QuantumScale
Single Cell RNA – Medium

Protocol

Legal Notices

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Table of Contents

Required Materials

Consumables and Reagents Manufactured by ScaleBio

Kits required for this protocol:

- QuantumScale Single Cell RNA Kit - Medium (PN 1258683)
  - QuantumScale Single Cell RNA - Small/Medium, Module 1 (PN 1255822)
  - QuantumScale Single Cell RNA - Medium, Module 2 (PN 1263436)
  - QuantumScale Single Cell RNA - Medium, Module 3 (PN 1263437)
  - QuantumScale Single Cell RNA – Small/Medium, RT Module (PN 1321199)
- QuantumScale Library Index Kit-I-1 (PN 1258495 for Illumina libraries)

Kit Module	Consumable	Part Number	Qty	Cap Color	Storage Temp
Module 1	Quantum Barcoding Plate	1254544	1	—	RT
Module 1	Plate Wetting Buffer	1254719	1	Clear	RT
Module 3	Plate Blocking Buffer	1254720	1	Clear	–20°C
Module 3	QS RT Barcode Plate 24 (Plate)	1254721	1	—	–20°C
Module 3	RT Enhancer	1314356	1	Blue	–20°C
Module 3	RT Enzyme Mix	1254722	1	Blue	–20°C
Module 3	RT Buffer	1254723	1	Blue	–20°C
Module 3	RT Additive	1254724	1	Blue	–20°C
Module 2	RT Stop Solution	1254726	1	Blue	4°C
Module 2	Cell Wash Buffer	1254728	1	White	4°C
Module 2	Quantum Barcoding Beads-RNA-8 (Plate)	1254730	1	—	4°C
Module 3	RC Enzyme	1254732	1	White	–20°C
Module 3	Cleanup Enzyme	1254734	1	White	–20°C
Module 3	RC and Cleanup Buffer	1254733	1	White	–20°C
Module 2	Bead Collection Buffer	1254739	1	White	4°C
Module 2	Bead Buffer	1254738	1	White	4°C
Module 1	Pre-RC Buffer	1316364	1	White	RT
Module 3	Ligation 1 Enzyme	1254741	1	Green	–20°C
Module 3	Ligation 1 Buffer	1254743	1	Green	–20°C
Module 2	Ligation 1 Wash Reagent	1254746	1	Green	4°C
Module 3	Oligo Blocking Enzyme	1254751	1	Green	–20°C
Module 3	Oligo Blocking Buffer	1254748	1	Green	–20°C

Kit Module	Consumable	Part Number	Qty	Cap Color	Storage Temp
Module 3	Oligo Blocking Additive	1279264	1	Green	–20°C
Module 3	Oligo Blocking Enhancer	1279265	1	Green	–20°C
Module 3	Second Strand Enzyme	1314369	1	Violet	–20°C
Module 3	Second Strand Buffer	1314371	1	Violet	–20°C
Module 3	Second Strand Additive	1314370	1	Violet	–20°C
Module 3	Second Strand Primer	1314372	1	Violet	–20°C
Module 3	QB Bead - RNA PCR MM	1314362	1	Yellow	–20°C
Module 3	QB Bead - RNA PCR Enhancer	1317035	1	Yellow	–20°C
Module 3	QB Bead - RNA PCR Primer	1317037	1	Yellow	–20°C
Module 2	SPRI Additive	1254779	1	Yellow	4°C
Module 1	Elution Buffer	1254781	1	Yellow	RT
Module 3	QS Index PCR MM	1254778	1	Pink	–20°C
QuantumScale Library Index Kit	QS RNA Index PCR Primer Tube	1255817	1	Pink	–20°C
QuantumScale Library Index Kit	QS ScalePlex Index Primer Tube	1255789	1	Blue	–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
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
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
NEBNext Library Quant Kit for Illumina[a]	NEB	E7630

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 15-mL tubes	Permagen	MSR50151 or MSR2X15 (or similar)
Magnetic stand for 1.5-mL tubes	Thermo Fisher	12321D
Magnetic stand for 0.2-mL strip tubes	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

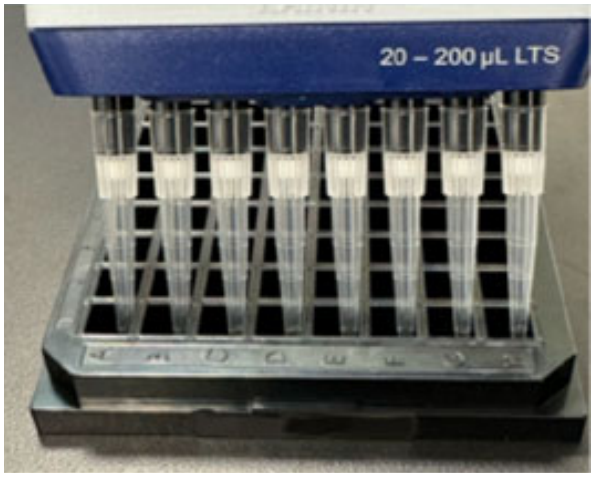
a. Required for quality control of library.

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.



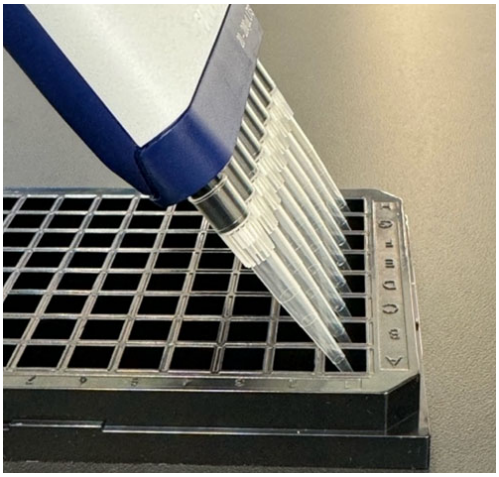


Figure 2: Pipetting into a well along the side wall.

