Tapestri® Single-Cell DNA + RNA Sequencing

IMPORTANT

- *Ensure instrument is using the current firmware.*
- *Vortex all reagents unless directed otherwise.*
- *Thaw -20 °C reagents on ice.*
- *Do not use the Lysis Mix or Cell Buffer provided in the Tapestri Single-Cell DNA Core reagent kit.*
- *Avoid sources of static and pipette slowly and carefully when handling emulsions.*
- *Provide debris-free cell suspension with > 80% viability.*
- *Final cell concentration is ~4,500 cells/µL.*
- *Always use a PCR skirt.*
- *Equilibrate Encapsulation Oil at RT for 30 minutes prior to use.*
- *RNaseZAP all work areas and pipettes; change gloves as needed.*

Prepare Cell Suspension

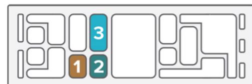
1. Use DPBS to dilute cells to **4,000 - 4,500 cells/µL** in a volume of at least 35 µL.

Encapsulate Cells

1. In a 0.2 mL PCR tube, preheat **7 µL of the RNA Reverse Primer Pool** to **70 °C for 5 minutes**, then store on ice for at least 10 minutes.
2. **Preheat the thermal cycler with the Lysis/RT protocol** (hold at 50 °C).
3. Mount the Base Plate onto the Tapestri Instrument.
4. Place a 0.2 mL emulsion-safe PCR tube into the middle of the slot at the left side of the Base Plate.
5. Place the DNA cartridge onto the base plate.
6. In a new tube, prepare **RNA Mix** as follows:

Reagent	Volume (µL)
RNA Enzyme Buffer (⬤)	12
RNA Reagent 1 (⬤)	3
RNA Reagent 2 (⬤)	5.4
RNA Reagent 3 (⬤)	3
RNA Reagent 4 (◯)	3
Nuclease-free water	23.3
Total Volume	**49.7**

7. Add **6 µL of the preheated RNA Reverse Primer Pool** (from **Step 1**) **to the RNA Mix** (total volume is 55.7 µL).
8. In a 0.2 mL PCR tube, combine **2 µL RNA Lysis Mix** (⬤) with **18 µL nuclease-free water**. Gently mix by pipetting up and down 5 times. *Do not vortex.* Store on ice.
9. Pipette **35 µL** of **Cell Suspension (4,500 cells/µL)** into **reservoir 2**.
10. Pipette **200 µL** of **Encapsulation Oil** into **reservoir 3**.



11. Add **3 µL of RNA Enzyme** (⬤) **to the RNA Mix** (total volume is 58.7 µL). Gently mix by pipetting up and down 5 times. *Do not vortex.*

IMPORTANT *Add the diluted RNA Lysis Mix to the RNA Mix immediately before loading the cartridge.*

12. **Add 1.3 µL of the diluted RNA Lysis Mix** (from **Step 11**) **to the RNA Mix** (total volume is 60 µL). Gently mix by pipetting up and down 5 times.
13. Pipette **55 µL** of **RNA Mix + RNA Lysis Mix** into **reservoir 1**.
14. **Apply DNA Gasket** on top of the cartridge and close the instrument lid.
15. Run the Cell Encapsulation program by pressing **Step 1: Encapsulation** on the Tapestri Instrument touch screen.
16. Once the program completes, press **DONE** and remove the cartridge and collection tube from the Base Plate.
17. Use a **gel loading tip** to carefully **remove excess oil from the bottom layer** of the tube, leaving a total of **100 µL** of emulsion + oil (approximately the middle point of the tube as it narrows to the conical base). *Proceed to the next step.*

Lyse Cells and Reverse Transcribe RNA

1. Immediately place the sample tube into the preheated thermocycler and **resume the Lysis/RT protocol**.

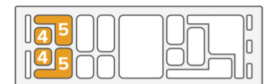
Step	Temperature	Time
1	50 °C	120 min
2	80 °C	10 min
3	4 °C	HOLD

2. Once complete, store the lysed and digested sample at **4 °C** until required in the next step.

Barcode Cells

PRIME THE DNA CARTRIDGE (20-50 MINS BEFORE BARCODING)

1. Place eight emulsion-safe PCR tubes into the eight slots at the bottom of the Base Plate.
2. Mount the DNA Cartridge back onto the Base Plate.
3. Pipette **200 µL** of **Electrode Solution** into each **reservoir 4** and **500 µL** of **Electrode Solution** into each **reservoir 5**.



