0.04% Trypan Blue solution
- 10% TritonX-100
- Vertical mixer
- Thermal cycler
- Refrigerated centrifuge compatible with 96-Well plate

cDNA Amplification and Library Preparation (Post-PCR)

- Thermal cycler
- 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 (Beckman Coulter cat # A63880, A63881, A63882)
- 10mM Tris-HCl pH 8.0 or Elution Buffer (EB, Qiagen cat # 19086)
- 0.2-mL 8-tube PCR strip
- PCR tubes
- Refrigerated centrifuge compatible with a 96-Well plate

Important: The consumables used should be sterile and RNAse/DNAse-free certified.

3. Workflow and Timeline

Workflow	station	station	station
Single cell Partitioning	Pre-PCR Station	1 h	
mRNA Capture			
Reverse Transcription		1.5-2 h	4℃ Overnight
cDNA Amplification	Post-PCR Station	2 h	
Library Preparation		3 h	4℃ for 48 h or -20℃ for 1 week
		Total 8-9 h	-20℃ or -80℃ for 3 months

Figure 1. Workflow and timeline overview

4. Preparation

We recommend users to establish a “clean workstation” for all pre-PCR experiments that require cleanroom conditions, such as cell lysis, mRNA capture and reverse transcription. For RNA-related work, clean working surfaces and pipets with RNAse Away (or equivalent). Operators should wear masks and sterile gloves to avoid contamination and RNA degradation.

A second workstation (post-PCR) should be designed where cDNA amplification, purification and QC is performed, as well as library preparation and QC.

Important: All buffers need to be mixed before use. If a precipitate is present in buffers, gently pipet to mix the solution, ensuring that is clear before use.

5. Single-Cell Partitioning and mRNA Capture

Materials:

- Single cell suspension or Total RNA

User supplied:

- PBS

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

   Lysis Mix:

Component	1 RXN（μL） ×96-Well
ASF Lysis Buffer	450
100mM DTT	60
RNAse Inhibitor	30
ASF Lysis Enhancer	60
Total	600

2. Thaw the DR barcode beads. Vortex for 10s, centrifuge briefly and place on ice. Carefully remove the sealing film on the 96-Well plate.
3. Transfer 5μL of DR barcode beads accurately to the bottom of a new RNase-free 96-well plate, ensuring the correct well order and preventing cross-contamination.

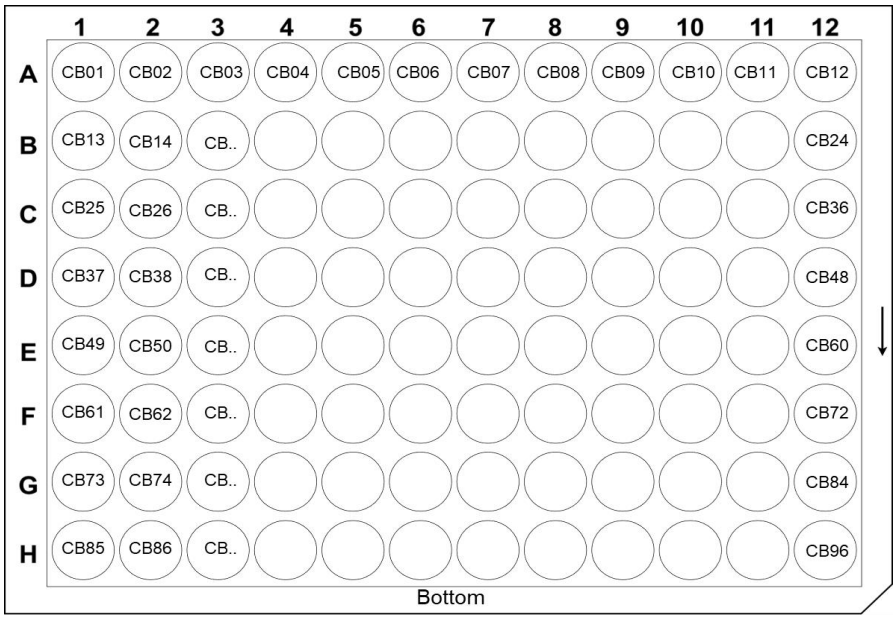


Figure 2. The arrangement order of DR barcode beads in a 96-well plate

4. Distribute the Lysis Mix into a 8-tube PCR strip (75 μL in each tube) and place it on ice.

5. Using a multichannel pipet, dispense 5 μL Lysis Mix to each well of the 96-Well plate containing the DR barcode beads.
6. The kit supports a total of 1-10000 cells or 10pg-100ng total RNA per well.