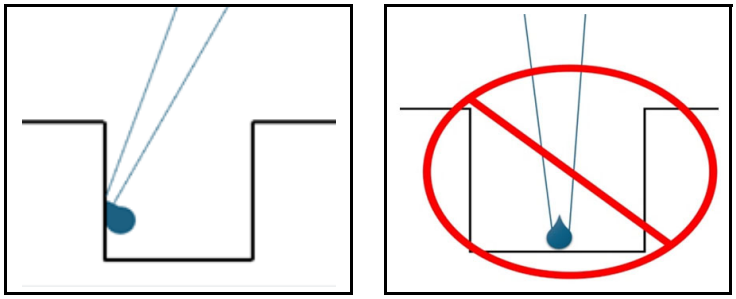
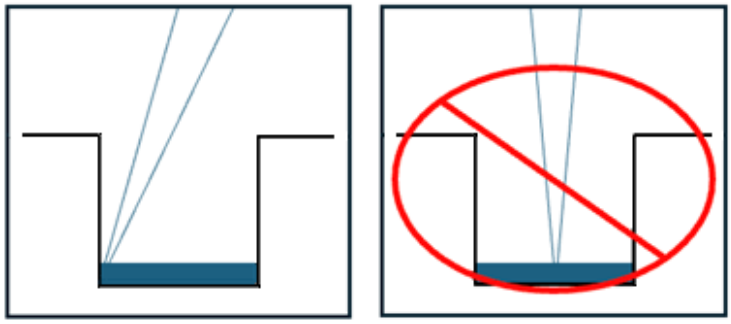
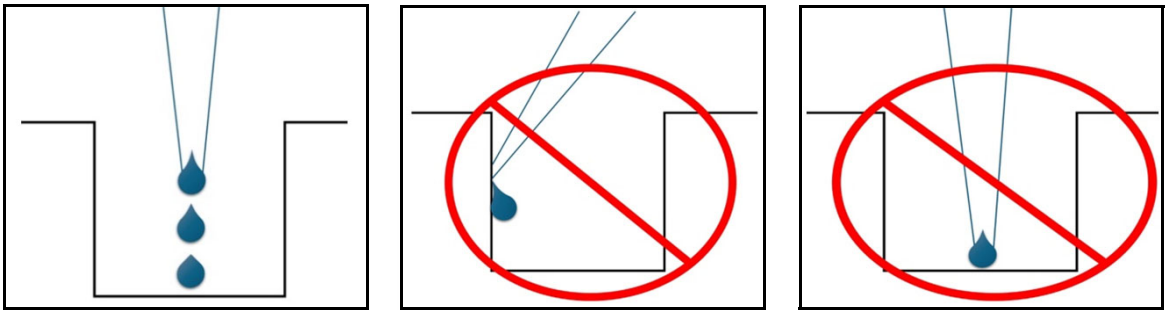


Figure 3: Pipetting out of a well from a selected corner (same corner every time)



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

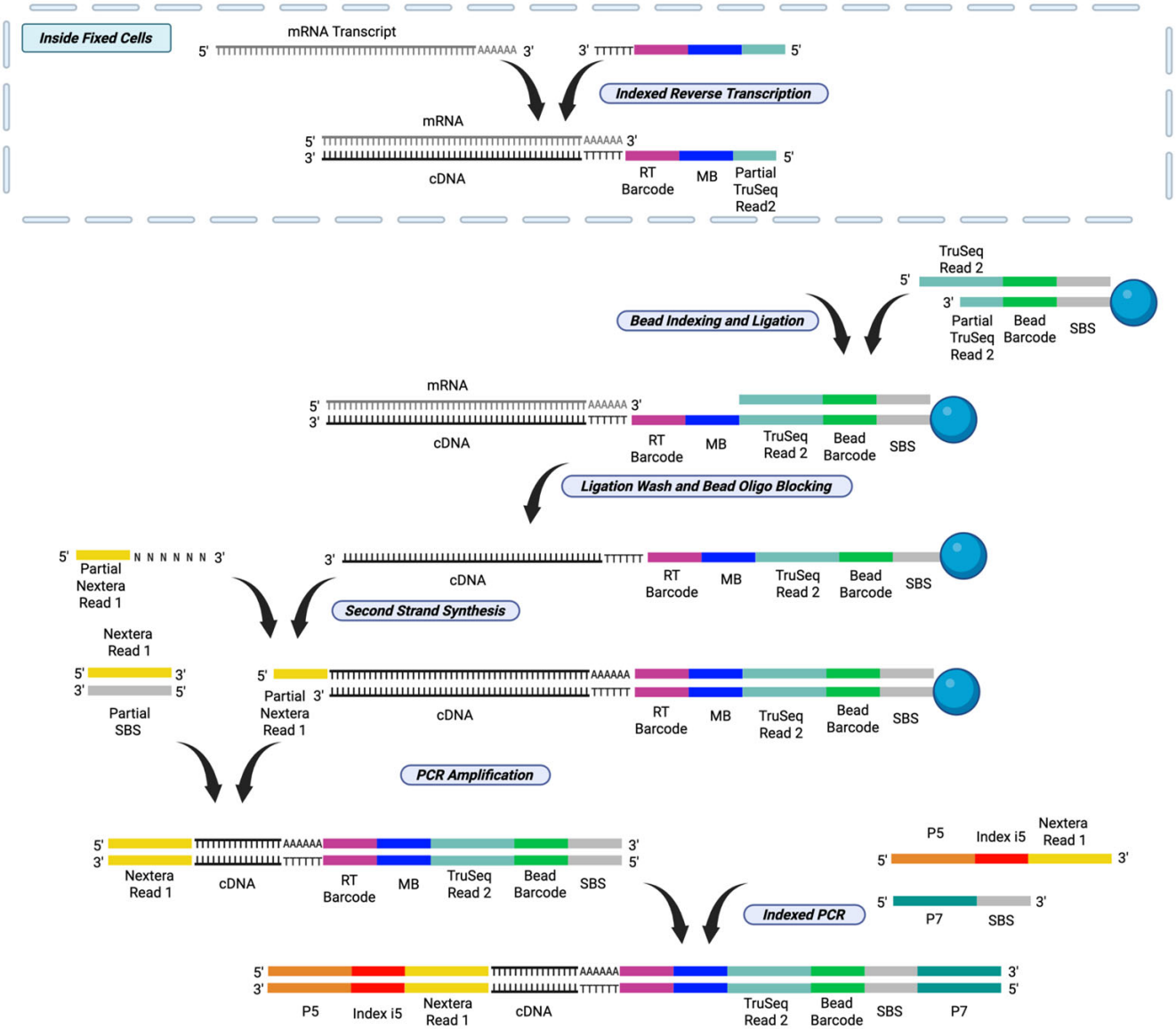
- Thaw and prepare reagent mixes in pre-amplification workspaces.
- Perform amplification in post-amplification workspaces.
- Perform PCR purification steps in post-amplification workspace.
- Never bring material or equipment from post-amplification workspaces into pre-amplification workspaces.
- Regularly 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.

