QuantumScale
Single Cell RNA – Large Kit

Protocol

Legal Notices

Document 1262598 Rev B, June 2025

Scale Biosciences
5601 Oberlin Dr., Suite 110
San Diego, CA 92121, United States
www.scale.bio
support@scale.bio

Disclaimer of Warranty

TO THE EXTENT PERMITTED BY APPLICABLE LAW, SCALEBIO PROVIDES THIS DOCUMENT "AS IS" WITHOUT WARRANTY OF ANY KIND, INCLUDING WITHOUT LIMITATION, ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NONINFRINGEMENT. IN NO EVENT WILL SCALEBIO BE LIABLE TO YOU OR ANY THIRD PARTY FOR ANY LOSS OR DAMAGE, DIRECT OR INDIRECT, FROM THE USE OF THIS DOCUMENT, INCLUDING WITHOUT LIMITATION, LOST PROFITS, LOST INVESTMENT, BUSINESS INTERRUPTION, GOODWILL, OR LOST DATA, EVEN IF SCALEBIO IS EXPRESSLY ADVISED IN ADVANCE OF THE POSSIBILITY OF SUCH LOSS OR DAMAGE. Any warranties applicable to the ScaleBio products are set forth in the Terms and Conditions accompanying such product and such Terms and Conditions are not modified in any way by the terms of this notice.

Trademark Information

ScaleBio may make reference to products or services provided by other companies using their brand names or company names solely for the purpose of clarity, and does not assert any ownership rights over those third-party marks or names. Images were created with BioRender.com.

Patent Information

ScaleBio products may be covered by one or more patents as indicated at https://scale.bio/legal-notice/.

Terms and Conditions

The use of the ScaleBio products described herein is subject to ScaleBio's Terms and Conditions that accompany the product, or such other terms as have been agreed to in writing between ScaleBio and the user.

Intended Use

All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Table of Contents

Required Materials

Consumables and Reagents Manufactured by ScaleBio

Kits required for this protocol:

- QuantumScale Single Cell RNA - Large Kit (PN 1258684)
  - QuantumScale Single Cell RNA - Large, Module 1 (PN 1255849)
  - QuantumScale Single Cell RNA - Large, Module 2 (PN 1255851)
  - QuantumScale Single Cell RNA - Large, Module 3 (PN 1255852)
  - QuantumScale Single Cell RNA – Large, RT Module (PN 1338459)
- QuantumScale Library Index Kit-I-12 (PN 1262550) for Illumina libraries
- QuantumScale Library Index Kit-U-12 (PN 1262554) for Ultima libraries

QuantumScale Single Cell RNA Kit - Large (PN 1258684) Consumables and Reagents

Kit Module	Consumable	Part Number	Qty	Cap Color	Storage Temp
RT Module	QS RT Barcode Plate 96 Large	1258435	1	—	–20°C
Module 1	Quantum Barcoding Plate	1254544	1	—	RT
Module 1	Plate Wetting Buffer	1258405	1	Clear	RT
Module 1	Elution Buffer	1258479	1	Yellow	RT
Module 1	Pre-RC Buffer	1321701	1	White	RT
Module 1	ScaleBio Sample Collection Funnel	936063	2	—	—
Module 2	SPRI Additive	1258478	1	Yellow	4°C
Module 2	RT Stop Solution	1258443	1	Blue	4°C
Module 2	Bead Buffer	1258456	1	White	4°C
Module 2	Cell Wash Buffer	1258445	1	White	4°C
Module 2	Bead Collection Buffer	1258457	1	White	4°C
Module 2	Quantum Barcoding Beads-RNA-96 (Plate)	1258446	1	—	4°C
Module 2	Ligation 1 Wash Reagent	1258464	1	White	4°C
Module 3	Plate Blocking Buffer	1258434	1	Clear	–20°C
Module 3	RT Enhancer	1321703	1	Blue	–20°C
Module 3	RT Enzyme Mix	1258436	1	Blue	–20°C
Module 3	RT Buffer	1258438	1	Blue	–20°C
Module 3	RT Additive	1258439	1	Blue	–20°C
Module 3	RC Enzyme	1258450	1	White	–20°C
Module 3	Cleanup Enzyme	1258453	1	White	–20°C

Kit Module	Consumable	Part Number	Qty	Cap Color	Storage Temp
Module 3	RC and Cleanup Buffer	1258451	1	White	–20°C
Module 3	Ligation 1 Enzyme	1258459	1	Green	–20°C
Module 3	Ligation 1 Buffer	1258461	1	Green	–20°C
Module 3	Oligo Blocking Enzyme	1258469	1	Green	–20°C
Module 3	Oligo Blocking Buffer	1258465	1	Green	–20°C
Module 3	Oligo Blocking Additive	1289210	1	Green	–20°C
Module 3	Oligo Blocking Enhancer	1289212	1	Green	–20°C
Module 3	Second Strand Enzyme	1321706	1	Violet	–20°C
Module 3	Second Strand Buffer	1321707	1	Violet	–20°C
Module 3	Second Strand Additive	1321708	1	Violet	–20°C
Module 3	Second Strand Primer	1321710	1	Violet	–20°C
Module 3	QB Bead - RNA PCR MM	1321834	1	Yellow	–20°C
Module 3	QB Bead - RNA PCR Enhancer	1321713	1	Yellow	–20°C
Module 3	QB Bead - RNA PCR Primer	1321712	1	Yellow	–20°C
Module 3	QS Index PCR MM	1258487	1	Pink	–20°C
	If sequencing using Illumina				
QuantumScale Library Index Kit – 12 - ILMN	QS RNA Index Primer Plate - ILMN	1258492	1	Pink (sticker)	–20°C
	QS ScalePlex Index Primer Plate - ILMN	1258490	1	Blue (sticker)	–20°C
	If sequencing using Ultima				
QuantumScale Library Index Kit – 12 - ULT	QS RNA Index Primer Plate - ULT	1258494	1	Pink (sticker)	–20°C
	QS ScalePlex Index Primer Plate - ULT	1258493	1	Blue (sticker)	–20°C

Consumables and Reagents Manufactured by Other Vendors

Consumable or Reagent	Supplier	Part Number
Nuclease-free water	Various	Various
Ethanol (pure)	Various	Various
SPRIselect	Beckman Coulter	B23317
Cell counting dye	Various	Various
Pipette tips (nuclease-free, filtered, low retention for P1000, P200, P20, P10)	Various	Various
25-mL Reservoirs	Integra	4317

Consumable or Reagent	Supplier	Part Number
0.2-mL PCR tube strips (nuclease-free)	Various	Various
1.5-mL DNA LoBind tubes	Eppendorf	022431021
2.0-mL DNA LoBind tubes	Eppendorf	022431048
15-mL conical tubes	Various	Various
50-mL conical tubes	Various	Various
96-well LoBind PCR plates	Eppendorf	0030129504
Microseal 'B' PCR Plate Sealing Film	Bio-Rad	MSB1001
Qubit dsDNA HS Assay Kit	Thermo Fisher	Q33231
HS D5000 ScreenTape (or equivalent kit for alternative fragment analyzer)[a]	Agilent	5067-5592

a. Required for quality control of library.

Recommended Equipment

Item	Supplier	Part Number
Pipettes (P1000, P200, P20, P10, P2)	Various	Various
Multi-channel pipettes (P200, P20, P10)	Various	Various
Magnetic stand for 1.5-mL tubes	Thermo Fisher	12321D
Magnetic stand for 2.0-mL tubes	Various	Various
Magnetic stand for 50-mL tubes	Permagen	MSR15150 or similar
Magnetic stand for 0.2-mL strip tubes	Various	Various
Magnetic stand (96-well compatible)	Various	Various
96-well aluminum cooler blocks	Various	Various
Vortex mixer	Various	Various
Microcentrifuge (1.5-mL tubes, 0.2-mL strip tubes)	Various	Various
Cell counter	Various	Various
Centrifuge with temperature control and swing-bucket rotor (1.5-mL tubes, 96-well plates)	Various	Various
Thermocycler with lid temperature control (0–105°C) for semi-skirted 96-well plates	Various	Various
Rotator/Orbital mixer	Various	Various
Shaker for semi-skirted 96-well plates (ThermoMixer)	Various	Various
Incubator (37°C)	Various	Various
Qubit 4 Fluorometer	Thermo Fisher	Q33238
4200 Tapestation Instrument (or equivalent system)[a]	Agilent	G2991BA

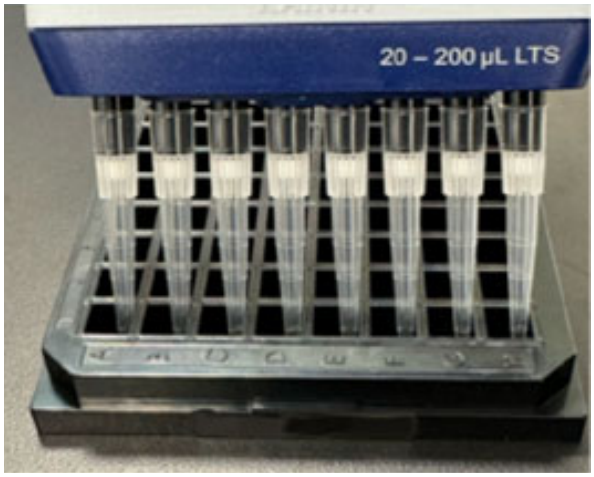
a. Required for quality control of library.

Best Practices

Assay Specific Methods of Pipetting

CAUTION: When adding liquid to the Quantum Barcoding Plate, ensure liquid is added to the side wall of a well, except when noted otherwise. When removing liquid from a well, always pipette from a selected corner of the well as shown in Figure 1. Ensure the same corner is used every time to avoid damaging the microwells.

Figure 1: The described method of pipetting in and out of a Quantum Barcoding Plate. The picture on the left shows the front view, and the picture on the right shows the side view of pipetting.



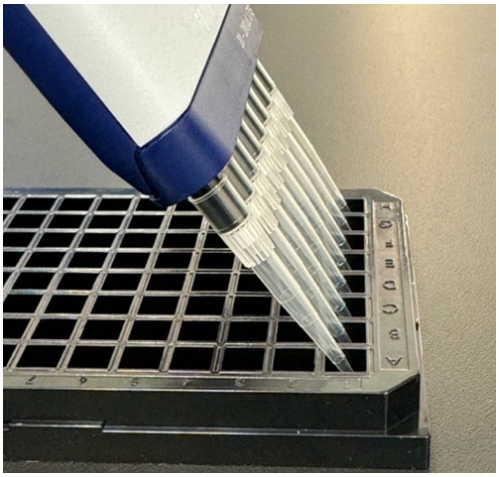


Figure 2: Pipetting into a well along the side wall.