Cell input

1) Single cell per well

Various methods can be used to aliquot single cells into the single wells of the PCR plate containing the Lysis Buffer and Barcoding Beads. Using a mouth pipette under a microscope or by flow cytometry.

2) Multiple cells per well

Dilute the cell suspension to the appropriate concentration, then dispense in the PCR plate containing the Lysis Buffer and Barcoding Beads using a pipette or other similar equipment.

The final volume in each well depends on the sample type, as referred in the table below.

Sample Type	Final Sample Volume	
Single cell	⩽1μL	
Multiple cell	Less than 50 cells	⩽1μL
	From 50 to 1000 cells	⩽5μL
Total RNA	⩽5μL	

7. Seal the PCR plate with sealing film, spin down briefly and binding on a vertical mixer rotating at 20-30 rpm for 15 min, as in figure 3.

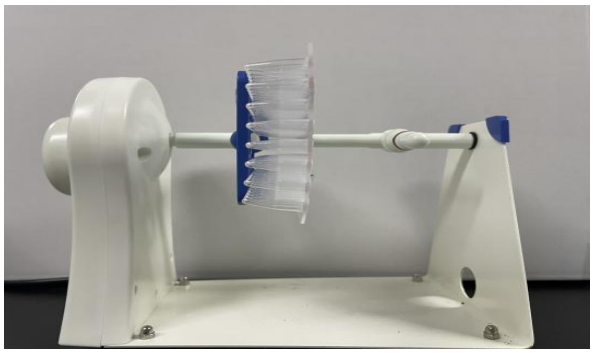


Figure 3. PCR plate binding on the vertical mixer

8. After the cell lysis, briefly spin down the PCR plate and store on ice for no more than one hour or place in the freezer at -80°C for no more than 3 days.

6. Reverse Transcription

Materials:

- Reverse transcription reagent

1. Preparation: thaw RT Master Mix, and ASF TS primer at room temperature, vortex for 10s, centrifuge briefly and place on ice. Prepare RT pre-Mix on ice according to the following table, mix well by vortex for 10s and spin down briefly.

Component	1 RXN(μL) × 96-Well
RT Master Mix	720
Reverse Transcriptase	60
RNase Inhibitor	30
Nuclease-free Water	264
10% TritonX-100	6
Total	1080

Note: Mix the reagent solution well to ensure that it is clear before use.

2. Set a PCR instrument, 42° C 90min, 70° C 15min, 4° C hold, heat lid 80° C (preheating in advance).
3. Briefly spin down the 96-Well PCR plate containing the lysed cells and place it on ice. If the plate was at -80° C, place it at room temperature until the contents are completely thawed.
4. Add 90 μL of pre-chilled Wash Buffer B + 0.05% TritonX-100 to each well of the 96-Well PCR plate, place it in the magnetic separation rack, and carefully aspirate the supernatant after the liquid turns clear (about 1 minute).

Wash Buffer B + 0.05% TritonX-100:

Component	1 RXN(μL) × 96-Well
Wash Buffer B	9950
10% TritonX-100	50
Total	10000

5. Distribute the RT pre-Mix into a 8-tube PCR strip (130 μL in each tube) and place it on ice.
6. Using a multichannel pipet, dispense 9 μL RT pre-Mix to each well of the 96-Well plate. Then add 1 μL of the ASF TS primer to each well, mix well and centrifuge briefly.