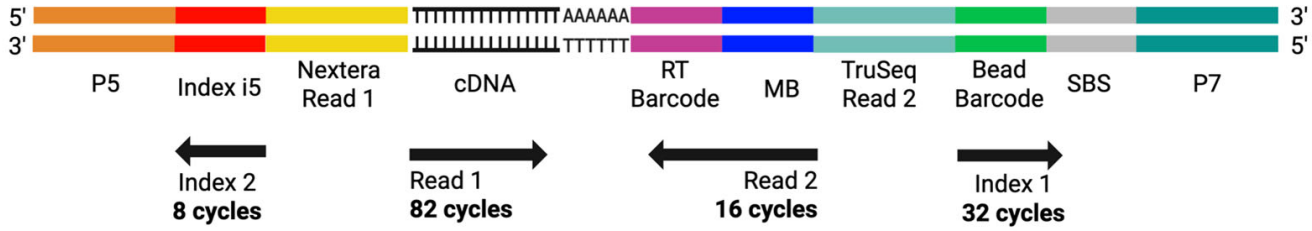
Next cDNA together with the linked bead oligo is amplified by a semi-suppressive PCR reaction. Final RNA library PCR is performed to add the Illumina P5 and P7 adaptors and i5 index. If using ScalePlex, ScalePlex libraries are enriched with a separate PCR reaction.



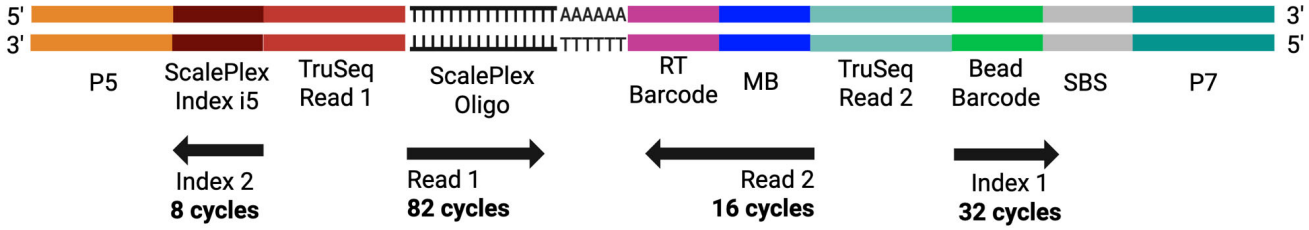
SBS – Scale Bead Sequence
P5/P7 – Illumina Adapters P5/P7

After SPRI purification of each library, the libraries are ready for sequencing. Note that this is the final library structure for Illumina libraries.

RNA Library – ILMN



ScalePlex Library – ILMN



Step 1: Preparing the Quantum Barcoding Plate

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

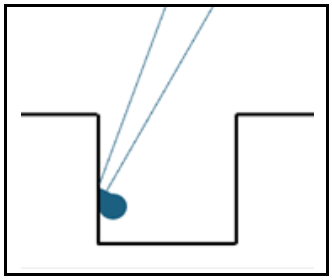
Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 1	Quantum Barcoding Plate	—	RT	RT	⊗	⊗
Module 1	Plate Wetting Buffer	Clear	RT	RT	⊗	⊗
Module 3	Plate Blocking Buffer	—	–20°C	RT	⊗	⊗
Other Vendors	Microseal 'B' PCR Plate Sealing Film					

Before you Begin

- Set a swing bucket centrifuge for 96-well plates to room temperature.
- Use only the first column (A1–H1) of the Quantum Barcoding Plate.

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. Add **100 µL** of Plate Wetting Buffer to the first column (A1–H1) 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

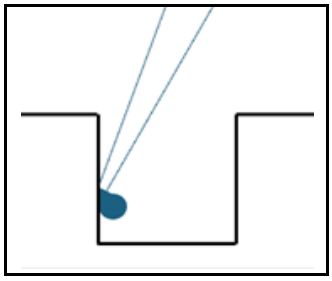
Temperature	Time
Room temperature (18–25°C)	>12 h

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.

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 residue liquid on a paper towel.

   Inspect that the majority of liquid is removed and plate is free of debris.
9. Invert to mix the Plate Blocking Buffer tube and add **150 µL** Plate Blocking Buffer into each well of the Quantum Barcoding Plate along the side wall (first column only), 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 Step 2: Initial Distribution and Reverse Transcription.

Step 2: Initial Distribution and Reverse Transcription

NOTE: Before starting this step 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 step.

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 3	QS RT Barcode Plate 24	—	–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	✗	✗
Other Vendors	Nuclease-free water					
	Microseal 'B' PCR Plate Sealing Film					
	P200 wide-bore pipette tips					
	Cell counting dye					
	2-mL DNA LoBind 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. Centrifuge the thawed QS RT Barcode Plate 24 at **500 x g** for **1 minute** at **4°C** and place on a 96-well metal block on ice.
2. 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**.
3. Unseal the QS RT Barcode Plate 24 on ice. A total of 24 RT reactions are performed.
4. Dilute RT Enhancer by mixing **14 µL** RT Enhancer with **42 µL** Cell Wash Buffer.
5. Add **2 µL** of diluted RT Enhancer into each barcode well of the QS RT Barcode Plate (first 3 columns).
6. On ice, use P200 wide-bore pipette tips to gently mix the cells/nuclei, and dilute with Cell Wash Buffer to achieve a concentration of 2800 cells/µL. Make sure the cells/nuclei are mixed well before loading.