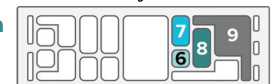
4. **Apply DNA Gasket** on top of the cartridge and close the instrument lid.
5. Run the Priming program by pressing **Step 2: Barcoding** on the Tapestri Instrument touch screen.
6. In the meantime, place the Barcoding Oil and Barcoding Beads (⬤) at **room temperature (protect Barcoding Beads from light)** and prepare **300 µL Barcode Mix:**

Reagent	Volume (µL)
Barcoding Mix	291.9
DNA Forward Primer Pool (◯)	2.5
DNA Reverse Primer Pool (⬤)	3.1
RNA Forward Primer Pool (◯)	2.5
Total Volume	**300**

7. Briefly vortex the Barcode Mix, quick-spin to collect the contents, and store on ice.

LOAD THE DNA CARTRIDGE

8. Quick-spin **Barcoding Beads** (⬤) to collect the contents. Take **67 µL** of the prepared Barcode Mix and add it to the Barcoding Beads tube.
9. Retrieve the lysed and digested sample from the thermocycler.
10. Slowly pipette the entire **cell encapsulation sample (~100 µL)**, including any oil at the bottom, into **reservoir 6**.



11. Vortex **Barcoding Beads** (⬤) at full speed for **1 minute**.
12. Pipette **250 µL** of **Barcoding Beads** (⬤) into **reservoir 7.**
13. Pipette **200 µL** of **Barcode Mix** into **reservoir 8**.
14. Pipette **1.25 mL** of **Barcoding Oil** into **reservoir 9**.
15. **Apply DNA Gasket** on top of the cartridge and close the instrument lid.
16. Run the Barcoding program by pressing **NEXT** on the Tapestri Instrument touch screen.
17. Once the program completes, press **DONE** and remove the Base Plate together with the cartridge and the eight collection tubes.
18. Remove the DNA Cartridge from the Base Plate.
19. Use a **gel loading tip** to carefully **remove excess oil from the bottom layer** of all eight tubes, leaving a total of **100 µL** of emulsion + oil (approximately the middle point of the tube as it narrows to the conical base) per tube.

Targeted PCR Amplification

1. Transfer the sample tubes to the thermocycler, and run the Targeted PCR protocol:

		Amplicon Number	20-100	101-200	201-300	>300	
Step	**Ramp**	**Temp.**	**Time**	**Time**	**Time**	**Time**	**Cycle**
1	4 °C/s	98 °C	6 min	6 min	6 min	6 min	
2	1 °C/s	95 °C	30 sec	30 sec	30 sec	30 sec	10
3		72 °C	10 sec	10 sec	10 sec	10 sec	
4		61 °C	3 min	4.5 min	6 min	9 min	
5		72 °C	20 sec	20 sec	20 sec	20 sec	
6	1 °C/s	95 °C	30 sec	30 sec	30 sec	30 sec	10
7		72 °C	10 sec	10 sec	10 sec	10 sec	
8		48 °C	3 min	4.5 min	6 min	9 min	
9		72 °C	20 sec	20 sec	20 sec	20 sec	
10	4 °C/s	72 °C	2 min	2 min	2 min	2 min	
11		4 °C	HOLD	HOLD	HOLD	HOLD	

IMPORTANT *Make sure to set up two separate 10 cycle programs, each with a ramp rate of 1 °C/s.*

BREAK EMULSIONS

2. Add **10 μL** of **Extraction Agent** (●) to each tube. Briefly vortex, then spin down for **20 seconds**.
3. **Add 45 μL of nuclease-free water** to each tube. Briefly vortex, then spin down for **10 seconds**.
4. Pipette the total volume of the **aqueous top layer from each tube** into one new 1.5 mL DNA LoBind Eppendorf tube. Spin down at **3,000 x g for 5 minutes** at room temperature.
5. Pipette **336 μL of the aqueous top layer** into a new 1.5 mL DNA LoBind Eppendorf tube. *Do not transfer any oil or Barcoding Beads. If needed, add nuclease-free water to achieve a total volume of 336 μL.*
6. Proceed to the next step or store at -20 °C.

Digest PCR Product

1. Add **40 μL** DNA Clean Up Buffer (●) and **24 μL DNA Clean Up Enzyme** (●) to the sample tube for a total of 400 μL.
2. Briefly vortex and spin down the tube.
3. Transfer tube to a thermo mixer or heat block and incubate at **37 °C** for **60 minutes**.
4. Store at room temperature and continue to the next step.

