Targeted Single-Cell DNA Sequencing using the Tapestri® Platform v3

IMPORTANT

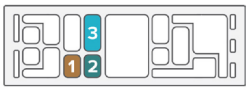
- *Ensure instrument is using the current v3 firmware.*
- *Vortex all reagents unless directed otherwise.*
- *Thaw -20 °C reagents on ice.*
- *Avoid sources of static and pipette slowly and carefully when handling emulsions.*
- *Provide debris-free cell suspension with > 80% viability.*
- *Final cell concentration is 2,800 - 3,200 cells/µL.*
- *Always use a PCR skirt.*
- *Equilibrate Encapsulation Oil for 30 minutes prior to use.*

Prepare Single Cell Suspension

1. Using Cell Buffer dilute cells to ~ 3,000 cells/µL in a total volume of at least 50 µL.

Encapsulate Cells

1. Mount the Base Plate onto the Tapestri Instrument.
2. Place 0.2 mL emulsion-safe PCR tube into the middle of the slot at the left side of the Base Plate.
3. Place the DNA cartridge onto the base plate.
4. In a new tube, prepare **Lysis Mix** by adding **5.1 µL of Reverse Primer Pool** (●) into **65 µL of Lysis Buffer** (●).



5. Pipette **60 µL of Lysis Mix** into **reservoir 1**.
6. Pipette **35 µL of Cell Suspension (3,000 cells/µL)** into **reservoir 2**.
7. Pipette **200 µL** of **Encapsulation Oil** into **reservoir 3**.
8. **Apply DNA Gasket** on top of the cartridge and close the instrument lid.
9. Run the Cell Encapsulation program by pressing Step 1: Encapsulation on the Tapestri Instrument touch screen.
10. Once program completes, press **DONE** and remove the cartridge and collection tube from the Base Plate.
11. 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).

Lysis + Protease Digest

1. Place the sample tube into the thermocycler and run the Lysis/Digest protocol.

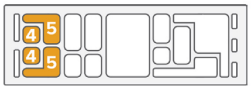
Step	Temperature	Time
1	50 °C	60 min
2	80 °C	10 min
3	4 °C	HOLD

2. Once complete, store the lysed and digested sample at **4 °C** until required in the next step. Proceed within 24 hours.

Barcode Cells

PRIME THE DNA CARTRIDGE

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 instrument lid.
5. Run the Priming program by pressing **Step 2: Barcoding** on the Tapestri Instrument touch screen.
6. In the meantime, retrieve the **Barcoding Oil** and **Barcoding Beads** (●) and **leave at room temperature (protect from light)** and prepare **300 µL Barcode Mix:**

Reagent	Volume (µL)
Barcoding Mix	295
Forward Primer Pool (●)	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 contents. Take **67 µL** of the prepared Barcode Mix and add it to the barcoding bead 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**. Pipette **250 µL** of **Barcoding Beads** (●) into **reservoir 7.**
12. Pipette **200 µL** of **Barcode Mix** into **reservoir 8**.
13. Pipette **1.25 mL** of **Barcoding Oil** into **reservoir 9**.
14. **Apply DNA Gasket** on top of the cartridge and close the instrument lid.
15. Run the Cell Barcoding program by pressing **NEXT** on the Tapestri Instrument touch screen.
16. Once the program completes, press **DONE** and remove the Base Plate together with the cartridge and the eight collection tubes.
17. Remove the DNA Cartridge from the Base Plate.
18. 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 based on the table located on the next page

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 and spin for **20 seconds**.

3. Incubate at room temperature for **3 minutes**.
4. **Add 45 µL of nuclease-free water** to each tube.
5. Briefly vortex and spin for **10 seconds**.
6. Pipette **42 µL of the aqueous top layer** from each tube into one new 1.5 mL DNA LoBind Eppendorf tube (**pool contents from all eight tubes**). Total volume will be 336 µL. **Do not transfer any oil or Barcoding Beads**.
7. Proceed to 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

NOTE *Equilibrate AMPure XP reagent to room temperature. Prepare 5 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**.
1. Add **400 µL of nuclease-free water** to tube (total volume = 800 µL). Quantify volume using the pipette, if necessary, add more water to adjust volume to 800 µl.
2. Thoroughly vortex AMPure XP reagent at high speed immediately prior to usage.
3. Add **576 µL (0.72X) of AMPure XP reagent** to tube. Vortex for **5 seconds** and quick-spin to collect contents.
4. Incubate tube at room temperature for **5 minutes**.
5. Place on magnet and wait **5 minutes** for the beads to separate from solution.
6. Without removing the tube from the magnet, remove the clear liquid from tube and discard.
7. Carefully add **1 mL** of the freshly prepared **80% ethanol**, wait **30 seconds**. Without removing the tube from the magnet, remove the ethanol without disturbing the AMPure XP beads.
8. Repeat **Step 7** once, for a total of two washes.
9. Keeping the tube on the magnet, remove all residual ethanol from tube without disturbing the beads.
10. Dry AMPure bead pellets 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 **110 µL** of nuclease-free water into 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 solutions are clear.
14. Transfer **100 µL of purified PCR product** to a new 0.2 mL PCR tube.
15. Add **76 µL (0.76X) of AMPure XP reagent** to the tube with eluted PCR product (176 µL total). Vortex for **5 seconds** and quick-spin to collect the contents.
16. Incubate tube at room temperature for **5 minutes**.
17. Place on magnet, wait **5 minutes** for the beads to separate from the solution.
18. Without removing the tube from the magnet, remove the supernatant and discard
19. Carefully add **200 µL** of the freshly prepared **80% ethanol**, wait **30 seconds**. Without removing the tube from the magnet, remove the ethanol without disturbing the AMPure XP beads
20. Repeat **Step 19** once, for a total of two washes
21. Keeping the tube on the magnet, remove all residual ethanol from tube without disturbing the beads.
22. Dry AMPure bead pellets in the tube on the magnet by incubating at **room temperature for 5 minutes**. Avoid overdrying the beads.
23. Remove the tube from the magnet. Add **110 µL** of nuclease-free water into tube. Vortex and quick-spin to collect the contents.
24. Incubate the tube at **room temperature for 2 minutes**.
25. Place the tube onto the magnet and wait for at least **2 minutes** or until the solutions are clear.
26. Transfer **100 µL of purified PCR product** to a new 0.2 mL PCR tube.
27. Store the purified PCR product on ice and proceed to the next step, or store at -20 °C long term.