7. Immediately distribute 5µL of cells/nuclei to each well of the first three columns (column 1, 2, 3) of the QS RT Barcode Plate 24 that contains RT Barcode Primer.

CAUTION: DO NOT pipette mix. 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.

8. Seal the QS RT Barcode Plate 24 and place it on a plate shaker.
9. Shake the plate at **2000 rpm** for **30 seconds**.
10. Centrifuge the plate at **100 x g** for **30 seconds** at **4°C**.
11. Incubate the QS RT Barcode Plate 24 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

12. Upon completion, **immediately** remove the QS RT Barcode Plate 24 from the thermocycler and place on the pre-chilled metal block **on ice**.
13. Incubate **on ice** for **2 minutes**.
14. Start Program 4 on a thermocycler and hold at **4°C** with a lid temperature of **65°C**.
15. **On ice**, prepare the Reverse Transcription Master Mix.

Table 1: Reverse Transcription Master Mix

Reagent	Volume (µL)
Nuclease-free water	70
RT Buffer Conc.	112
RT Additive	28
RT Enhancer	14
RT Enzyme Mix	28
Total volume	**252**

16. **On ice**, slowly pipette mix the RT Master Mix until the solution is homogeneous and briefly spin down.
17. Aliquot **82 µL** of the RT Master Mix into three tubes of an 8-tube strip **on ice**.

18. **On ice**, unseal the QS RT Barcode Plate 24 and distribute **9 µL** of the RT Master Mix into each well of column 1, 2, and 3 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.

19. Seal the QS RT Barcode Plate 24 with an adhesive seal and place it on a plate shaker.
20. Shake the plate at **2000 rpm** for **30 seconds**.
21. Centrifuge the plate at **100 x g** for **30 seconds** at **4°C** and place **on ice**.
22. Incubate the QS RT Barcode Plate 24 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	∞

23. Once the thermocycler program is completed, take out the QS RT Barcode Plate 24 and shake the plate at **2000 rpm** for **30 seconds**. Unseal the QS RT Barcode Plate 24, place on ice, then add **100 µL** of cold RT Stop Solution into each well of the **first column** of QS RT Barcode Plate 24.
24. Use an 8-channel pipette to pool the RT reaction from columns 2 and 3 to column 1. To increase the cell/nuclei recovery, pipette mix before taking out the RT reaction.
25. Pool the RT reaction with RT Stop buffer from the first column into a new 1.5-mL DNA LoBind tube on ice.
26. Spin the RT cells/nuclei in the 1.5-mL tube at **500 x g** for **8 minutes**.
27. Remove the tube from the centrifuge and remove the supernatant, leaving 50 µL.

    Best practice is to fill another 1.5-mL tube with 50 µL and use that tube as a reference point.
28. Add **1200 µL** of Cell Wash Buffer to resuspend cells/nuclei for a total volume of 1250 µL.
29. (Optional) Take **10 µL** for cell counting. Keep the rest of the cell/nuclei suspension **on ice** for cell loading.
30. Proceed directly to Step 3: Cell/Nuclei Loading.

Step 3: Cell/Nuclei Loading

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

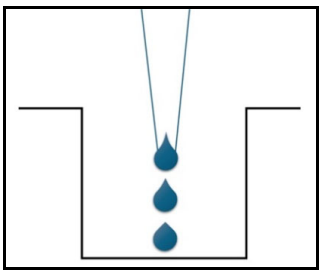
Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 2	Cell Wash Buffer	White	4°C	On ice	⊗	⊗
Other Vendors	Microseal 'B' PCR Plate Sealing Film					

Before you Begin

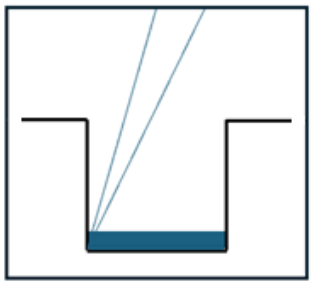
- Set a swing bucket centrifuge for 96-well plates to 4°C.

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. Note that only the first column (8 wells) is used.
2. Add **150 µL** of cell/nuclei solution with a 200-µL pipette tip to the center of each well of the first column of the Quantum Barcoding Plate without touching the bottom of the plate, as shown. If there is residual cell/nuclei solution in the tube, distribute it evenly into 8 wells.



3. Place the plate lid on the Quantum Barcoding Plate and incubate for **5 minutes** on a flat surface.
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 Step 4: Bead Loading.

Step 4: Bead Loading

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

Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 2	Quantum Barcoding Beads-RNA-8 (Plate)	—	4°C	On ice	✗	✓
Module 2	Cell Wash Buffer	White	4°C	On ice	✗	✗
Other Vendors	Microseal 'B' PCR Plate Sealing Film					

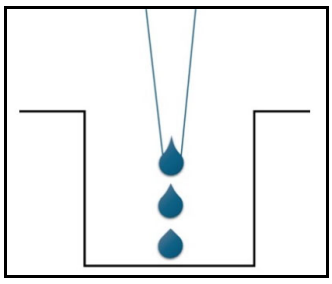
Before you Begin

- Note that only the first column (8 wells) contains Quantum Barcoding Beads-RNA-8 solution.

Procedure

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

1. Invert the Quantum Barcoding Beads Plate 2–3 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.
2. Pipette the whole volume (~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. Continue to incubate for **2 minutes** after shaking.

   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 in Figure 3.
6. Proceed directly to Step 5: cDNA Release and Capture.