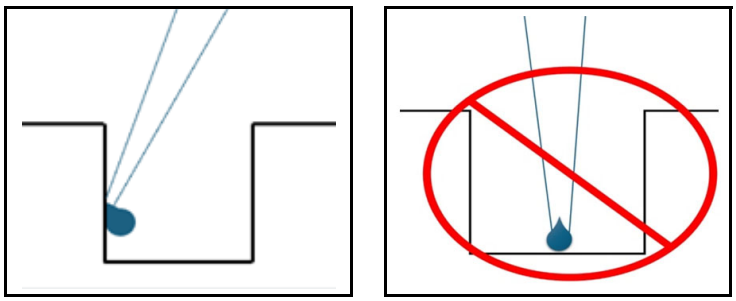
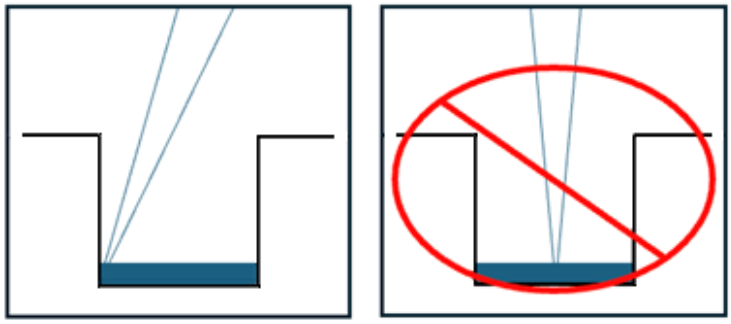
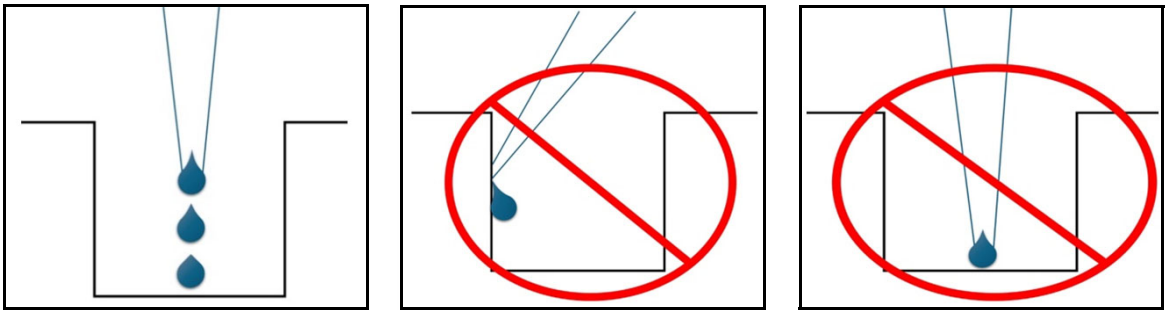


Figure 3: Pipetting out of a well from a selected corner (same corner every time)



CAUTION: When loading cells/nuclei and Quantum Barcoding Beads into the Quantum Barcoding Plate ensure that you are **pipetting slowly over the center of each well** to allow for even distribution across the entire well, as shown in Figure 4.

Figure 4: The described method of loading cells/nuclei and Quantum Barcoding Beads into the Quantum Barcoding Plate.



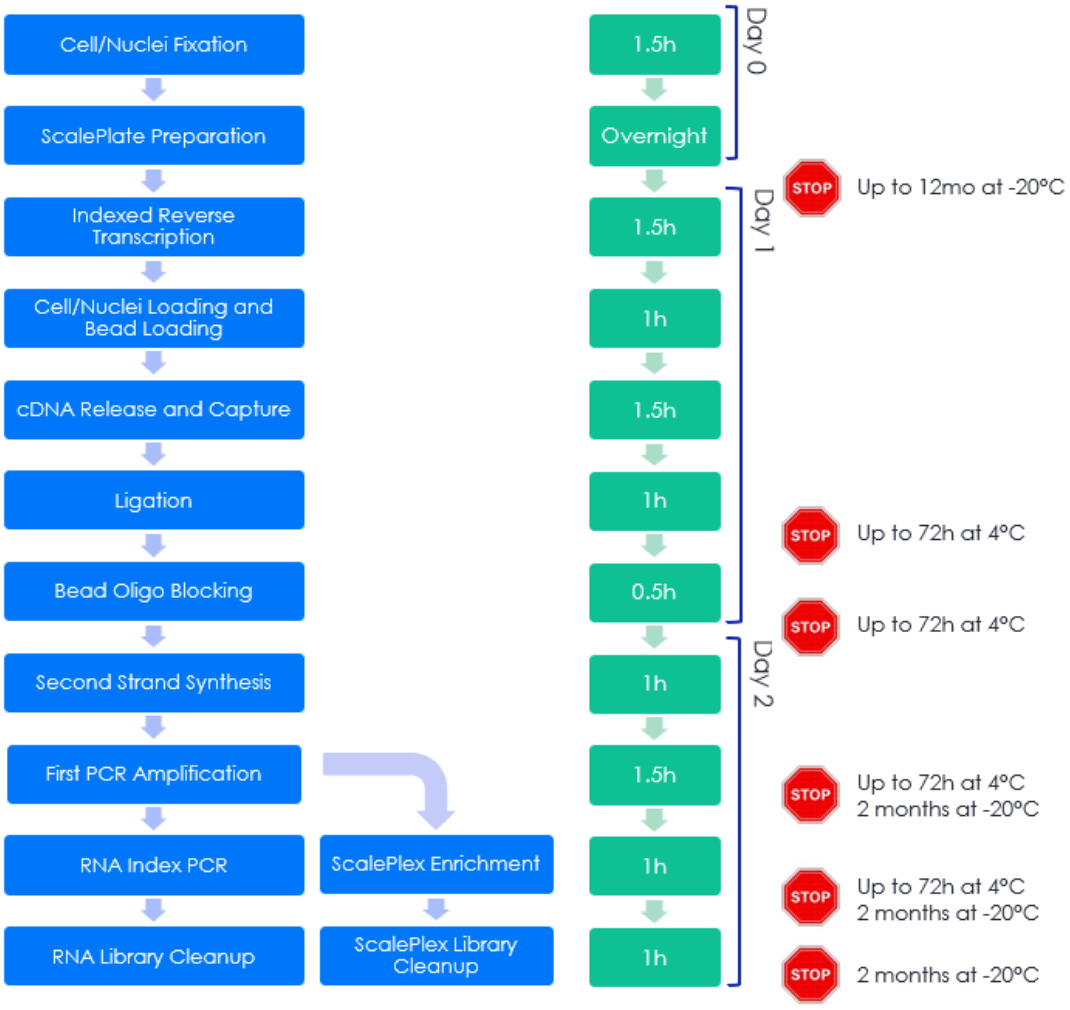
General Laboratory Best Practices

- Calibrate and service pipettes every 12 months to ensure accurate sample volume transfer at each step.
- Store all reagents at the storage conditions recommended by the supplier.
- Unless otherwise specified, thaw reagents on ice.
- Never reuse pipette tips or tubes.
- Keep pipette tip boxes, reagent containers, and sample tubes closed when not in use.
- Wear suitable protective clothing, eyewear, and gloves.

Prevention of Amplicon Cross-contamination in Sequencing Libraries

- Thaw and prepare reagent mixes in pre-amplification workspaces.
- Perform amplification in post-amplification workspaces.
- Perform PCR purification steps in post-amplification workspace.
- Never bring material or equipment from post-amplification workspaces into pre-amplification workspaces.
- Regularly clean post-amplification workspaces with a 10% bleach solution.

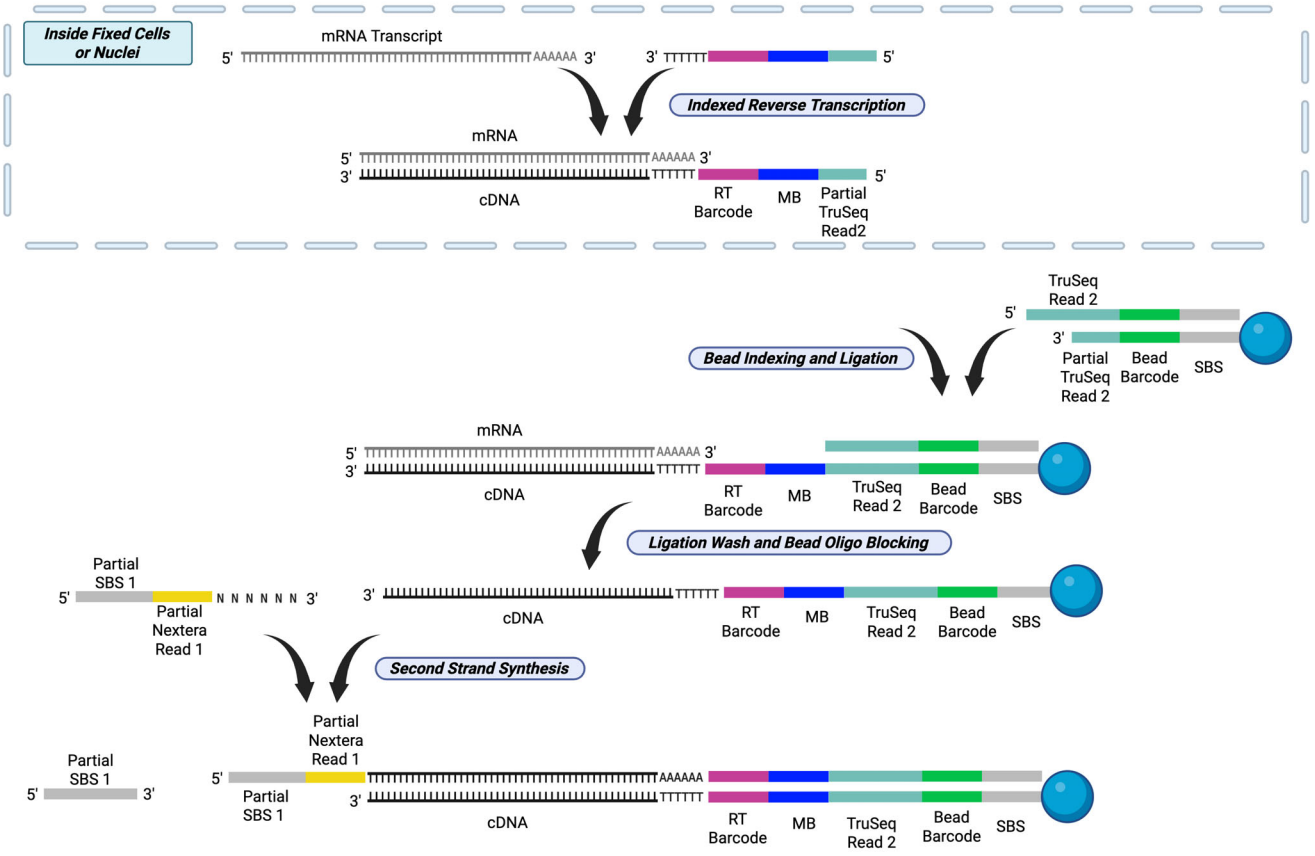
Workflow Diagram



Assay Introduction

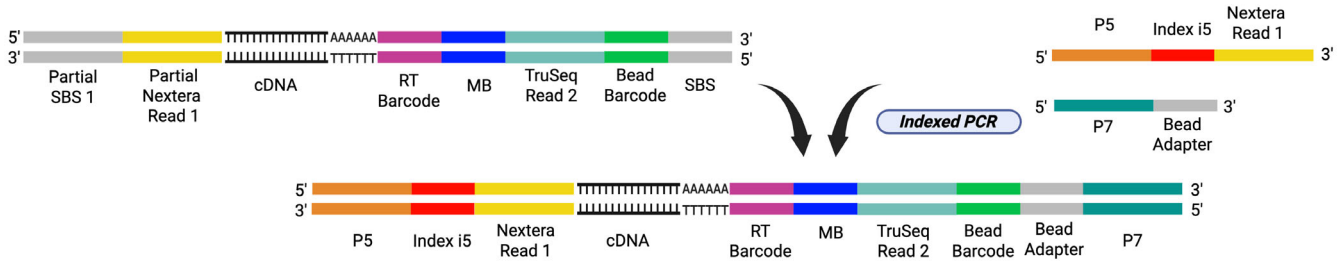
Fixed cells are used in the in situ Reverse Transcription (RT) with poly-dT and Multi-Specific Primers (MSP) carrying a partial TruSeq Read 2 adaptor sequence, the RT Barcode and the Molecular Barcode (MB). RT reactions with distinct RT barcodes are performed, then the cells/nuclei are pooled and loaded into the Quantum Barcoding Plate, followed by bead loading. The cDNA molecules are then released from the cells/ nuclei and captured on beads carrying barcoded oligos. A ligation reaction is carried out to link the cDNA with the bead oligos. The beads are then washed to generate single stranded cDNA linked with the bead oligo, and then the bead oligo is blocked. Second strand synthesis is performed with a random primer carrying the partial SBS (Scale Bead Sequence) sequence which is used as PCR handle for amplification.