Library PCR

1. In a Pre-PCR area in a new 0.2 mL PCR tube add 15 µL of the Targeted DNA PCR product (**Step 26**, Clean Up PCR Product).
2. Add Library Mix (●) and DNA Library Index Primer (●) as follows:

Reagent	Volume
Library Mix (●)	25
DNA Library Index Primer (●)	10
Targeted DNA PCR product	15
Total Volume	**50**

3. Vortex and quick-spin tube to collect contents.
4. Transfer sample tube to a thermocycler and run the Library PCR protocol:

Step	Temperature	Time	Cycle
1	95 °C	3 min	
2	98 °C	20 sec	10
3	62 °C	20 sec	
4	72 °C	45 sec	
5	72 °C	2 min	
6	4 °C	HOLD	

Clean Up Library PCR Product

NOTE *Equilibrate AMPure XP reagent to room temperature. Prepare 10 mL fresh 80% ethanol using nuclease-free water.*

1. Add **50 µL of nuclease-free water** to the sample tube (DNA Library).
2. Add **69 µL (0.69X) of AMPure XP reagent** to the 100 µL sample tube. Vortex for **10 seconds** and quick-spin to collect the contents.
3. Incubate the tube at **room temperature for 5 minutes**.
4. Place on the magnet, wait **2 minutes** for the beads to separate from the solution.
5. Without removing the tube from the magnet, remove the supernatant and discard.
6. Carefully add **200 µL** of freshly prepared **80% ethanol**, wait **30 seconds**, and remove the ethanol without disturbing the AMPure XP beads.
7. Repeat **Step 6** once for a total of two washes.
8. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the AMPure XP beads.
9. Dry AMPure bead pellets in the tube on the magnet by incubating at room temperature for **2 minutes**. Avoid overdrying the beads.
10. Remove the tube from the magnet and add **110 µL of nuclease-free water**. Vortex and quick-spin to collect the contents.
11. Incubate for **2 minutes**.
12. Place the tubes on the magnet and wait **2 minutes** or until the solution is clear.
13. Transfer **100 µL** of purified PCR product from the tube to a new 0.2 mL PCR tube.
14. Add **72 µL (0.72X) of AMPure XP reagent** to the 100 µL sample tube.
15. Incubate the tube at **room temperature for 5 minutes**.
16. Place on the magnet, wait **2 minutes** for the beads to separate from the solution.

17. Without removing the tube from the magnet, remove the supernatant and discard.
18. Carefully add **200 µL** of freshly prepared **80% ethanol**, wait **30 seconds**, and remove the ethanol without disturbing the AMPure XP beads.
19. Repeat **Step 18** once for a total of two washes.
20. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the AMPure XP beads.
21. Dry AMPure bead pellets in the tube on the magnet by incubating at **room temperature for 2 minutes**. Avoid overdrying the beads.
22. Remove the tube from the magnet and add **12 µL of nuclease-free water**. Vortex and quick-spin to collect the contents.
23. Incubate the tube at **room temperature for 2 minutes**.
24. Place the tube onto the magnet and wait for at least **2 minutes** or until the solution is clear.
25. Transfer **10 µL** of purified PCR product from the tube to a new 0.2 mL PCR tube or 1.5 mL DNA LoBind Eppendorf tube. Store at -20 °C for long-term storage.

Quantify and Pool Library

1. Dilute the sample 10X and run 1 µL of the sample on a High-Sensitivity Bioanalyzer chip or equivalent. Quantify the sample using Qubit or equivalent. Normalize libraries to 5 nM and pool using the Tapestri Sample Pooling tool PN40676.
   Contact support@missionbio.com if final libraries are < 2.0 ng/µL

IMPORTANT *Refer to the Tapestri Single-Cell DNA Sequencing v3 User Guide (MB05-0017) for additional information on how to accurately quantify Tapestri libraries that include large size off-target products.*

Sequence Tapestri Single-cell DNA and Protein Libraries

IMPORTANT

- *The final DNA library consists of target-specific amplicons ranging from 350 - 550 bp with a peak at 460 bp.*
- *The final Protein library consists of target-specific amplicons ranging from 230 - 270 bp with a peak at 250 bp.*
- *Refer to the Tapestri Single-Cell DNA Sequencing v3 User Guide (MB05-0017) for sequencing recommendations.*

Firmware Update

IMPORTANT *Firmware update MUST be completed prior to beginning Tapestri Single-Cell DNA v3*

1. Turn on Tapestri instrument.
2. From home screen, tap  icon to enter the help screen.
3. Tap the middle of the screen three times, three buttons will appear.
4. Insert designated USB into instrument.
5. Press Update Firmware to upload new firmware. This process may take several seconds. Once firmware is uploaded, the instrument reboots, idicating successful update.
6. If “update unsuccessful” pop up appears, please contact support@missionbio.com.