Step 5: cDNA Release and Capture

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

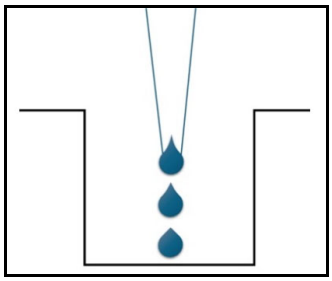
Source	Material	Cap Color	Take From	Thaw At	Brief Vortex	Brief Spin
Module 3	RC Enzyme	White	–20°C	On ice	✗	✓
Module 3	Cleanup Enzyme	White	–20°C	On ice	✗	✓
Module 3	RC and Cleanup Buffer	White	–20°C	On ice	✗	✓
Module 3	Pre-RC Buffer	White	RT	RT	✗	✗

Before you Begin

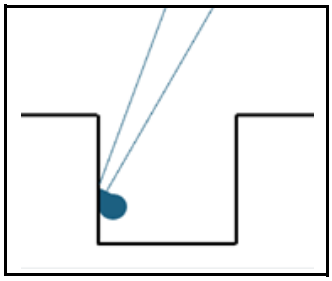
- Pre-heat an incubator that will fit the Quantum Barcoding Plate to 37°C.

Procedure

1. Remove the lid from the Quantum Barcoding Plate and add **100 µL** of Pre-RC buffer to each well.



2. Incubate at **37°C** for **10 minutes**.
3. Remove the Pre-RC Buffer.
4. Gently add **150 µL** of Cell Wash Buffer by dispensing the liquid on the side walls of the wells, as shown.



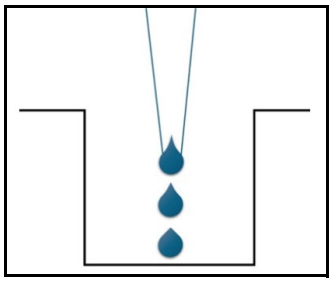
5. Pipette mix two times from a selected corner of the well and remove and discard all solution.

6. Prepare the RC Solution in a 1.5-mL DNA LoBind tube at **room temperature** according to Table 2. Pipette mix RC Solution 5 times.

Table 2: RC Solution

Reagent	Volume (µL)
RC and Cleanup Buffer	460
RC Enzyme	40
Total volume	**500**

7. 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 place the plate in an incubator according to Program 5.

Program 5: RC Incubation

Temperature	Time
37°C	30 min

9. About **5 minutes** before the RC Incubation ends, prepare the RC Cleanup Solution according to Table 3. Pipette mix RC Cleanup Solution 5 times.

Table 3: RC Cleanup Solution

Reagent	Volume (µL)
RC and Cleanup Buffer	225
Cleanup Enzyme	25
Total volume	**250**

10. Remove the Quantum Barcoding Plate from the incubator, remove the lid.
11. Add **25 µL** of RC Cleanup Solution to the side of each well and mix by moving the plate in a circular motion 5 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 Step 6: Bead Collection.

Step 6: Bead Collection

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

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	⊗	⊗
Other Vendors	Magnetic stand (15-mL compatible) Magnetic stand (1.5-mL 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 and add **200 µL** of Bead Collection Buffer to each well. Note that only the first column is used.
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. Use a 200-µL pipette and set to 150 µL to pipette mix the bead solution. Take out the beads and pool in a 5-mL tube. You need to pipette twice to take out all bead solution.
4. Add **100 µL** of Bead Buffer to each well (first column) of the Quantum Barcoding Plate and pipette mix two to three times to dislodge the residue beads.
5. Take out the Bead Buffer with beads and add to the 5-mL tube.
6. Place the tube on a magnetic stand.
7. Once the solution is clear, carefully remove and discard the supernatant without touching the beads.

   NOTE: Waiting for the supernatant to become clear takes 3–5 minutes. Ensure that the solution is clear after waiting 3–5 minutes.

8. Remove the tube from the magnetic stand and resuspend the Quantum Barcoding Beads with **1 mL** of Bead Buffer.
9. Transfer to a 1.5-mL tube, briefly centrifuge in a microcentrifuge, and place on the magnetic stand.
10. Once the solution is clear, carefully remove and discard the supernatant without pipetting the beads.
11. Wash the beads two more times with **200 µL** of Bead Buffer for a total of **three washes**. Remove the tube from the magnetic stand and resuspend the beads during each wash.
12. Proceed directly to Step 7: Bead Ligation.

Step 7: Bead Ligation

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

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	Green	4°C	RT	✗	✗
Other Vendors	Magnetic stand (1.5-mL compatible) Nuclease-free water					

Procedure

1. On the magnetic stand, carefully remove and discard the supernatant without pipetting 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	23.5
Nuclease-free water	164
Ligation 1 Enzyme	2.5
Total volume	**190**

3. Resuspend the Quantum Barcoding Beads in the Ligation Master Mix (about 190 µL).
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. A thermomixer can also be used with settings at 18–25°C and 1500 rpm. If you use a thermomixer, a 2-mL tube with a round bottom is recommended because it facilitates mixing.

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 **200 µL** of Bead Buffer and invert several times to resuspend.
10. Briefly spin the tube, and place it on a magnetic stand.
11. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.
12. Remove the tube from the magnetic stand.
13. Add **200 µL** of Ligation 1 Wash Reagent and invert several times to resuspend.
14. Incubate at **room temperature** for **5 minutes** on a rotator/orbital mixer. Make sure the beads do not settle down during the reaction. A thermomixer can also be used with settings at 25°C and 1500 rpm. If you use a thermomixer, a 2-mL tube with a round bottom is recommended because it facilitates mixing.
15. Spin the tube, and place it on a magnetic stand.
16. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.
17. Repeat steps 13–16 **two times** for a total of **three Ligation 1 Wash Reagent washes**.
18. Add **200 µL** of **Bead Buffer** and invert several times to resuspend.

NOTE: Perform the remaining washes with Bead Buffer.