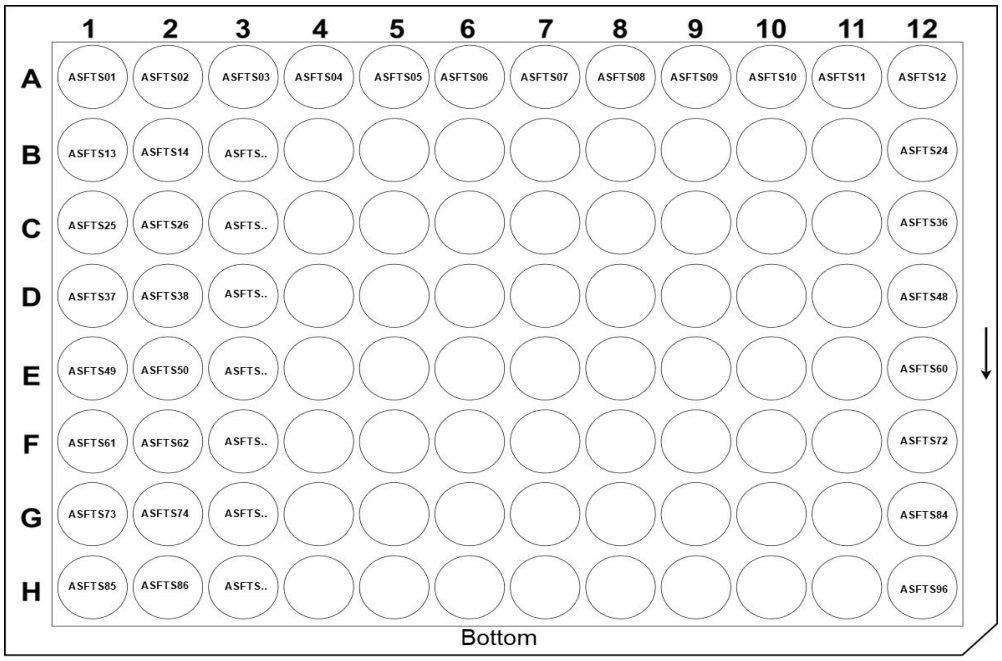


Figure 4. The arrangement order of ASF TS primer in a 96-well plate

Important: This step needs to be carried out quickly to prevent the Well Barcoding Beads from drying out. It is recommended to use a multichannel pipette for the operation. After removing the supernatant (Wash Buffer B + 0.05% TritonX-100) from each column, promptly add the “RT pre-Mix” to the corresponding wells.

96 kinds of ASF TS primer correspond to 96 kinds of Barcoding Beads. Do not disturb the corresponding relation.

7. Place in a pre-set PCR instrument in step 2, and start the reverse transcription.

Note: Stop point, the RT product can be stored at 4°C for 15 hours.

7. cDNA Amplification

7.1 cDNA Amplification

Materials:

- Amplification Master Mix
- ASF Amplification Primer
- Amplification Enzyme
- Reverse transcription product (from previous step)

User supplied:

- 0.2-mL 8-tube PCR strip
- Nuclease-free water
- DynaMag™-2 Magnetic rack

1. Thaw the Amplification Master Mix and ASF Amplification Primer at room temperature, vortex, and place on ice.
2. Prepare the PCR Mix on ice according to the table below. Add the reagents in the order listed, vortex, centrifuge briefly and keep on ice.

   PCR Mix:

Component	1 RXN(μL) ×1
Amplification Master Mix	516
ASF Amplification Primer	96
Amplification Enzyme	24
Nuclease-free water	564
Total	1200

3. After reverse transcription (RT) is complete, remove the 96-Well plate from the PCR instrument, briefly spin it down, remove the seal, and transfer all the RT products from the wells to a new 2 mL centrifuge tube.

   Important: Try to aspirate all the liquid and magnetic beads, avoiding excessive foaming caused by repeated pipetting. Use 100 μL of nuclease-free water to wash each well sequentially, and finally add the water into the 2-mL centrifuge tube, if necessary.

4. Place the 2-mL tube containing the RT products on the magnetic stand. After the solution clears, remove the supernatant, add 1 ml of nuclease-free water, and gently shake to mix. Return the tube to the magnetic stand, remove the supernatant after the solution clears and the magnetic beads have accumulated by the magnet (about 1 minute)

5. Add 1200 μL of the PCR mix to the 2-mL tube, thoroughly mix with the magnetic beads, and distribute into 3 sets of 8-tube PCR strips, 50 μL per tube, for a total of 24 tubes.
6. Place the 8-tube PCR strip in the preheated thermal cycler. Perform PCR amplification using the following program:

Lid Temperature 105℃	Reaction Volume 50μL	Run Time
Step	Temperature	Time (mm:ss)
1	95°C	03:00
2 cycles = 4	98°C	00:20
	60°C	00:45
	72°C	03:00
3 *cycles = 8~14	98°C	00:20
	65°C	00:20
	72°C	03:00
4	72°C	05:00
5	4°C	Hold

The selection of the number of PCR cycles can be selected based on the following table:

Per well	PCR *cycle
1-5 cells or 10-50 pg Total RNA	16
5-50 cells or 50-500 pg Total RNA	13
50-100 cells or 500-1000 pg Total RNA	10
100-500 cells or 1-5 ng Total RNA	9
500-1000ls or 5-10 ng Total RNA	8

7. 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.