Next cDNA together with the linked bead oligo is amplified by a semi-suppressive PCR reaction.



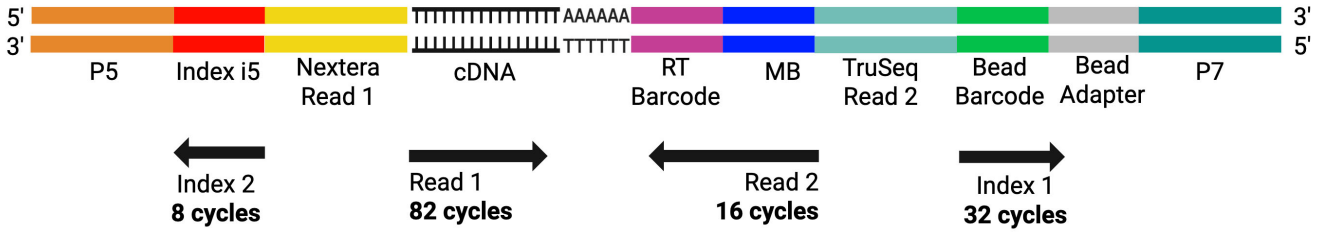
Illumina Libraries

Final RNA library PCR is performed to add the Illumina P5 and P7 adaptors and i5 index. If using ScalePlex, ScalePlex libraries are enriched with a separate PCR reaction.

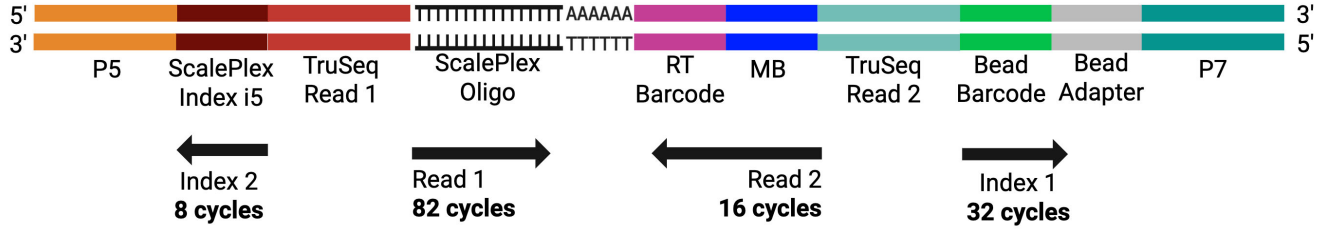


After SPRI purification of each library, the libraries are ready for sequencing.

RNA Library – ILMN

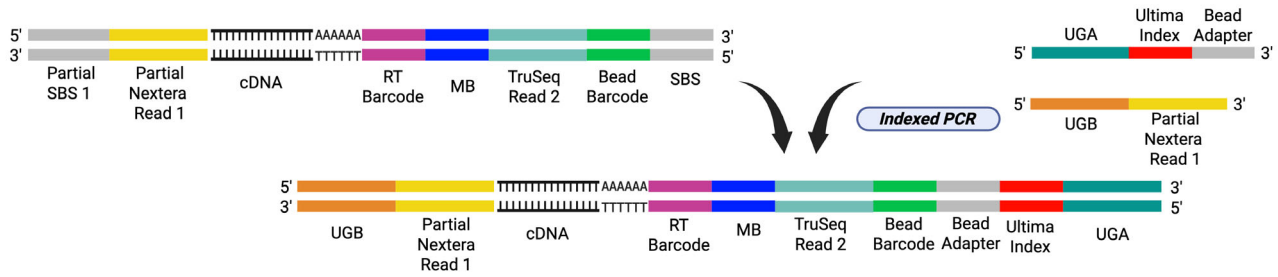


ScalePlex Library – ILMN



Ultima Libraries

Final RNA library PCR is performed to add the Ultima UGA and UGB adaptors and Ultima index. If using ScalePlex, ScalePlex libraries are enriched with a separate PCR reaction.



After SPRI purification of each library, the libraries are ready for sequencing.

RNA Library – ULT

ScalePlex Library – ULT

Section 1: Preparing the Quantum Barcoding Plate

Review the following table to prepare reagents before starting this section.

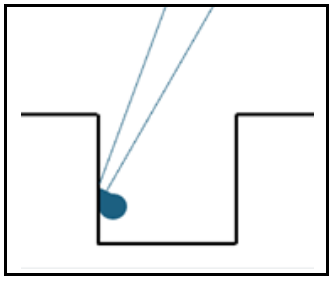
Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 1	Quantum Barcoding Plate	—	RT	RT	⊗	⊗
Module 1	Plate Wetting Buffer	Clear	RT	RT	⊗	⊗
Module 3	Plate Blocking Buffer	—	–20°C	RT	⊗	⊗
Other Vendors	Microseal 'B' PCR Plate Sealing Film					

Before you Begin

- Set a swing bucket centrifuge for 96-well plates to room temperature.

Procedure

1. Unpack the Quantum Barcoding Plate. Set the plastic lid aside on a clean surface; the lid will be used throughout the protocol.
2. Pour the Plate Wetting Buffer into a 25-mL reservoir, and add **100 µL** of Plate Wetting Buffer to each well of the Quantum Barcoding Plate by dispensing the liquid on the side walls, as shown.



3. Seal the plate with an adhesive seal and place the plate lid on the plate.
4. Centrifuge the plate at **300 x g** for **5 minutes** at **room temperature**.

5. Incubate the Quantum Barcoding Plate on the bench according to Program 1.

Program 1: Plate Preparation

Temperature	Time
Room temperature (18–25°C)	>12 h

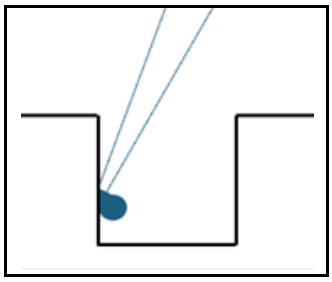
CAUTION: Ensure that you wait at least 12 hours for plate preparation. Do not proceed immediately.

NOTE: For longer storage time (up to 2 months), place the Quantum Barcoding Plate with Plate Wetting Buffer in the wells at 4°C after the 12-hour room temperature incubation.

NOTE: If bubbles are observed at the edges/corner of the Quantum Barcoding Plate wells, proceed with plate preparation. If bubbles are observed in the center of the Quantum Barcoding Plate, stop and contact support@scale.bio.

6. Centrifuge the plate at **300 x g** for **30 seconds** at **room temperature**.
7. Remove the plate lid, unseal the Quantum Barcoding Plate and discard the seal.
8. Invert the Plate Wetting Buffer over a sink and shake the plate to discard the Plate Wetting Buffer. Blot the residual liquid on a paper towel.

   Inspect and ensure that the majority of liquid is removed and plate is free of debris.
9. Invert to mix the Plate Blocking Buffer tube, pour the Plate Blocking Buffer into a 25-mL reservoir, and add **150 µL** Plate Blocking Buffer into each well of the Quantum Barcoding Plate along the side wall, as shown.



10. Place the lid back on the Quantum Barcoding Plate.
11. Incubate the Quantum Barcoding Plate on the bench according to Program 2.

Program 2: Plate Blocking

Temperature	Time
Room temperature (18–25°C)	30 min

12. Store the Quantum Barcoding Plate with Plate Blocking Buffer in the wells for a maximum of **72 hours** at **4°C**, or proceed Section 2: Initial Distribution and Reverse Transcription.

Section 2: Initial Distribution and Reverse Transcription

NOTE: Before starting this section make sure cells/nuclei are fixed with the ScaleBio Sample Fixation Kit (PN 2020001) or the ScalePlex Fixation Kit v2 (PN 1269724).

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
RT Module	QS RT Barcode Plate 96	—	–20°C	On ice	✗	✓
Module 3	RT Enhancer	Blue	–20°C	On ice	✗	✓
Module 3	RT Enzyme Mix	Blue	–20°C	On ice	✗	✓
Module 3	RT Buffer	Blue	–20°C	On ice	✓	✓
Module 3	RT Additive	Blue	–20°C	On ice	✓	✓
Module 2	RT Stop Solution	Blue	4°C	On ice	✗	✗
Module 2	Cell Wash Buffer	White	4°C	On ice	✗	✗
Module 1	ScalePlex Sample Collection Funnel	—	—	—	—	—
Other Vendors	Nuclease-free water					
	Microseal 'B' PCR Plate Sealing Film					
	P1000 wide-bore pipette tips					
	Cell counting dye					
	2-mL DNA LoBind tubes					
	15-mL conical tubes					

Before you Begin

- If frozen, thaw cells/nuclei on ice. Do not vortex.
- Fully chill a 96-well metal block on ice.
- Set a swing bucket centrifuge for 96-well plates to 4°C.
- Start Program 3 on a thermocycler and hold at 55°C with a lid temperature of 65°C.

Procedure

1. Determine the concentration of the cell/nuclei suspension prepared with the Sample Fixation Kit or ScalePlex Fixation Kit using cell counting equipment. Keep the cell/nuclei suspension **on ice**.
2. On ice, use wide-bore pipette tips to gently mix the cells/nuclei, and dilute with Cell Wash Buffer to achieve a concentration of 9000 cells/µL. Make sure the cells/nuclei are mixed well before loading.
3. Centrifuge the thawed QS RT Barcode Plate 96 at **300 x g** for **30 seconds** at **4°C** and place on a 96-well metal block on ice.
4. Unseal the QS RT Barcode Plate 96 on ice.

5. Dilute RT Enhancer by mixing **65 µL** RT Enhancer with **195 µL** Cell Wash Buffer.

   NOTE: Do not discard the remaining RT Enhancer stock. It will be used in Table 1 for making RT Master Mix.

6. Distribute **30 µL** of the diluted enhancer to 8 wells of a strip tube.
7. Using a multi-channel pipette, add **2 µL** of diluted RT Enhancer into each barcode well of the QS RT Barcode Plate.

   CAUTION: DO NOT pipette mix. **Change pipette tips** between wells to avoid cross-contamination.