19. Spin the tube, and place it on a magnetic stand.
20. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.
21. Remove the tube from the magnetic stand.
22. Repeat steps 18–21 **three times** for a total of **four Bead Buffer washes**. Note that the beads can be stored in **200 µL** of Bead Buffer (final wash) at **4°C**.
23. Store the Quantum Barcoding Beads, or proceed to Step 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.

Step 8: Bead Oligo Blocking

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

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 (1.5-mL compatible)					

Before You Begin

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

Procedure

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

Table 5: Bead Oligo Blocking Master Mix

Reagent	Volume (µL)
Nuclease-free water	138
Oligo Blocking Buffer	20
Oligo Blocking Enhancer	20
Oligo Blocking Additive	8
Oligo Blocking Enzyme	4
Total volume	**190**

2. Place the beads on a magnetic stand and let them separate.
3. Once separated, remove and discard the Bead Buffer on the magnetic stand.
4. Resuspend the Quantum Barcoding Beads in the Bead Oligo Blocking Master Mix (about 190 µL).

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. A thermomixer can also be used with settings at 37°C and 1200 rpm. If you use a thermomixer, a 2-mL tube with a round bottom is recommended because it facilitates mixing.

6. After the incubation is complete, place the 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. Remove the tube from the magnetic stand.
9. Add **200 µL** of Bead Buffer and invert several times to resuspend.
10. Spin the tube and place it on the magnetic stand.
11. Once the solution is clear, carefully remove and discard the supernatant without disturbing the beads.
12. Remove the tube from the magnetic stand.
13. Repeat steps 8–11 **one more time** for a total of **two Bead Buffer washes**. Remove the tube from the magnetic stand and resuspend the beads during each wash.
14. Resuspend the beads in **200 µL** of Bead Buffer.
15. Proceed to Step 9: Second Strand Synthesis.

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.

Step 9: Second Strand Synthesis

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

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 (1.5-mL compatible)					

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 according to Table 6.

Table 6: Second Strand Synthesis Master Mix

Reagent	Volume (µL)
Nuclease-free water	184
Second Strand Buffer	24
Second Strand Primer	8
Second Strand Additive	8
Total volume	**224**

2. Place the beads on a magnetic stand and let them separate.
3. Once separated, remove the buffer from the beads on the magnetic stand.
4. Resuspend the beads in **224 µL** of Second Strand Synthesis Master Mix.
5. Aliquot **28 µL** of Second Strand Synthesis Master Mix with beads into each tube of PCR strips (total of eight tubes).
6. Incubate the tubes in the thermocycler at **95°C** for **3 minutes**.
7. Immediately place the reaction on ice for **3 minutes**.
8. Add **2 µL** of Second Strand Enzyme into each reaction.
9. Shake the strip tubes at **2000 rpm** for **30 seconds**.

10. Incubate the tubes on a rotator/orbital mixer according to Program 8.

    Alternatively, you can use a thermomixer at 1500 rpm according to Program 8.

    Program 8: Second Strand Synthesis

Temperature	Reaction Volume
37°C	30 min

11. Inactivate the reaction in a thermocycler mixer at **95°C** for **3 minutes**.
12. Incubate the tubes **on ice** for **5 minutes**.
13. Proceed directly to Step 10: First PCR Amplification.

Step 10: First PCR Amplification

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

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)					

Procedure

1. Prepare First PCR Amplification Master Mix according to Table 7

Table 7: First PCR Amplification Master Mix

Reagent	Volume (µL)
QB Bead Primers	16
QB Enhancer	185
QB Master Mix	440
Total volume	**641**

2. Add **70 µL** of First PCR Amplification Master Mix into each Second Strand Synthesis reaction.

3. Place the strip tube 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 4–7 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)	7
If uncertain about your RNA content of your sample	6

4. After PCR is complete, take out the PCR tubes and place them on a magnetic stand.
5. Remove **25 µL** of First PCR Amplification product from each PCR reaction and pool into one new tube (total 200 µL).
6. Proceed to Step 11: Post-PCR SPRI Cleanup and QC.

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

Step 11: Post-PCR SPRI Cleanup and QC

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

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

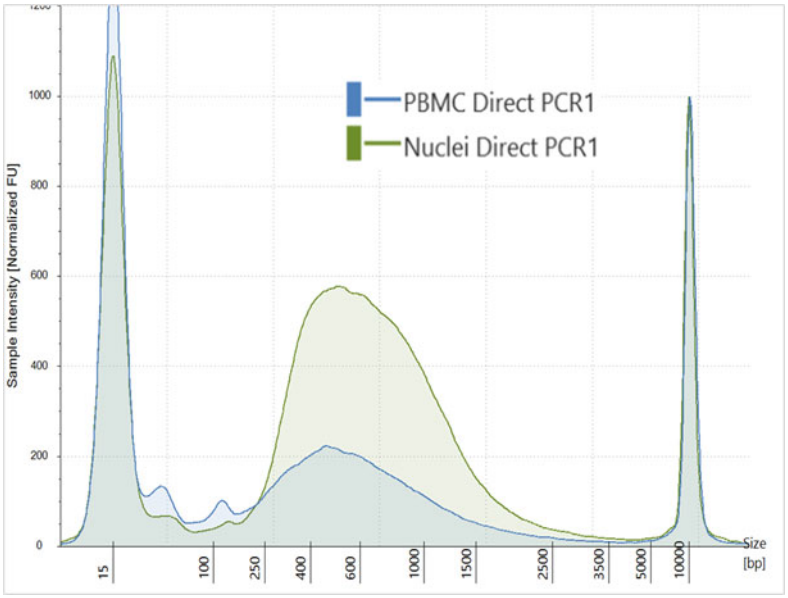
- Prepare 500 µL of fresh 80% ethanol.

Procedure

1. To the 200 µL of pooled First PCR Amplification product, add **8 µL** of SPRI Additive for SPRI cleanup for a final volume of 208 µL.
2. Vortex the SPRIselect beads until they appear homogeneous in color.
3. Add **166 µL** of SPRI beads (0.8X) 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 the 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** of 80% ethanol wash for a total of two washes.
11. Briefly spin the tube 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 tube from the magnetic stand and add **43 µL** of Elution Buffer.