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

7.2 cDNA Purification

Materials:

- Amplified cDNA (from previous step)
- Purification Beads Wash Buffer

User supplied:

- 15-mL conical tubes
- Absolute ethanol
- Nuclease-free water
- 10mM Tris-HCl pH 8.0 or Elution Buffer
- AMPure
- DynaMag™-2 Magnetic rack

Important: Vortex the AMPure beads thoroughly before use.

Bring the AMPure beads to room temperature by incubating ~1 mL aliquot for 30 minutes or until they reach room temperature.

Pipet the AMPure beads slowly to ensure the accuracy of the retrieved volume.

During the AMPure cleanup, the tubes containing the magnetic beads should not be rotated once placed on the magnetic stand.

1. Prepare 5 mL of 80% ethanol per reaction.
2. Pooling the cDNA:

   1) Briefly centrifuge the 8-tube PCR strips, collect all the PCR products into a new 2.0-mL tube.

   2) Calculate the volume of AMPure magnetic beads equivalent to 0.6x the total volume of the amplified cDNA. For example, if the volume of the measured product is 1200 µL, then use 0.6x1200=720 µL of AMPure beads.

   3) Vortex for 15 s, mix well, and incubate at room temperature for 5 min.
3. Centrifuge the tube briefly and place on the magnetic rack for 5 minutes or until the liquid is clear (Figure 5).

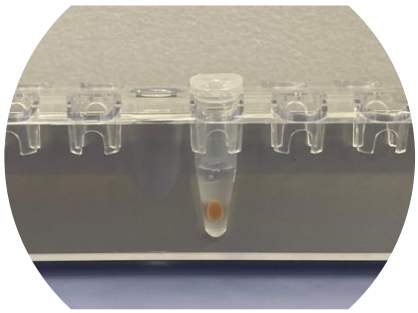


Figure 5. Clearing of the liquid with AMPure beads on magnetic stand

4. Carefully remove the supernatant without disturbing the beads.
5. Remove the tube from the magnetic stand and add 800 µL Purification Beads Wash Buffer and vortex 15 seconds until the beads are fully resuspended. Incubate at room temperature for 5 minutes.

6. Centrifuge the tube briefly and place on the magnetic rack for 5 minutes or until the liquid is clear.
7. Carefully remove the supernatant without disturbing the beads.
8. Keep the tube on the magnetic stand and add 800 µL of freshly prepared 80% ethanol to wash the magnetic beads.
9. Incubate at room temperature for 30 seconds, and carefully aspirate the supernatant without disrupting the beads.
10. Repeat the 80% ethanol wash one more time (steps 8-9).
11. Centrifuge briefly and return the tube on the magnetic stand.
12. Remove excess of ethanol using a fine pipet tip.
13. Keep the lid open to dry the beads for about 2 minutes or until the beads are not shiny anymore (no more than 5 minutes).
14. Remove the tube from the magnetic stand and add 20 µL Elution Buffer (10mM Tris-HCl pH 8.0). Vortex to mix.
15. Incubate at room temperature for 5 minutes. Centrifuge briefly, and place back on the magnetic stand until the liquid is clear.
16. Transfer the supernatant containing the purified cDNA from the control tube to a new 1.5-mL tube. The cDNA from the treated group were transferred to another 1.5mL tube.
17. The cDNA can be stored at 4°C for 72 hours or at -20°C for one month.

7.3 cDNA QC

Materials:

- Purified cDNA (from previous step)

User supplied:

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

1. Take an aliquot of the cDNA to measure the concentration using QuBit™ 4 Fluorometer.
2. Take an aliquot of the cDNA to measure the fragment size profile using Agilent Fragment Analyzer 5200 or equivalent. Dilute the cDNA following the fragment analyzer recommendations.
3. The ideal cDNA should meet the following criteria:
    a. The size of the main peak should be between 900-2000bp;
    b. Fragments between 1000bp-5000bp should represent more than 15% of the total molecules.

c. Fragments below 300bp should represent less than 40% of the total molecules.