8. Pipette mix the cell/nuclei suspension and immediately distribute **5 µL** of cells/nuclei to each well of the QS RT Barcode Plate 96 that contains RT Barcode Primer.

   CAUTION: Change pipette tips between wells to avoid cross-contamination.

   NOTE: Write down the well position of individual samples for downstream data analysis. This is the first level RT Barcode.

9. Seal the QS RT Barcode Plate 96 and place it on a plate shaker.
10. Shake the plate at **2000 rpm** for **30 seconds**.
11. Centrifuge the plate at **100 x g** for **30 seconds** at **4°C**.
12. Incubate the QS RT Barcode Plate 96 in a **pre-heated** thermocycler according to Program 3.

Program 3: RNA Denaturation

Lid Temperature	Reaction Volume
65°C	11 µL
Temperature	**Time**
55°C	∞
Skip the HOLD step after placing the plate in the thermocycler.	
55°C	3 min

13. Upon completion, **immediately** remove the QS RT Barcode Plate 96 from the thermocycler and place on the pre-chilled metal block **on ice**.
14. Incubate **on ice** for **2 minutes**.
15. Start Program 4 on a thermocycler and hold at **4°C** with a lid temperature of **65°C**.

16. **On ice**, prepare the Reverse Transcription Master Mix.

Table 1: Reverse Transcription Master Mix

Reagent	Volume (µL)
Nuclease-free water	275
RT Buffer	440
RT Additive	110
RT Enhancer (stock)	55
RT Enzyme Mix	110
Total volume	**990**

17. **On ice**, slowly pipette mix the RT Master Mix until the solution is homogeneous and briefly spin down.
18. Aliquot **120 µL** of the RT Master Mix into each tube of an 8-tube strip **on ice**.
19. **On ice**, unseal the QS RT Barcode Plate 96 and distribute **9 µL** of the RT Master Mix into each well of the plate, dispensing the RT Master Mix to the bottom of the wells.

CAUTION: DO NOT pipette mix. **Change pipette tips** between wells to avoid cross-contamination.

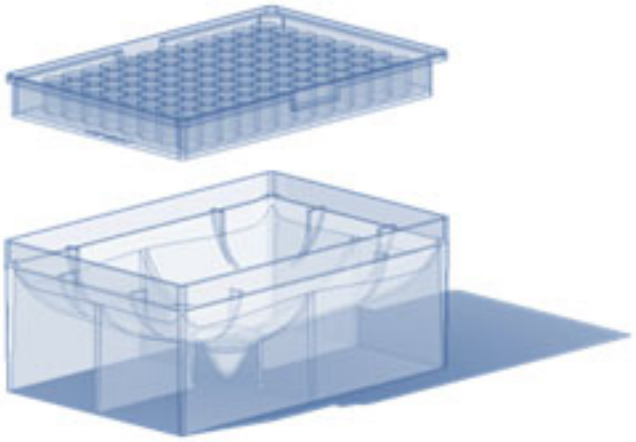
20. Seal the QS RT Barcode Plate 96 with an adhesive seal and place it on a plate shaker.
21. Shake the plate at **2000 rpm** for **30 seconds**.
22. Centrifuge the plate at **100 x g** for **30 seconds** at **4°C** and place **on ice**.
23. Incubate the QS RT Barcode Plate 96 in a **pre-cooled** thermocycler according to Program 4.

Program 4: Gradient Reverse Transcription

Lid Temperature	Reaction Volume
65°C	20 µL
Temperature	**Time**
4°C	∞
Skip the HOLD step after placing the plate in the thermocycler.	
4°C	2 min
10°C	2 min
20°C	2 min
30°C	2 min
40°C	2 min
50°C	2 min
55°C	10 min
4°C	∞

24. Unpack a ScaleBio Sample Collection funnel and place **on ice**. Do not touch the inside of the collection funnel.
25. Add **6400 µL** cold RT Stop Solution into the bottom of the collection funnel.
26. Once the thermocycler program is completed, take off the QS RT Barcode Plate 96 and shake the plate at **2000 rpm** for **30 seconds**.
27. Unseal the QS RT Barcode Plate 96, quickly invert it, and place it in the collection funnel as shown in Figure 5.

    Figure 5: ScaleBio Sample Collection Funnel and Plate Assembly



28. Centrifuge the collection funnel and plate assembly with an appropriately weighted balance at **300 x g** for **2 minutes** at **4°C**.
29. Discard the QS RT Barcode Plate 96. Transfer pooled material (**~8320 µL**) from the collection funnel into a new 15-mL DNA LoBind tube **on ice**.
30. Add **5 mL** of Cell Wash Buffer to the collection funnel, rinsing the sides of the funnel by pipetting up and down to collect the residual liquid and any remaining cells. Transfer to the 15-mL DNA LoBind tube **on ice**.
31. Centrifuge the tube at **500 x g** for **8 minutes** at **4°C** in a swing bucket centrifuge.
32. Carefully remove the supernatant without disturbing the cell pellet, leaving **100 µL** of residual volume. Flick to resuspend the pellet in the supernatant.
33. Add **15.2 mL** of Cell Wash Buffer to resuspend cells/nuclei. Pipette mix.
34. (Optional) Take **10 µL** for cell counting. Keep the rest of the cell/nuclei suspension **on ice** for cell loading.
35. **On ice** and using P1000 wide-bore pipette tips, transfer cell solution into a 25-mL reservoir.
36. Proceed directly to Section 3: Cell/Nuclei Loading.

Section 3: Cell/Nuclei Loading

Review the following table to prepare reagents before starting this section.

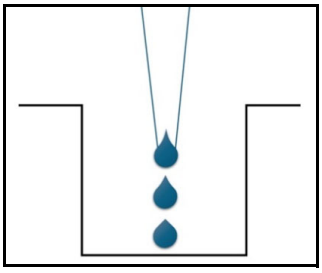
Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 1	Quantum Barcoding Plate (filled with Plate Blocking Buffer)	—	RT	RT	⊗	⊗

Before you Begin

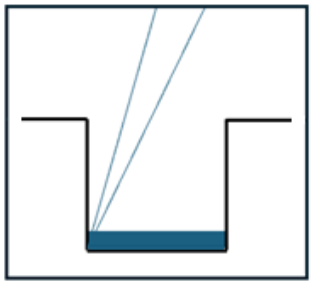
- Set a swing bucket centrifuge for 96-well plates to 4°C.

Procedure

1. Invert the Quantum Barcoding Plate with Plate Blocking Buffer over a sink and shake the plate to discard the Plate Blocking Buffer. Blot the residual liquid on a paper towel. Inspect that the majority of the liquid is removed and the plate is free of debris.
2. Add **150 µL** of cell/nuclei solution with a 200-µL pipette tip to the center of each well of the Quantum Barcoding Plate without touching the bottom of the plate, as shown.



3. Place the plate lid on the Quantum Barcoding Plate and incubate for **5 minutes** on a flat surface at room temperature.
4. Centrifuge the Quantum Barcoding Plate at **500 x g** for **3 minutes** at **4°C**.
5. Gently remove and discard all supernatant from the wells by pipetting from a selected corner of the well, as shown.



6. Proceed directly to Section 4: Bead Loading.

Section 4: Bead Loading

Review the following table to prepare reagents before starting this section.

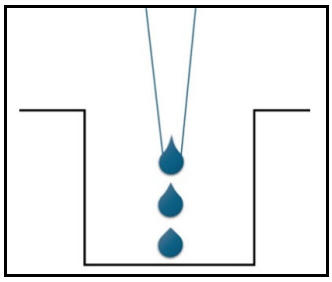
Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 2	Quantum Barcoding Beads-RNA-96 (Plate)	—	4°C	On ice	✗	✓

Procedure

1. Invert the Quantum Barcoding Beads Plate two to three times to wash off beads stuck to the foil. Centrifuge the Quantum Barcoding Beads Plate at **300 x g** for **30 seconds** at **4°C**. Remove the sealing foil and inspect to ensure no beads are stuck to the foil.

   CAUTION: Quantum Barcoding Beads will be at the bottom of the wells. Pipette mix the beads solution well before pipetting beads out.

2. Pipette 100 µL of Quantum Barcoding Beads with a multi-channel pipette up and down to mix. Take **100 µL** of the beads and pipette to the center of each well of the Quantum Barcoding Plate without touching the bottom of the plate, as shown. Do not pipette vigorously to avoid bubbles.

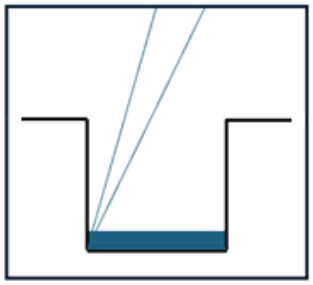


3. Place the plate lid on the Quantum Barcoding Plate and incubate for **5 minutes** on a flat surface at **room temperature**. After **3 minutes**, gently shake and swirl the plate horizontally to uniformly distribute the beads.

   IMPORTANT: The 5-minute incubation is critical to ensure efficient beads loading. Gently shaking the plate after a 3-minute incubation ensures uniform distribution of the beads.

4. Centrifuge the plate at **500 x g** for **3 minutes** at **4°C**.

5. Set the pipette at 160 µL and gently remove and discard all supernatant from the wells by pipetting from a selected corner (same corner) of the wells, as shown.



6. Proceed directly to Section 5: cDNA Release and Capture.

Section 5: cDNA Release and Capture

Review the following table to prepare reagents before starting this section.

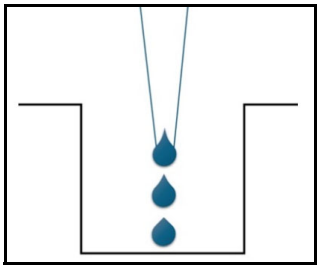
Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 3	RC Enzyme	White	–20°C	On ice	✗	✓
Module 3	Cleanup Enzyme	White	–20°C	On ice	✗	✓
Module 3	RC and Cleanup Buffer	White	–20°C	On ice	✗	✓
Module 1	Pre-RC Buffer	White	RT	RT	✗	✗
Module 2	Cell Wash Buffer	White	4°C	On ice	✗	✗
Other Vendors	25-mL reservoirs 5-mL DNA LoBind tubes 15-mL conical tubes					

Before you Begin

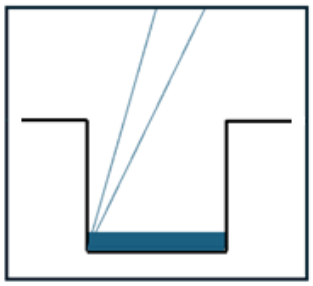
- Pre-heat to 37°C an incubator that will fit the Quantum Barcoding Plate.

Procedure

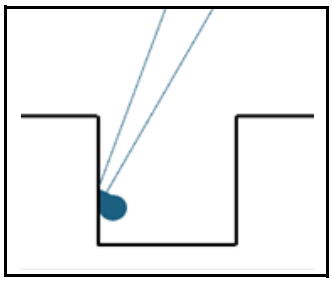
1. Remove the lid from the Quantum Barcoding Plate, invert to mix the Pre-RC Buffer tube, and pour into a 25-mL reservoir. Use a multichannel pipette to add **100 µL** of Pre-RC buffer to each well, as shown.