15. Pipette mix to resuspend the beads in solution.
16. Incubate at **room temperature** for **2 minutes**.
17. Place the tube back on the magnetic stand and wait until the solution is clear.
18. Transfer supernatant into a fresh PCR 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 5.
20. Quantify the SPRIselect bead purified product with the Qubit dsDNA HS Assay Kit.
21. Dilute an aliquot of your sample to 1 ng/µL in at least **40 µL** with nuclease free water.
22. Transfer **40 µL** of the 1-ng/µL aliquot (40 ng total) to a fresh PCR tube.
23. Store the sample at **4°C** or proceed directly to Step 12: RNA Index PCR. If using ScalePlex fixed samples, the remaining First Amplification Product will be used in Step 14: ScalePlex Enrichment PCR.

Figure 5: Representative First PCR Amplification Product Traces on Tapestation



NOTE: Samples will be low yield at this point in the protocol.

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

Step 12: RNA Index PCR

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

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 PCR Primer Tube	Pink	–20°C	On ice	✓	✓
Other Vendors	Nuclease-free water					

Procedure

1. Prepare RNA Index PCR Reaction Mix according to the Table 8.

Table 8: RNA Index PCR Reaction Mix

Reagent	Volume (µL)
Nuclease-free water	25
Purified First PCR Amplification Product	40
QS Index PCR Primer Tube	10
QS RNA Index PCR MM	25
Total volume	**100**

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

2. Pipette mix and briefly spin down in a microcentrifuge.
3. Incubate the RNA Index PCR reaction 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 Step 13: Final Library Cleanup.

Step 13: Final Library Cleanup

NOTE: This is two sequential 0.7X SPRI cleanups.

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

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 (96-well compatible)					
	Qubit dsDNA HS Assay Kit					
	D5000 ScreenTape Kit (or equivalent)					

Before you Begin

- Prepare 1 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 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 tube 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 side of the tube 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 tube to collect the residual ethanol at the bottom of the tube 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 pellet appears matte instead of glossy, but not cracked.

13. Remove the tube from the magnetic stand and 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 tube back on the magnet and wait until the solution is clear.
17. Transfer **50 µL** of supernatant into fresh PCR tube.

SPRI Cleanup 2

1. Add **35 µL** of SPRIselect beads (0.7x) to 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 tube 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 tube 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 tube to collect the residual ethanol at the bottom of the tube and place it 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 pellet appears matte instead of glossy, but not cracked.
12. Remove the tube from the magnetic stand and 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 tube back on the magnetic stand and wait until the solution is clear.
16. Carefully transfer **40 µL** of eluted library to a fresh PCR tube.
17. Proceed to Step 16: Final Library QC.

Step 14: ScalePlex Enrichment PCR

NOTE: Perform this step if ScalePlex Fixation was used.

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

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 PCR Primer Tube	Blue	–20°C	On ice	✓	✓
Other Vendors	Nuclease-free water					

Procedure

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

Table 9: ScalePlex PCR Reaction Mix

Reagent	Volume (µL)
Purified First PCR Amplification product	40
Nuclease-free water	25
QS Index PCR MM	25
QS ScalePlex PCR Primer Tube	10
Total volume	**100**

4. Pipette mix and briefly spin down in a microcentrifuge.

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 Step 15: ScalePlex Library Cleanup.

Step 15: ScalePlex Library Cleanup

NOTE: Perform this step if ScalePlex Fixation was used.

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

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 (96-well compatible)					
	Qubit dsDNA HS Assay Kit					
	D5000 ScreenTape Kit (or equivalent)					

Before you Begin

- Prepare 500 µL of fresh 80% ethanol.

Procedure

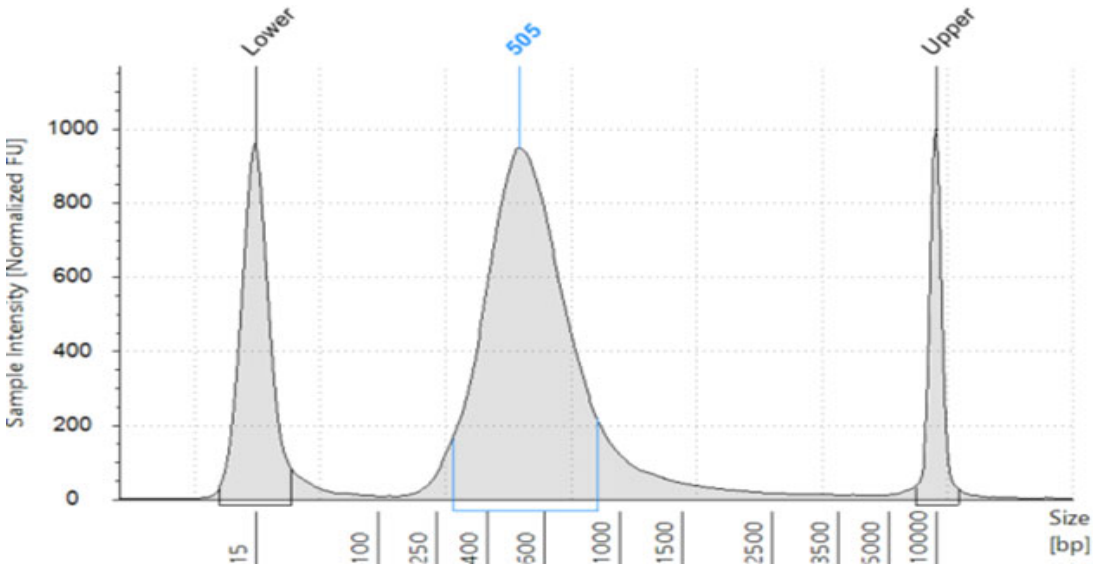
1. Vortex the SPRIselect beads well until they appear homogeneous in color.
2. Add **100 µL** of SPRIselect beads (1.0X) to the ligation product and pipette mix until the solution is homogeneous.
3. Incubate at **room temperature** for **5 minutes**.
4. Briefly spin and place the tube on a magnetic stand 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 side of the tube 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 tube to collect the residual ethanol at the bottom of the tube and place it 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 pellet appears matte instead of glossy but not cracked.
13. Remove the tube from the magnetic stand and 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 tube back on the magnet and wait until the solution is clear.
17. Transfer **50 µL** of supernatant into fresh PCR tube.
18. Proceed to Step 17: ScalePlex Library QC.