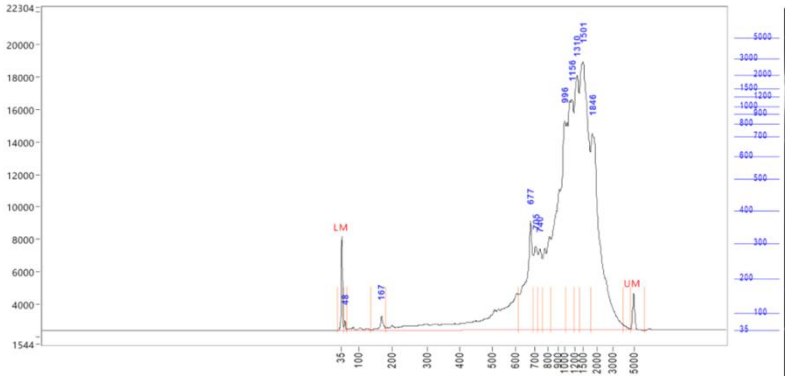


Figure 6. QC of cDNA on Agilent Fragment Analyzer 5200

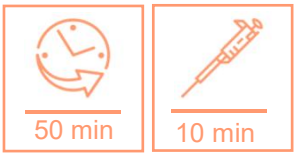
4. Optional: To reduce the presence of small fragments, perform a second AMPure purification according to the table below:

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

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.

8. Library Preparation

8.1 Fragmentation



Materials:

- Fragmentation Buffer V3
- Fragmentation Enzyme Mix V3
- 1xTE
- Purified cDNA (from previous step)

User supplied:

- PCR tubes

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

Lid Temperature 75℃		Reaction Volume 35 µL
Step	Temperature	Time
1	37°C	0:10:00
2	65°C	0:30:00
3	4°C	Hold

2. Thaw the Fragmentation Buffer V3 at room temperature. Ensure that the buffer is completely thawed, vortex, centrifuge, and place on ice. If a precipitate is seen, mix by pipetting up and down and vortex.
3. Vortex the Fragmentation Enzyme Mix V3 before use.
4. Dilute the cDNA with 1xTE according to the following recommendations to a total volume of 26 µL:
   - If the total amount of cDNA is less than 10 ng, use all the cDNA.
   - If the total amount of cDNA is between 10 and 50 ng, use 10 ng as input.
   - If the total amount of cDNA is above 50 ng, use 50 ng as the input.
5. In a labeled PCR tube, prepare the Fragmentation Reaction on ice according to the table below. Add the reagents in the order listed, vortex, centrifuge briefly and keep on ice.

Fragmentation Reaction:

Component	Volume(µL)
Diluted cDNA (10ng or 50ng)	26
Fragmentation Buffer V3	7
Fragmentation Enzyme Mix V3	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.

8.2 Adapter Ligation

Materials:

- Adapter
- Ligation Booster
- Ligation Mix
  - Fragmentation product (from previous step)

1. On a thermal cycler, set up the **Ligation program** according to the following table. The lid temperature of the thermal cycler should be **off**.

Lid Temperature OFF		Reaction Volume 70µL
Step	Temperature	Time
1	20°C	0:15:00
2	4°C	Hold

2. Thaw the Adapter and Ligation Booster at room temperature, mix well, and keep on ice.
3. Mix the Ligation Mix by pipetting up and down at least 10 times. Centrifuge briefly and keep on ice.
4. On ice, add the Adapter Ligation reagents directly to the fragmentated cDNA:

Adapter Ligation*:

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

** It is not recommended to prepare a mastermix*

***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 in the pre-cooled (20°C) thermal cycler and proceed with the Ligation program.
6. Once the thermocycler reaches 4°C, proceed to the purification of the adapter-ligated cDNA.

8.3 Purification of the Adapter-Ligated cDNA

Materials:

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

User supplied:

- PCR tubes
- AMPure
- Nuclease-free water
- Absolute ethanol
- DynaMag™-2 Magnetic rack

Important: Vortex the AMPure beads thoroughly before use.

Bring the AMPure beads to room temperature by incubating ~1 mL aliquot for 30 minutes or until they reach room temperature.

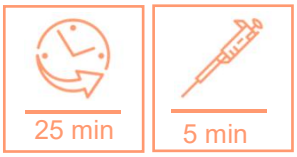
Pipet the AMPure beads slowly to ensure the accuracy of the retrieved volume.

During the AMPure cleanup, the tubes containing the magnetic beads should not be rotated once placed on the magnetic stand.