2. Incubate at **37°C** for **10 minutes**.
3. Remove the Pre-RC Buffer from a selected corner, as shown.



4. Pipette Cell Wash Buffer into a 25-mL reservoir and use a multichannel pipette to gently add **150 µL** of Cell Wash Buffer by dispensing the liquid on the side walls of the wells, as shown.

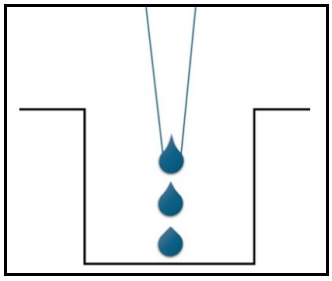


5. Pipette mix two times from a selected corner of the well, dispensing liquid on the side walls of the well, and remove and discard all solution.
6. Prepare the RC Solution in a 15-mL conical tube at **room temperature** according to Table 2. Pipette mix RC Solution five times with a pipette setting at 500 µL.

Table 2: RC Solution

Reagent	Volume (µL)
RC and Cleanup Buffer	5060
RC Enzyme	440
Total volume	**5500**

7. Pipette into a 25-mL reservoir and dispense **50 µL** of RC Solution to each well loaded with cells/nuclei and beads, as shown.



8. Place a plastic lid on the Quantum Barcoding Plate and incubate the plate according to Program 5.

Program 5: RC Incubation

Temperature	Time
37°C	30 min

9. Prepare the RC Cleanup Solution in a 5-mL tube **on ice**, according to Table 3. Pipette mix the RC Cleanup Solution five times with a pipette setting at 500 µL.

Table 3: RC Cleanup Solution

Reagent	Volume (µL)
RC and Cleanup Buffer	2475
Cleanup Enzyme	275
Total volume	**2750**

10. Remove the Quantum Barcoding Plate from the incubator and remove the plastic lid.
11. Pipette the RC Cleanup Solution into a 25-mL reservoir and distribute **25 µL** of RC Cleanup Solution dropwise to each well and mix by moving the plate in a circular motion five times on a flat surface.
12. Place a plastic lid on the Quantum Barcoding Plate and incubate at **room temperature** for **10 minutes**.
13. Proceed directly to Section 6: Bead Collection.

Section 6: Bead Collection

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 2	Bead Collection Buffer	Clear	4°C	On ice	⊗	⊗
Module 2	Bead Buffer	White	4°C	On ice	⊗	⊗
Module 1	ScalePlex Sample Collection Funnel	—	—	—	—	—
Other Vendors	Magnetic stand (50-mL tube compatible) 25-mL reservoirs Magnetic stand (1.5-mL tube compatible)					

Before you Begin

- Set a swing bucket centrifuge for 96-well plates to room temperature.

Procedure

1. Remove the lid from the Quantum Barcoding Plate. Pour Bead Collection Buffer into a 25-mL reservoir and add **200 µL** of Bead Collection Buffer to each well.
2. Centrifuge at **500 x g** for **3 minutes** at **room temperature** to dislodge the beads. The collection buffer will force the beads out of the microwells during this step.
3. Unpack a ScaleBio Sample Collection funnel and place on ice. Do not touch the inside of the collection funnel.
4. Place the collection funnel upside-down on top of the Quantum Barcoding Plate and quickly invert the assembly.
5. Centrifuge the collection funnel assembly with an appropriately weighted balance at **300 x g** for **30 seconds** at **room temperature**.
6. Carefully remove the upside-down Quantum Barcoding Plate from the collection funnel and place the plate right-side up on a benchtop. Be careful not to let the liquid in the funnel spill over.
7. Collect the pooled Quantum Barcoded Beads solution (~24 mL) from the collection funnel and transfer it to a 50-mL conical tube at **room temperature**.
8. Pipette 16 mL of Bead Buffer into a 25-mL reservoir, add **150 µL** of Bead Buffer to each well of the Quantum Barcoding Plate, and pipette mix two times to dislodge the residual beads.
9. Place the empty collection funnel upside down on top of the Quantum Barcoding Plate and quickly invert the assembly.
10. Centrifuge the collection funnel assembly at **300 x g** for **30 seconds** at **room temperature**.
11. Collect the pooled Quantum Barcoded Beads solution (~14.4 mL) from the collection funnel and add it to the conical tube from step 7.

12. Wash the now empty collection funnel with **2 mL** of Bead Buffer, rinsing the sides of the funnel, and add to the conical tube.
13. Place the 50-mL conical tube on a magnetic stand and let the beads separate for at least **10 minutes**.
14. Once the solution is clear, carefully and without touching the beads, remove and transfer the supernatant to a new 50-mL tube and allow to sit on a magnetic stand for **10 minutes**.

    NOTE: Waiting for the supernatant to become clear takes about 10 minutes. Ensure that the solution is completely clear before removing the supernatant.

15. While the second 50-mL conical tube sits on the magnetic stand for 10 minutes, resuspend the separated beads in the first 50-mL conical tube by adding **800 µL** of Bead Buffer to the tube, then pipetting the beads off the tube wall to resuspend.
16. Transfer the resuspended bead solution to a 2-mL DNA LoBind tube.
17. Add **400 µL** of Bead Buffer to the first 50-mL tube. Pipette along the tube walls to wash out the residual beads, then transfer the solution to the same 2-mL DNA LoBind tube.
18. Once the solution in the second tube is clear, remove and discard the supernatant from the second 50-mL conical tube.
19. Add **400 µL** of Bead Buffer to the second 50-mL tube. Pipette along the tube wall to wash out the residual beads, then transfer the solution to the same 2-mL DNA LoBind tube.
20. Briefly centrifuge the 2-mL in a microcentrifuge and place on the magnetic stand.
21. Once the solution is clear, carefully remove and discard the supernatant without pipetting out the beads.
22. Wash the beads two more times with **1 mL** of Bead Buffer for a total of **three washes**. Remove the tube from the magnetic stand and resuspend the beads during each wash.
23. Proceed directly to Section 7: Bead Ligation.

Section 7: Bead Ligation

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 3	Ligation 1 Enzyme	Green	–20°C	On ice	✗	✓
Module 3	Ligation 1 Buffer	Green	–20°C	On ice	✓	✓
Module 2	Bead Buffer	White	4°C	On ice	✗	✗
Module 2	Ligation 1 Wash Reagent	White	4°C	RT	✗	✗
Other Vendors	Magnetic stand (2-mL tube compatible) Magnetic stand (0.2-mL strip tube compatible) 25-mL reservoirs Nuclease-free water					

Procedure

1. On the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
2. Prepare the Ligation Master Mix **on ice** according to Table 4.

Table 4: Ligation Master Mix

Reagent	Volume (µL)
Ligation 1 Buffer	117.5
Nuclease-free water	820
Ligation 1 Enzyme	12.5
Total volume	**950**

3. Resuspend the Quantum Barcoding Beads in 950 µL of Ligation Master Mix.

4. Incubate the reaction mix on a rotator/orbital mixer according to Program 6. A longer incubation of up to 2 hours is acceptable.

Program 6: Ligation Incubation

Temperature	Time
18–25°C	30 min

CAUTION: Set the rotator/orbital mixer speed and rotation so the Quantum Barcoding Beads do not settle.

NOTE: A thermomixer can also be used with settings at 18–25°C and 1500 rpm.

5. After the incubation is complete, spin the tube briefly.
6. Place the tube containing the Ligation Master Mix on a magnetic stand.
7. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.
8. Remove the tube from the magnetic stand.
9. Add **800 µL** of Bead Buffer at **room temperature** and pipette the beads several times to resuspend.
10. Distribute the beads evenly into an 8-tube strip (100 µL/tube).
11. Briefly spin the PCR strip tube, and place them on a magnetic stand.
12. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.
13. Pour all the Ligation 1 Wash Reagent into a 25-mL reservoir at room temperature.
14. Remove the strip tube from the magnetic stand.
15. Add **200 µL** of Ligation 1 Wash Reagent at **room temperature** to each tube and invert several times to resuspend.
16. Incubate at **room temperature** for **5 minutes** on a rotator/orbital mixer. Make sure the beads do not settle down during the reaction.

NOTE: A thermomixer can also be used with settings at 25°C and 1500 rpm.

17. Spin the strip tube and place it on a magnetic stand.
18. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.
19. Repeat steps 15–18 **four times** for a total of **five Ligation 1 Wash Reagent washes** using **200 µL** of wash reagent for each wash.

NOTE: Perform the remaining washes with Bead Buffer.

20. Pipette 8 mL of Bead Buffer into a 25-mL reservoir.
21. Add **200 µL** of **Bead Buffer** to each well of the strip tube and pipette mix to resuspend.
22. Spin the strip tube and place it on a magnetic stand.
23. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.

24. Remove the strip tube from the magnetic stand.
25. Repeat steps 21–24 **three times** for a total of **four Bead Buffer washes**. Note that the beads can be stored in **200 µL** of Bead Buffer (fourth wash) at **4°C**.

NOTE: Do not discard the Bead Buffer from the fourth wash.

26. Store the Quantum Barcoding Beads, or proceed to Section 8: Bead Oligo Blocking.

Safe stopping point. After ligation, Quantum Barcoding Beads with cDNA can be stored in Bead Buffer at 4°C for up to 72 hours.

Section 8: Bead Oligo Blocking

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 3	Oligo Blocking Enzyme	Green	–20°C	On ice	✗	✓
Module 3	Oligo Blocking Buffer	Green	–20°C	On ice	✓	✓
Module 3	Oligo Blocking Additive	Green	–20°C	On ice	✓	✓
Module 3	Oligo Blocking Enhancer	Green	–20°C	On ice	✓	✓
Module 2	Bead Buffer	White	4°C	On ice	✗	✗
Other Vendors	Magnetic stand (0.2-mL strip tube compatible) Nuclease-free water 25-mL reservoirs					

Before You Begin

- Preheat the rotator/orbital mixer to 37°C.

Procedure

1. Prepare the Bead Oligo Blocking Master Mix according to Table 5 and pipette mix.

Table 5: Bead Oligo Blocking Master Mix

Reagent	Volume (µL)
Nuclease-free water	639
Oligo Blocking Buffer	93
Oligo Blocking Enhancer	93
Oligo Blocking Additive	37
Oligo Blocking Enzyme	18
Total volume	**880**

2. Place the strip tube with beads on a magnetic stand.
3. Once the solution is clear, remove and discard the Bead Buffer on the magnetic stand.
4. Resuspend each tube of the Quantum Barcoding Beads in 100 µL of Bead Oligo Blocking Master Mix and pipette mix.

5. Incubate the reaction mix on a rotator/orbital mixer according to Program 7.

Program 7: Bead Oligo Blocking Incubation

Temperature	Time
37°C	30 min

CAUTION: Set the rotator/orbital mixer speed and rotation so the Quantum Barcoding Beads do not settle.

NOTE: A thermomixer can also be used with settings at 37°C and 1500 rpm.

6. After the incubation is complete, place the strip tube containing Bead Oligo Blocking reaction mix on a magnetic stand.
7. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.
8. Pipette 5 mL of Bead Buffer into a 25-mL reservoir at room temperature.
9. Remove the strip tube from the magnetic stand.
10. Add **200 µL** of Bead Buffer at **room temperature** and pipette mix to resuspend.
11. Spin the strip tube and place it on the magnetic stand.
12. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.
13. Remove the strip tube from the magnetic stand.
14. Repeat steps 9–12 **one more time** for a total of **two Bead Buffer washes**. Remove the strip tube from the magnetic stand and resuspend the beads during each wash.
15. Resuspend the beads in each tube in **200 µL** of Bead Buffer.
16. Proceed to Section 9: Second Strand Synthesis.