Step 16: Final Library QC

1. Quantify the Final Library concentration with the Qubit™ 1X dsDNA HS Assay Kit.
2. Determine the size of the Final Library product using an Agilent Tapestation or equivalent fragment analyzer. Example traces are shown in Figure 6 (Tapestation).

Figure 6: Representative Final Library Traces on Tapestation



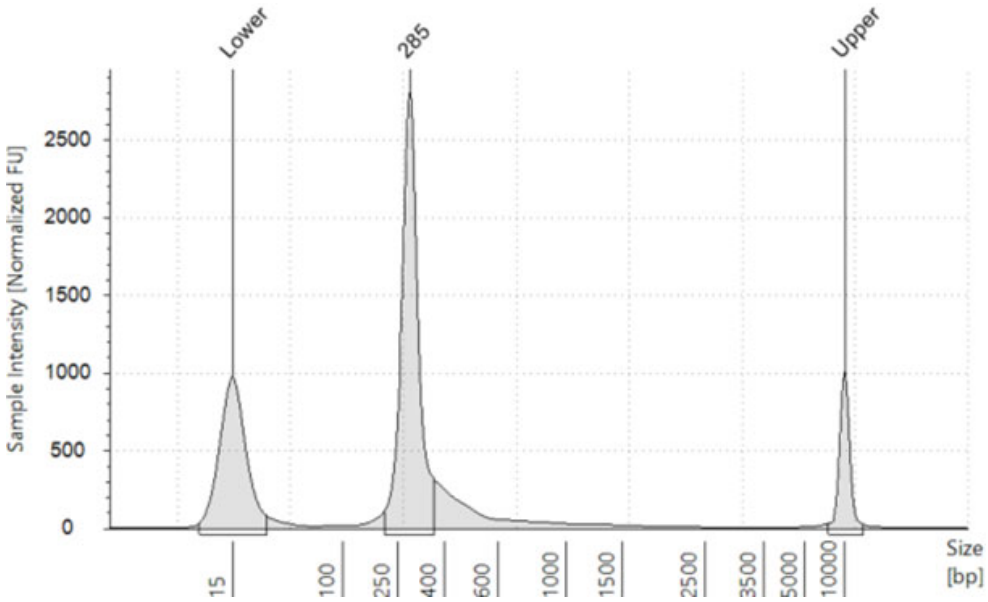
STOP **Safe stopping point.** Purified library can be stored at –20°C for up to 2 months.

Step 17: ScalePlex Library QC

NOTE: Perform this step if ScalePlex Fixation was used.

1. Quantify the Final Library concentration with the Qubit™ 1X dsDNA HS Assay Kit.
2. Determine the size of the Final Library product using an Agilent Tapestation or equivalent fragment analyzer. Example traces are shown in Figure 7 (Tapestation).

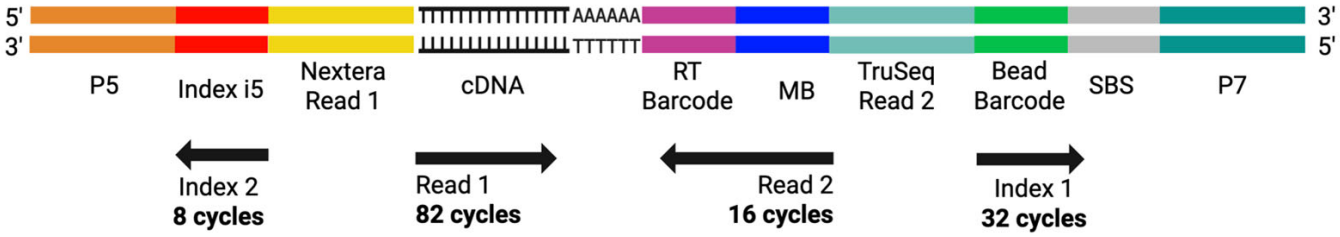
Figure 7: Representative Final Library Traces on Tapestation



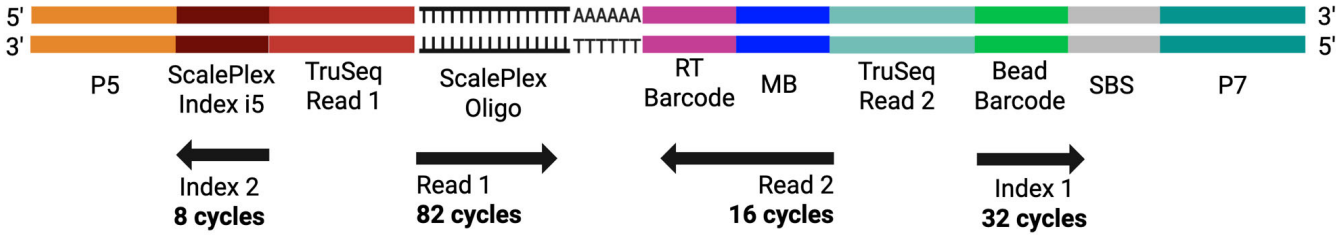
Safe stopping point. Purified library can be stored at –20°C for up to 2 months.

Step 18: Sequencing Parameters

RNA Library - ILMN



ScalePlex Library - ILMN



Sequence the library on an Illumina sequencer using the following parameters. Perform library dilution and clustering according to sequencing manufacturers parameters.

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

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.

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

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

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

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

Document Revision History

Document Revision	Revisions Date	Description of Change
1323029 Rev A	May 2025	Initial release