1. Prepare 0.5 mL of 80% ethanol and 35 μl 0.1xTE per reaction (1:10 dilution of 1 x TE with nuclease-free water).
2. Briefly spin down the adapter-ligated cDNA from the previous step and measure the volume with a pipette.

3. Calculate the volume of AMPure magnetic beads equivalent to 0.2x the total volume of the ligated product. For example, if the volume of the measured product is 68.5 µL, then 0.2x68.5=13.7 µL of AMPure magnetic beads should be used.
4. Vortex the AMPure beads until homogenized and add the appropriate 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 on the magnetic rack for 5 minutes or until the liquid is clear.
7. Carefully remove the supernatant without disturbing the beads.
8. Keep the tube on the magnetic stand and add 200 µL of freshly prepared 80% ethanol to wash the magnetic beads.
9. Incubate at room temperature for 30 seconds, and carefully aspirate the supernatant without disrupting the beads.
10. Repeat 80% ethanol wash one more time (steps 8-9).
11. Centrifuge briefly and return the tube to the magnetic stand.
12. Remove the excess ethanol using a fine pipet tip.
13. Keep the lid open to dry the beads for about 2 minutes or until the beads are not shiny anymore (no more than 5 minutes).
14. Remove the tube from the magnetic stand and add 17 µL of 0.1xTE. (1:10 dilution of 1 x TE with nuclease-free water). Vortex to mix.
15. Incubate at room temperature for 5 minutes. Centrifuge briefly, and place back on the magnetic stand 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.

8.4 Library Amplification



Materials:

- Library Amp Mix V3
- Indexing Primer Mix
- Purified adapter-ligated cDNA (from previous step)

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

Lid Temperature 105℃	Reaction Volume 50 μL	
Step	Temperature	Time
1	98°C	0:00:30
2(cycle number varies*)	98°C	0:00:10
	65°C	0:01:15
3	65°C	0:05:00
4	4°C	Hold

** The number of cycles should be selected based on the amount of cDNA input:*

cDNA input	Number of cycles
50ng	10
10ng	12

2. On ice, add the following reagents directly to the purified adapter-ligated product:

Component	Volume(μL)
Purified adapter-ligated product	15
Library Amp Mix V3	25
Indexing Primer Mix*	10
Total	50

** Choose a unique Indexing Primer Mix for each sample.*

3. Mix by pipetting up and down and centrifuge briefly.
4. Place the reaction in the thermocycler and run the library amplification program.
5. Proceed to the purification and size selection of the amplified library.

8.5 Purification and Size Selection of the Amplified Library

Materials:

- Amplified library (from previous step)

User supplied:

- Absolute ethanol
- Nuclease-free water
- AMPure
- DynaMag™-2 PCR Magnetic rack
- 10mM Tris-HCl pH 8.0 or Elution Buffer

Important: Vortex the AMPure beads thoroughly before use.

Bring the AMPure beads to room temperature by incubating ~1 mL aliquot for 30 minutes or until they reach room temperature.

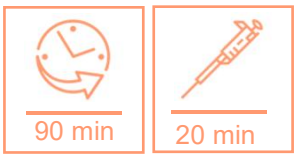
Pipet the AMPure beads slowly to ensure the accuracy of the retrieved volume.

During the AMPure cleanup, the tubes containing the magnetic beads should not be rotated once placed on the magnetic stand.

1. Prepare 0.5 mL of 80% ethanol per reaction.
2. Centrifuge briefly the amplified library and measure the volume with a pipette.
3. Calculate the volume of AMPure beads equivalent to 0.5x the total volume of the amplified libraries. For example, if the volume of the measured product is 50 µL, then use 0.5x50=25 µL of AMPure magnetic beads.
4. Vortex the AMPure beads until homogenized and add the appropriate volume to the amplified libraries.
5. Mix well by vortexing and incubate at room temperature for 5 minutes.
6. Centrifuge the tube briefly and place on the magnetic rack for 5 minutes or until the liquid is clear.
7. Transfer all of the supernatant containing the amplified library to a new tube.
8. Vortex the AMPure beads 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 aspirate the supernatant without disrupting the beads.
12. Keep the tube on the magnetic stand and add 200 µL of freshly prepared 80% ethanol to wash the magnetic beads.
13. Incubate at room temperature for 30 seconds, and carefully aspirate the supernatant without disrupting the beads.
14. Repeat 80% ethanol wash one more time (steps 12 and 13).
15. Centrifuge briefly and return the tube to the magnetic stand.
16. Remove the excess ethanol using a fine pipet tip.
17. Keep the lid open to dry the beads for about 2 minutes or until the beads are not shiny anymore (no more than 5 minutes).
18. Remove the tube from the magnetic stand and add 20 µL of Elution Buffer or 10mM Tris-HCl pH 8.0. Vortex to mix.