STOP

Safe stopping point. After Bead Oligo Blocking, Quantum Barcoding Beads with cDNA can be stored in Bead Buffer at 4°C for up to 72 hours.

Section 9: Second Strand Synthesis

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 3	Second Strand Enzyme	Violet	–20°C	On ice	✗	✓
Module 3	Second Strand Buffer	Violet	–20°C	On ice	✓	✓
Module 3	Second Strand Additive	Violet	–20°C	On ice	✗	✓
Module 3	Second Strand Primer	Violet	–20°C	On ice	✓	✓
Other Vendors	Nuclease-free water					
	Magnetic stand (0.2-mL strip tube compatible)					
	96-well LoBind PCR Plate					

Before you Begin

- Pre-heat the rotator/orbital mixer to 37°C.
- Pre-heat the thermocycler to 95°C.

Procedure

1. Prepare the Second Strand Synthesis Master Mix **on ice** according to Table 6 and pipette mix.

Table 6: Second Strand Synthesis Master Mix

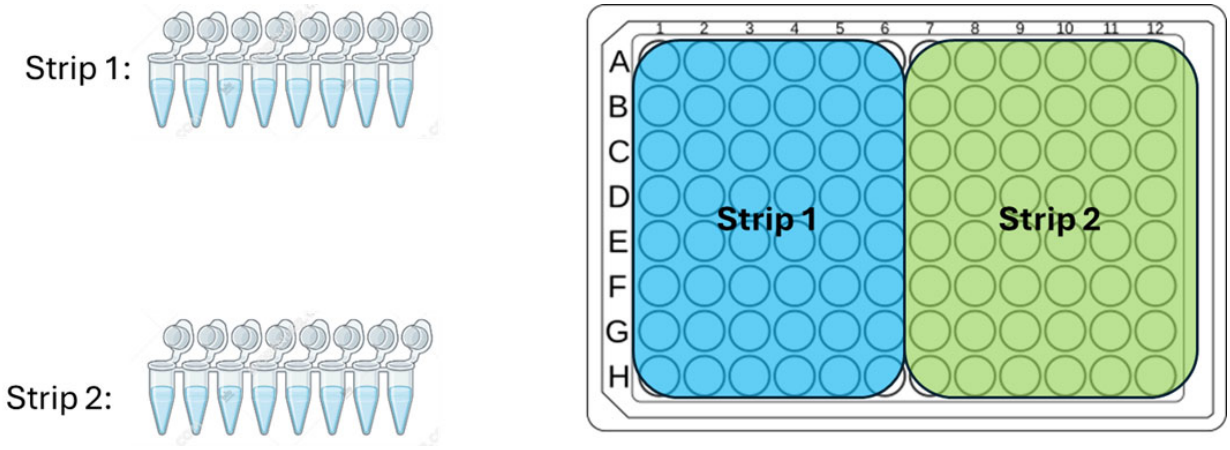
Reagent	Volume (µL)
Nuclease-free water	2530
Second Strand Buffer	330
Second Strand Primer	110
Second Strand Additive	110
Total volume	**3080**

2. Split the beads currently in an 8-well strip tube with 200 µL of Bead Wash Buffer into two 8-well strip tubes, taking **100 µL** into each tube.
3. Place the strip tubes on a magnetic stand and allow the beads to separate.
4. Once the solution is clear, remove the buffer from the beads on the magnetic stand.
5. Resuspend each tube of beads in **182 µL** of Second Strand Synthesis Master Mix.

6. Aliquot **28 µL** of Second Strand Synthesis Master Mix with beads into each well of a 96-well plate as shown in Figure 6: Strip 1–Columns 1–6 and Strip 2–Columns 7–12. Any excess beads can be distributed in a few wells of the plate to ensure all beads are recovered. This will not affect the reaction.

NOTE: Pipette mix between aliquots to ensure that the beads stay in suspension.

Figure 6: Plate Layout Example



7. Seal the 96-well plate and incubate in the thermocycler at **95°C** for **3 minutes**.
8. Immediately place the reaction on ice for **3 minutes**.
9. Spin at **100 x g** for **30 seconds**.
10. Remove the plate seal and distribute **30 µL** of the Second Strand Enzyme into a new 8-well PCR strip tube.
11. From the distributed second strand enzyme, take **2 µL** of Second Strand Enzyme and transfer into each reaction well using a multichannel pipette.
12. Seal the 96-well plate and shake at **2000 rpm** for **30 seconds**.
13. Incubate the plate on a thermomixer at 37°C and 1500 rpm according to Program 8.

Program 8: Second Strand Synthesis

Temperature	Reaction Volume
37°C	30 min

14. Inactivate the reaction in a thermocycler at **95°C** for **3 minutes**.
15. Incubate the plate **on ice** for **5 minutes**.
16. Proceed directly to Section 10: First PCR Amplification.

Section 10: First PCR Amplification

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 3	QB Bead - RNA PCR MM	Yellow	–20°C	On ice	✗	✓
Module 3	QB Bead - RNA PCR Primer	Yellow	–20°C	On ice	✓	✓
Module 3	QB Bead - RNA PCR Enhancer	Yellow	–20°C	On ice	✗	✓
Other Vendors	Microseal 'B' PCR Plate Sealing Film Magnetic stand (96-well compatible) 25-mL reservoir					

Procedure

1. Prepare First PCR Amplification Master Mix **on ice** according to Table 7 and pipette mix.

   Table 7: First PCR Amplification Master Mix

Reagent	Volume (µL)
QB Bead - RNA PCR MM	5500
QB Bead - RNA PCR Enhancer	2310
QB Bead - RNA PCR Primer	204
Total volume	**8014**

2. Invert the First PCR Amplification Master Mix several times to ensure that the reaction components are mixed.
3. Pipette the First PCR Amplification Master Mix into a clean 25-mL reservoir.
4. Remove the seal and transfer **70 µL** of First PCR Amplification Master Mix into each Second Strand Synthesis reaction using a multichannel pipette.

   NOTE: The wells of the 96-well plate will have beads on the sides of each well after the Second Strand reaction. These beads must be pipetted back into the well during the addition of the First PCR Amplification Master Mix.

5. Seal the 96-well plate and shake at **2000 rpm** for **30 seconds**.

6. Place the 96-well plate in the thermocycler and run Program 9.

Program 9: First PCR Amplification

Lid Temperature		Reaction Volume	
105°C		100 µL	
Step	**Temperature**	**Time**	**Cycles**
1	98°C	2 min	1
2	98°C	10 sec	Stage 1 5 cycles (steps 2–4)
3	55°C	20 sec	
4	68°C	40 sec	
5	98°C	10 sec	Stage 2 4–8 cycles (steps 5–7)
6	60°C	10 sec	
7	68°C	40 sec	
8	68°C	1 min	1
9	10°C	∞	1

NOTE: The number of cycles for this PCR stage is determined based on the RNA content. See the following table for recommendations for selected samples.

Cell Types	Stage 2 Cycle Numbers
Large cells (high RNA content)	4
Brain nuclei	5
Small cells and other nuclei (low RNA content)	8
If uncertain about the RNA content of your sample	7

7. After PCR is complete, place the 96-well plate on a magnetic stand.
8. Remove **12 µL** of First PCR Amplification product from each PCR reaction and pool into one new 8-tube strip tube (total 144 µL in each tube).
9. Proceed to Section 11: Post-PCR SPRI Cleanup and QC.

Safe stopping point. First PCR Amplification product can be stored at 4°C for up to 72 hours.

For Long-Term Storage: Transfer the remaining 88 µL of First PCR Amplification product to new strip tubes and store at –20°C for up to 2 months.

Section 11: Post-PCR SPRI Cleanup and QC

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 2	SPRI Additive	Yellow	4°C	RT	✗	✓
Module 1	Elution Buffer	Yellow	RT	RT	✓	✓
Other Vendors	Nuclease-free water					
	SPRIselect beads					
	Ethanol (pure)					
	0.2-mL PCR tube strips (nuclease-free)					
	2.0-mL DNA LoBind tubes					
	Magnetic stand (0.2-mL strip tube compatible)					
	Qubit dsDNA HS Assay Kit					
	D5000 ScreenTape Kit (or equivalent)					

Before you Begin

- Prepare 4 mL of fresh 80% ethanol.

Procedure

1. To each tube of 144 µL of pooled First PCR Amplification product, add **6 µL** of SPRI Additive for SPRI cleanup for a final volume of 150 µL per tube.
2. Vortex the SPRIselect beads until they appear homogeneous in color.
3. Add **120 µL** of SPRI beads (0.8X) to each tube and pipette mix until the solution is homogeneous.
4. Incubate at **room temperature** for **5 minutes**.
5. Briefly spin and place on a magnetic stand for **2 minutes**.
6. Carefully remove and discard the superantant without disturbing the beads.
7. Add **200 µL** of 80% ethanol to the side of each tube opposite the pellet.
8. Incubate for **30 seconds**.
9. Carefully remove and discard the supernatant without disturbing the beads.
10. Repeat the **200-µL** 80% ethanol wash for a total of two washes.
11. Briefly spin the tubes to collect the residual ethanol at the bottom of the tube and place it back on the magnetic stand.
12. Carefully remove any residual 80% ethanol without disturbing the beads.
13. Air dry the beads for **1 minute** or until the bead pellet appears matte instead of glossy, but not cracked.

14. Remove the strip tube from the magnetic stand and add **43 µL** of Elution Buffer.

    NOTE: Do not discard the remaining Elution Buffer. It will be used in subsequent sections of the protocol.

15. Pipette mix to resuspend the beads in solution.

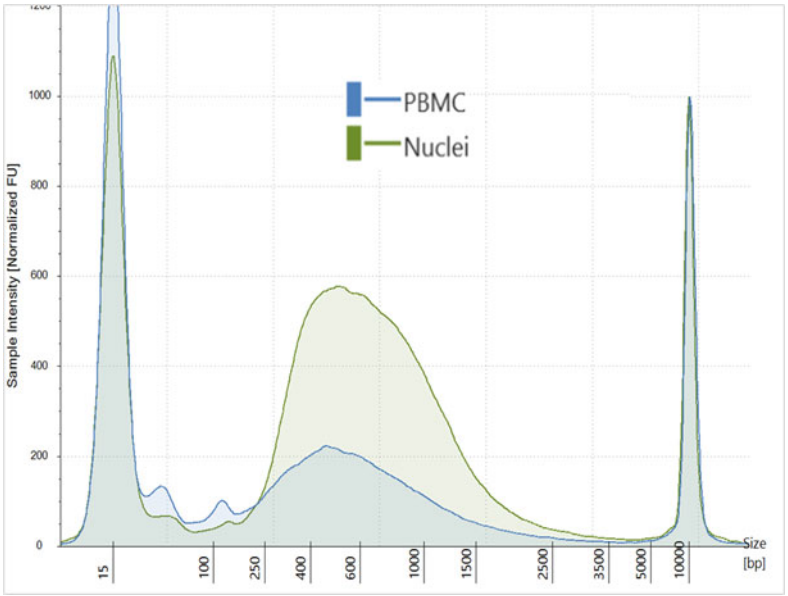
    NOTE: Beads may clump at this step with Elution Buffer, but it is acceptable to proceed.