Clean Up PCR Product

AMPURE XP PURIFICATION

NOTE *Equilibrate AMPure XP reagent to room temperature. Prepare 15 mL fresh 80% ethanol using nuclease-free water.*

1. Spin down sample tube for **20 seconds. If a pellet is visible, transfer clear aqueous solution to a new tube**, being careful not to disturb the pellet. Add **nuclease-free water** to achieve a **total volume of 400 μL**. *Discard the remaining tube containing the pellet.*
2. Thoroughly **vortex AMPure XP reagent for 45 seconds** at high speed immediately prior to usage.
3. Add **288 μL (0.72X) of AMPure XP reagent** to the tube. **Vortex for 5 seconds** and quick-spin to collect the contents.
4. Incubate the tube at **room temperature for 5 minutes**.
5. Place the tube onto the magnet and wait **5 minutes** for the beads to separate from the solution.
6. Without removing the tube from the magnet, remove the supernatant and discard.
7. Carefully add **1 mL** of the freshly prepared **80% ethanol**; wait **30 seconds**. With the tube on the magnet, remove the ethanol without disturbing the AMPure XP beads and discard.
8. Repeat **Step 7** once, for a total of two washes.
9. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the beads.
10. Dry AMPure XP beads in the tube on the magnet by incubating at **room temperature for 5 minutes**. *Avoid overdrying the beads.*
11. Remove the tube from the magnet. Add **70 μL** of nuclease-free water into the tube. Vortex and quick-spin to collect the contents.
12. Incubate the tube at **room temperature for 2 minutes**.
13. Place the tube onto the magnet and wait for at least **2 minutes**, or until the solution is clear.
14. Transfer **65 μL of purified PCR product** to a new 0.2 mL PCR tube.

GEL EXTRACTION

NOTE *Equilibrate Qubit dsDNA Quantification reagents to room temperature. Thaw DNA sizing ladder and store on ice.*

1. Follow Qubit protocol using 1-2 μL of the purified PCR product to verify DNA yield. If the **concentration is > 7 ng/μL, add 20 μL of nuclease-free water to the sample tube**.
2. Load the first and last wells of a **2% Agarose E-gel (SYBR™ II Gold DNA Stain) with 20 μL of DNA sizing ladder**.
3. Load **20 μL of the purified PCR product** into three wells if no dilution was performed in **Step 1**, and four wells if dilution was performed. *If running multiple samples, leave an empty well between samples.*
4. Add 20 μL of nuclease-free water to any empty wells.
5. Run the program for 1-2% E-gel for **15 min** until the bands of the ladder between 200 and 500 bp are well separated.
6. **Visualize bands and mark squares** on the back of the gel cassette **between 250-450 bp**. Capture a picture of the gel and export via USB.
7. Weigh a 1.5 mL DNA LoBind Eppendorf tube and record the weight. *If the sample was run in four wells of the gel, two 1.5 mL tubes will be needed; record the weight of each tube.*
8. **Excise gel fragments between 250-450 bp** using a scalpel and transfer them to the **1.5 mL tube**. *If using two 1.5 mL tubes, divide the gel fragments between the two tubes.*
9. Capture a picture of the gel post-excision and export via USB.
10. **Recover DNA using the Zymoclean Gel DNA Recovery Kit** according to the manufacturer's instructions. Elute in **110 μL of DNA Elution buffer**.
11. Transfer **100 μL of the eluted product** to a new 1.5 mL DNA LoBind Eppendorf tube and add **400 μL of nuclease-free water**
12. Add **460 μL (0.92x) of AMPure XP reagent** to the tube. **Vortex for 5 seconds** and quick-spin to collect the contents.
13. Incubate the tube at **room temperature for 5 minutes**.
14. Place the tube onto the magnet and wait **5 minutes** for the beads to separate from the solution.
15. Without removing the tube from the magnet, remove the supernatant and discard.
16. Carefully add **1 mL** of the freshly prepared **80% ethanol**; wait **30 seconds**. With the tube on the magnet, remove the ethanol without disturbing the AMPure XP beads and discard.
17. Repeat **Step 15** once, for a total of two washes.
18. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the beads.
19. Dry AMPure XP beads in the tube on the magnet by incubating at **room temperature for 5 minutes**. *Avoid overdrying the beads.*
20. Remove the tube from the magnet. Add **55 μL** of nuclease-free water into the tube. Vortex and quick-spin to collect the contents.
21. Incubate the tube at **room temperature for 2 minutes**.
22. Place the tube onto the magnet and wait for at least **2 minutes**, or until the solution is clear.
23. Transfer **50 μL of purified PCR product** to a new 0.2 mL PCR tube.
24. Store the purified PCR product on ice and proceed to the next step, or store at -20 °C long term.