19. Incubate at room temperature for 5 minutes. Centrifuge briefly, and place back on the magnetic stand 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 three months.

8.6 QC of the Library



Materials:

- Purified library (from previous step)

User supplied:

- QuBit™ Assay Tubes
- QuBit™ 1x dsDNA HS assay kit
- Agilent Fragment Analyzer 5200 (Agilent cat # M5310AA) or equivalent

1. Take an aliquot (1 μL) of the library to measure the concentration using QuBit™ 4 Fluorometer.
2. Take an aliquot (2 to 5 ng/μl) of the library to measure the fragment size profile using Agilent Fragment Analyzer 5200 or equivalent. Dilute the library following the fragment analyzer recommendations.
3. The ideal library should meet the following criteria (Figure 7):
   a. The main peak should lie between 400-700bp when the region analyzed is set between 300bp and 2000bp.
   b. Fragments between 900bp and 1500bp should account for less than 10% of the molecules.
   c. Fragments below 300bp should account for less than 10% of the molecules.
4. If a peak is observed around 200bp, an extra AMPure purification using a bead ratio of 0.8x the volume of your sample should be performed.
5. Qualified libraries can be sent directly for sequencing (PE150) or can be stored at -20°C or -80°C for three months.

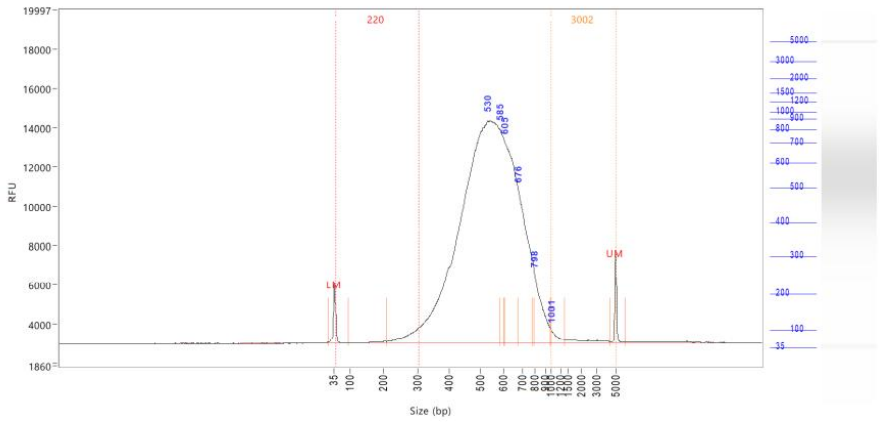


Figure 7. QC of the NGS library on Agilent Fragment Analyzer 5200

Appendix: Library structure

3’-library structure:

5’-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATACGCGGA[9bp barcode] [12bp UMI (N_{12})] [dT_{17}VN] [insert]AGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGTTATCTCGTATGCCGTCTTCTGCTTG-3’

5’-library structure:

5’-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT[6 bp barcode]NNNWWGGG [insert] AGATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTTTATCTCGTATGCCGTCTTCTGCTTG-3’

P5 sequence
TruSeq read1 sequencing primer
Linker
Read2 sequencing primer
5’-library index
P7 sequence

Appendix: Frequently Asked Questions (FAQ)

1.How to select single cells?

Cells can be picked manually under a microscope or labeled with target cells sorted by flow cytometry.

1) Select single cells under the microscope: Single cells with intact cell morphology and good activity should be selected. Aspirate the cells of interest from the initial suspension with a capillary. Transfer to new buffer and wash once or twice. Place the isolated cells in the lysis buffer, The volume of the liquid brought in does not exceed 1μl.

2) Flow cytometry sorting single cells: Cells are sorted according to the marker of the cells of interest.

2. What is the recommended sequencing parameters for single cell full-length transcription samples?

It is recommended to sequence 300,000~700,000 reads per well on average.

Singleron Biotechnologies GmbH
Phone: +49 (0) 221 16824777
Email: info@singleronbio.com
Website: www.singleronbio.com
Address: Gottfried-Hagen-Strasse 60, 51105 Cologne, Germany