16. Incubate at **room temperature** for **2 minutes**.
17. Place the tubes back on the magnetic stand and wait until the solution is clear.
18. Transfer supernatant into a fresh strip tube.
19. (Optional) Determine the size of the First PCR Amplification product using an Agilent Tapestation or equivalent fragment analyzer. Example traces are shown in Figure 7.
20. Quantify the SPRIselect bead purified products with the Qubit dsDNA HS Assay Kit.

    NOTE: Samples will be low yield at this point. If the yield is too low to move forward, a second pooling and purification can be run to provide more material for downstream indexing reactions.

21. Dilute an aliquot of each sample to 1 ng/µL in at least **40 µL** with nuclease free water.
22. Transfer **40 µL** of each 1-ng/µL aliquot (40 ng total) to a fresh strip tube.
23. Store the sample at **4°C** or proceed directly to Section 12: RNA Index PCR. If using ScalePlex fixed samples, the remaining First Amplification product will be used in Section 14: ScalePlex Enrichment PCR.

Figure 7: Representative First PCR Amplification Product Traces on Tapestation



NOTE: First PCR Amplification product yields a variable trace. Actual trace will vary based on individual samples.

STOP **Safe stopping point.** Samples can be stored at –20°C for up to 2 months.

Section 12: RNA Index PCR

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 3	QS Index PCR MM	Pink	–20°C	On ice	✗	✓
QuantumScale Library Index Kit	QS RNA Index Primer Plate	Pink (sticker)	–20°C	On ice	✓	✓
Other Vendors	Nuclease-free water					

Procedure

1. Prepare eight RNA Index PCR Reaction Mixes (one per tube of Purified First PCR Amplification product) according to the Table 8. Use a different primer from the QS Index PCR Primer Plate (wells A1–A12) for each RNA Index PCR Reaction Mix.

   NOTE: The QS RNA Index Primer Plate has a **pink sticker**.

   NOTE: There are 12 RNA Index Primers provided in the Library Index Kit. Select 8 RNA Index Primers and note which ones were used.

   Table 8: RNA Index PCR Reaction Mix

Reagent	Volume (µL)
Purified First PCR Amplification product (diluted 1 ng/µL product)	40
Nuclease-free water	25
QS RNA Index PCR MM	25
QS RNA Index Primer (A1–A12)	10
Total volume	**100**

   CAUTION: If using ScalePlex samples, do not throw away the QS Index PCR MM tube, as it will be used in Section 14: ScalePlex Enrichment PCR.

2. Pipette mix and briefly spin down.

3. Incubate the RNA Index PCR reactions in a thermocycler according to Program 10.

Program 10: RNA Index PCR

Lid Temperature		Reaction Volume	
105°C		100 µL	
Step	**Temperature**	**Time**	**Cycles**
1	98°C	45 sec	1
2	98°C	15 sec	7 cycles
3	60°C	30 sec	
4	72°C	40 sec	
5	72°C	1 min	1
6	10°C	∞	1

4. Once the PCR Program 10 is finished, proceed to Section 13: Final Library Cleanup.

Safe stopping point. Index PCR product can be stored at 4°C for up to 72 hours or at –20°C for up to 2 months.

Section 13: Final Library Cleanup

NOTE: This is two sequential 0.7X SPRI cleanups.

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 1	Elution Buffer	Yellow	RT	RT	✓	✓
Other Vendors	Nuclease-free water					
	SPRIselect beads					
	Ethanol (pure)					
	0.2-mL PCR tube strips (nuclease-free)					
	2.0-mL DNA LoBind tubes					
	Magnetic stand (0.2-mL strip tube compatible)					
	Qubit dsDNA HS Assay Kit					
	D5000 ScreenTape Kit (or equivalent)					

Before you Begin

- Prepare 8 mL of fresh 80% ethanol.

Procedure

SPRI Cleanup 1

1. Vortex the SPRIselect beads well until they appear homogeneous in color.
2. Add **70 µL** of SPRIselect beads (0.7X) to each tube of the RNA Index PCR product and pipette mix until the solution is homogeneous.
3. Incubate at **room temperature** for **5 minutes**.
4. Briefly spin and place the tubes on a magnetic stand for **2 minutes** or until the solution is clear.
5. Remove and discard the supernatant.
6. Add **200 µL** of 80% ethanol to the sides of the tubes opposite the pellet.
7. Incubate for **30 seconds**.
8. Carefully remove and discard the supernatant without disturbing the beads.
9. Repeat the **200 µL** 80% ethanol wash for a total of two washes.
10. Briefly spin the tubes to collect the residual ethanol at the bottom of the tubes and place it back on the magnetic stand.
11. Carefully remove any residual 80% ethanol without disturbing the beads.

12. Air dry the beads for **1 minute** or until the bead pellets appears matte instead of glossy, but not cracked.
13. Remove the tubes from the magnetic stand and immediately add **52 µL** of Elution Buffer.
14. Pipette mix to resuspend the beads in solution.
15. Incubate at **room temperature** for **2 minutes**.
16. Place the tubes back on the magnetic stand and wait until the solution is clear.
17. Transfer **50 µL** of supernatant into fresh 0.2-mL strip tubes.

SPRI Cleanup 2

1. Add **35 µL** of SPRIselect beads (0.7x) to each tube of the cleaned PCR product and pipette mix until the solution is homogeneous.
2. Incubate at **room temperature** for **5 minutes**.
3. Briefly spin and place the tubes on a magnetic stand for **2 minutes** or until the solution is clear.
4. Remove and discard the supernatant.
5. Add **200 µL** of 80% ethanol to the side of the tubes opposite the pellet.
6. Incubate for **30 seconds**.
7. Carefully remove and discard the supernatant without disturbing the beads.
8. Repeat the **200 µL** 80% ethanol wash for a total of two washes.
9. Briefly spin the tubes to collect the residual ethanol at the bottom of the tubes and place them back on the magnetic stand.
10. Carefully remove residual 80% ethanol without disturbing the beads.
11. Air dry the beads for **1 minute** or until the bead pellets appears matte instead of glossy, but not cracked.
12. Remove the tubes from the magnetic stand and immediately add **42 µL** of Elution Buffer.
13. Pipette mix to resuspend the beads in solution.
14. Incubate at **room temperature** for **2 minutes**.
15. Place the tubes back on the magnetic stand and wait until the solution is clear.
16. Carefully transfer **40 µL** of eluted library to fresh 0.2-mL strip tubes.
17. Proceed to Section 16: Final Library QC.

Safe stopping point. Purified library can be stored at –20°C for up to 2 months.

Section 14: ScalePlex Enrichment PCR

NOTE: Perform this section if ScalePlex Fixation was used.

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 3	QS Index PCR MM	Pink	–20°C	On ice	✗	✓
QuantumScale Library Index Kit	QS ScalePlex Index Primer Plate	Blue (sticker)	–20°C	On ice	✓	✓
Other Vendors	Nuclease-free water					

Procedure

1. Take the purified First PCR Amplification product from Section 11: Post-PCR SPRI Cleanup and QC and quantify.
2. Dilute an aliquot of each to 0.5 ng/µL in **40 µL** with nuclease-free water.
3. **On ice**, prepare eight ScalePlex Enrichment PCR Reaction Mixes by combining the components specified in Table 9.

   NOTE: The QS ScalePlex Index Primer Plate has a **blue sticker**.

   NOTE: There are 12 ScalePlex Index PCR Primers provided in the Library Index Kit. Select the eight ScalePlex Index PCR Primers that align with the RNA Index PCR Primers used in Step 12 and note which ones were used.

   Table 9: ScalePlex PCR Reaction Mix

Reagent	Volume (µL)
Purified First PCR Amplification product (diluted 0.5 ng/µL product)	40
Nuclease-free water	25
QS Index PCR MM	25
QS ScalePlex Index Primer (A1–A12)	10
Total volume	**100**

4. Pipette mix and briefly spin down.

5. Incubate the ScalePlex PCR reaction in a thermocycler according to Program 11.

Program 11: ScalePlex Enrichment PCR

Lid Temperature		Reaction Volume	
105°C		100 µL	
Step	**Temperature**	**Time**	**Cycles**
1	98°C	45 sec	1
2	98°C	15 sec	Stage 1
3	63°C	30 sec	5 cycles
4	72°C	30 sec	(steps 2–4)
5	98°C	15 sec	Stage 2
6	72°C	30 sec	7 cycles (steps 5–6)
7	72°C	1 min	1
8	10°C	Hold	1

6. Once the PCR reaction is complete, proceed directly to Section 15: ScalePlex Library Cleanup.

Safe stopping point. ScalePlex Index PCR product can be stored at 4°C for up to 72 hours or at –20°C for up to 2 months.

Section 15: ScalePlex Library Cleanup

NOTE: Perform this section if ScalePlex Fixation was used.

Review the following table to prepare reagents before starting this section.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 1	Elution Buffer	Yellow	RT	RT	✓	✓
Other Vendors	Nuclease-free water					
	SPRIselect beads					
	Ethanol (pure)					
	Magnetic stand (0.2-mL strip tube compatible)					
	0.2-mL PCR tube strips (nuclease-free)					
	Qubit dsDNA HS Assay Kit					
	D5000 ScreenTape Kit (or equivalent)					

Before you Begin

- Prepare 500 µL of fresh 80% ethanol.

Procedure

1. Vortex the SPRIselect beads well until they appear homogeneous in color.
2. Add **100 µL** of SPRIselect beads (1.0X) to each tube of the ScalePlex Index PCR product and pipette mix until the solution is homogeneous.
3. Incubate at **room temperature** for **5 minutes**.
4. Briefly spin and place the tubes on a magnetic stand for **2 minutes** or until the solution is clear.
5. Carefully remove and discard the supernatant without disturbing the beads.
6. Add **200 µL** of 80% ethanol to the sides of the tubes opposite the pellet.
7. Incubate for **30 seconds**.
8. Carefully remove and discard the supernatant without disturbing the beads.
9. Repeat the **200 µL** 80% ethanol wash for a total of **two washes**.
10. Briefly spin the tubes to collect the residual ethanol at the bottom of the tubes and place them back on the magnetic stand.
11. Carefully remove residual 80% ethanol without disturbing the beads.
12. Air dry the beads for **1 minute** or until the bead pellets appears matte instead of glossy but not cracked.
13. Remove the tubes from the magnetic stand and immediately add **53 µL** of Elution Buffer.
14. Pipette mix to resuspend the beads in solution.