Library PCR

1. Set up two different Library PCR reactions, one for the DNA Library and one for the RNA library, as follows:

IMPORTANT *Ensure DNA Library Index Primers (●) are used for DNA and RNA Index Primers (●) are used for RNA.*

	DNA	RNA
Reagent	**Volume [µL]**	
Library Mix (●)	25	25
DNA Library Index Primer (●)	10	-
RNA Library Index Primer (●)	-	10
Gel-extracted PCR product	15	15
Total Volume	**50**	**50**

2. Vortex and quick-spin tube to collect the contents.
3. Transfer sample tubes to separate thermocyclers and run the following Library PCR protocols:

Step	Temperature	Time	Cycle
1	95 °C	3 min	
2	98 °C	20 sec	**10** for DNA **15** for RNA
3	**62 °C** for DNA **65 °C** for RNA	20 sec	
4	72 °C	45 sec	
5	72 °C	2 min	
6	4 °C	HOLD	

Clean Up Library PCR Products

NOTE *Equilibrate AMPure XP reagent to room temperature and thoroughly vortex for 15 seconds at high speed.*

1. Transfer the DNA and RNA PCR products to separate 1.5 mL DNA LoBind Eppendorf tubes. Add **450 µL of nuclease-free water** to each tube. *Total volume is 500 µL in each tube.*
2. Add **345 µL (0.69X) of AMPure XP reagent** to the tubes. Vortex for **5 seconds** and quick-spin to collect the contents.
3. Incubate the tubes at **room temperature for 5 minutes**.
4. Place the tubes onto the magnet and wait **5 minutes** for the beads to separate from the solution.
5. Without removing the tubes from the magnet, remove the supernatant and discard.
6. Carefully add **1 mL** of the freshly prepared **80% ethanol**; wait **30 seconds**. With the tubes on the magnet, remove the ethanol without disturbing the AMPure XP beads and discard.
7. Repeat **Step 6** once, for a total of two washes.
8. Keeping the tubes on the magnet, remove all residual ethanol from the tubes without disturbing the beads.
9. Dry AMPure XP beads in the tubes on the magnet by incubating at **room temperature for 5 minutes**. *Avoid overdrying the beads.*
10. Remove the tubes from the magnet. Add **510 µL** of nuclease-free water into the tubes. Vortex and quick-spin to collect the contents.
11. Incubate the tubes at **room temperature for 2 minutes**.
12. Place the tubes onto the magnet and wait for at least **2 minutes**, or until the solution is clear.
13. Transfer **500 µL of each purified PCR product** to separate 1.5 mL DNA LoBind Eppendorf tubes.
14. Add **345 µL (0.69X) of AMPure XP reagent** to the tubes. Vortex for **5 seconds** and quick-spin to collect the contents.
15. Incubate the tubes at **room temperature for 5 minutes**.
16. Place the tubes onto the magnet and wait **5 minutes** for the beads to separate from the solution.
17. Without removing the tubes from the magnet, remove the supernatant and discard.
18. Carefully add **1 mL** of the freshly prepared **80% ethanol**; wait **30 seconds**. With the tubes on the magnet, remove the ethanol without disturbing the AMPure XP beads and discard.
19. Repeat **Step 18** once, for a total of two washes.
20. Keeping the tubes on the magnet, remove all residual ethanol from the tubes without disturbing the beads.
21. Dry AMPure XP beads in the tubes on the magnet by incubating at **room temperature for 5 minutes**. *Avoid overdrying the beads.*
22. Remove the tubes from the magnet. Add **12 µL** of nuclease-free water into the tube. Vortex and quick-spin to collect the contents.
23. Incubate the tubes at **room temperature for 2 minutes**.
24. Place the tubes onto the magnet and wait for at least **2 minutes**, or until the solution is clear.
25. Transfer **10 µL of each purified PCR product** to separate 0.2 mL PCR tubes or 1.5 mL DNA LoBind Eppendorf tubes. Store at -20 °C.

Quantify and Pool Libraries

1. Quantify the library using Qubit or equivalent. Determine the size distribution of the library on a Bioanalyzer High-Sensitivity chip or equivalent. Normalize and pool libraries using the Tapestri Sample Pooling tool (PN40676). *Contact support@missionbio.com if final libraries are < 1 ng/µL.*

Sequence Tapestri Single-cell DNA and RNA Libraries

IMPORTANT

- *The final DNA library consists of target-specific amplicons ranging from 350 - 550 bp with a peak at ~460 bp.*
- *The final RNA library consists of target-specific amplicons ranging from 350 - 550 bp with a peak at ~460 bp.*
- *Refer to the Tapestri Single-Cell DNA + RNA Sequencing User Guide (MB05-0045) for sequencing recommendations.*

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