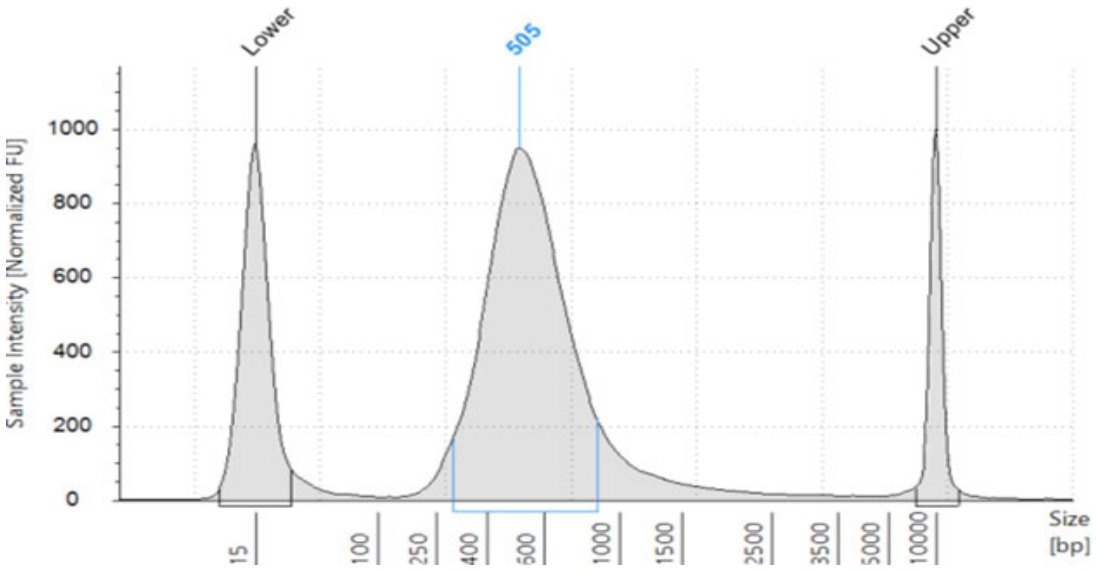
15. Incubate at **room temperature** for **2 minutes**.
16. Place the tubes back on the magnetic stand and wait until the solution is clear.
17. Transfer **50 µL** of supernatant into fresh PCR tube.
18. Proceed to Section 17: ScalePlex Library QC.

Safe stopping point. Purified library can be stored at –20°C for up to 2 months.

Section 16: Final Library QC

1. Quantify each RNA Final Library product to determine the concentration with the Qubit™ 1X dsDNA HS Assay Kit.
2. Determine the size of each Final Library product using an Agilent Tapestation or equivalent fragment analyzer. Example traces are shown in Figure 8 (Tapestation).

Figure 8: Representative Final Library Traces on Tapestation



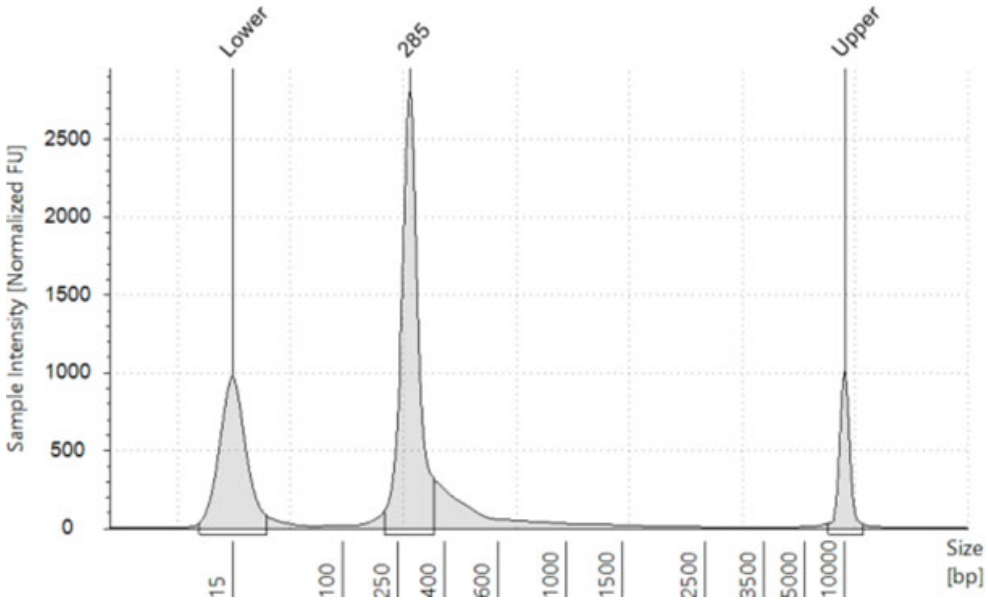
Safe stopping point. Purified library can be stored at –20°C for up to 2 months.

Section 17: ScalePlex Library QC

NOTE: Perform this section if ScalePlex Fixation was used.

1. Quantify each ScalePlex Final Library product to determine the concentration with the Qubit™ 1X dsDNA HS Assay Kit.
2. Determine the size of each Final Library product using an Agilent Tapestation or equivalent fragment analyzer. Example traces are shown in Figure 9 (Tapestation).

Figure 9: Representative Final Library Traces on Tapestation

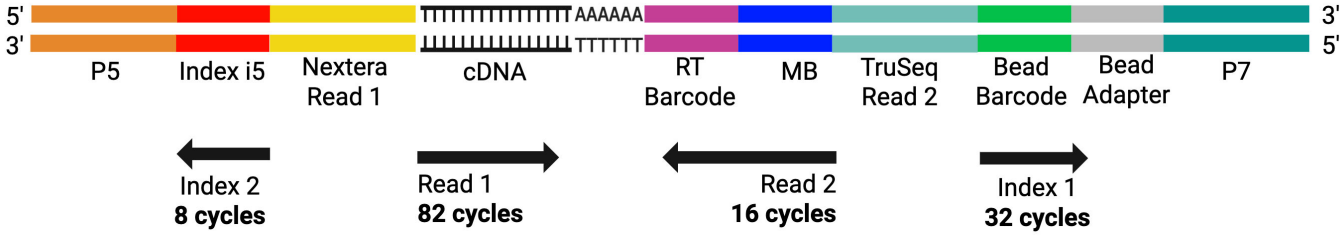


Safe stopping point. Purified library can be stored at –20°C for up to 2 months.

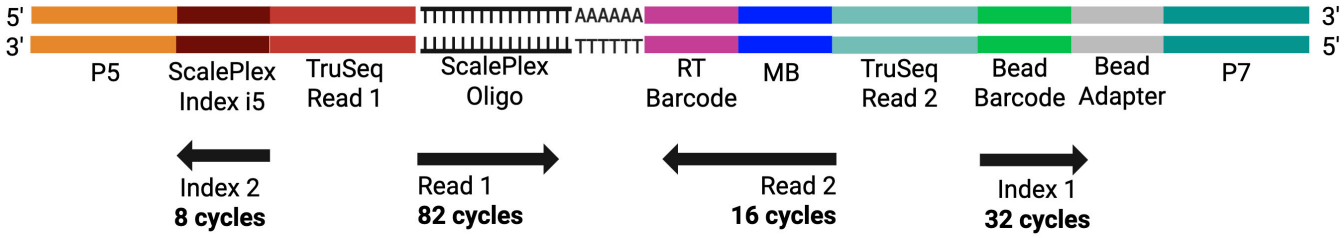
Section 18: Sequencing Parameters

The following sequencing guidance is for Illumina sequencing only. For Ultima sequencing guidance, contact support@scale.bio.

RNA Library - ILMN



ScalePlex Library - ILMN



Sequencing Guidance with Custom Read Configuration

Note that these libraries require a custom read configuration using the following override cycles:

OverrideCycles: Y82;I8U24;I8;Y16

This configuration ensures that the 32 cycles of Index 1 are fully captured. During demultiplexing, the first 8 bases of Index 1 are used to generate the FASTQ files, and the remaining 24 bases are retained for downstream analysis in the ScaleBio Seq Suite: RNA pipeline.

To ensure correct demultiplexing and data processing, please download and use the QuantumScale Single Cell RNA Samplesheets for Large kits available on the ScaleBio support website. These sheets include the required OverrideCycles settings and will help guide your analysis.

Read	Length	Purpose
Read 1	≤82 cycles	Transcript
Read 2	16 cycles	RT Barcode (96 different barcodes)
		MB (Molecular Barcode)
Index 1	32 cycles	Bead Barcode (~880k different barcodes)
		8 bases for FASTQ generation
		24 bases for demultiplexing
Index 2	8 cycles	Index PCR Barcode (1 barcode)

Library Loading for Sequencing

Sequencing Depth
We recommend a minimum sequencing depth of **20,000 read pairs per cell/nucleus** for the RNA library and **2,000 read pairs per cell/nucleus** for the ScalePlex Library.

When loading the libraries onto the flow cell, spike in the ScalePlex library at a 1:10 ratio to the RNA library. This ratio will achieve the recommended sequencing depths.

Recommended Final Loading Concentrations	
NovaSeq X (25B)	180 pM
NovaSeq X (10B)	160 pM
NextSeq 2000 (XLEAP-SBS)	600 pM (on-board denaturation)
PhiX	1% (optional)

CAUTION: To ensure correct sample demultiplexing, sublibraries processed by multiple ScaleBio Single Cell RNA Sequencing Kits using the same Index PCR Primers cannot be pooled nor sequenced together on the same lane of a flow cell.

Note that the QuantumScale Single Cell RNA Kit - Large must use the ScaleBio Seq Suite: RNA v2.0 or later for data analysis.

For implementation of the ScaleBio RNA Bioinformatics Pipeline (ScaleBio Seq Suite), contact your local Field Application Scientist or support@scale.bio.

Bioinformatics Analysis

For downstream bioinformatics analysis, please use the samplesheets available on the ScaleBio support website. These sheets include the OverrideCycles settings and other important parameters to ensure accurate processing of your data. Note that the individual FASTQ files generated do not correspond to different samples loaded into the QuantumScale assay. Sample-level demultiplexing is done by the ScaleBio Seq Suite: RNA software.

Additionally, for large QuantumScale kits using the **QS RNA Index Primer Plate** (ILMN or ULT), libraries will be designated on the sample barcode table for libIndex2 depending on which RNA Index Primers were used. The same applies for ScalePlex libraries for scalePlexLibIndex2. This ensures that they are processed correctly during demultiplexing and subsequent analysis steps. Twelve RNA and ScalePlex Index Primers are provided in the Library Index Kits, and eight are selected for library preparation.

QS RNA Index Primer Plate Position	libIndex2 (sample barcode table)	Corresponding QS ScalePlex Index Primer Plate Position	ScalePlexLibIndex2 (sample barcode table)
A1	QSR-1	A1	QSR-1-SCALEPLEX
A2	QSR-2	A2	QSR-2-SCALEPLEX
A3	QSR-3	A3	QSR-3-SCALEPLEX
A4	QSR-4	A4	QSR-4-SCALEPLEX

QS RNA Index Primer Plate Position	libIndex2 (sample barcode table)	Corresponding QS ScalePlex Index Primer Plate Position	ScalePlexLibIndex2 (sample barcode table)
A5	QSR-5	A5	QSR-5-SCALEPLEX
A6	QSR-6	A6	QSR-6-SCALEPLEX
A7	QSR-7	A7	QSR-7-SCALEPLEX
A8	QSR-8	A8	QSR-8-SCALEPLEX
A9	QSR-9	A9	QSR-9-SCALEPLEX
A10	QSR-10	A10	QSR-10-SCALEPLEX
A11	QSR-11	A11	QSR-11-SCALEPLEX
A12	QSR-12	A12	QSR-12-SCALEPLEX

Document Revision History

Document Revision	Revisions Date	Description of Change
1262598 Rev B	June 2025	Clarifications to workflow.
1262598 Rev A	March 2025	Initial release