Tapestri® Single-Cell DNA + CpG Methylation Sequencing v3

IMPORTANT

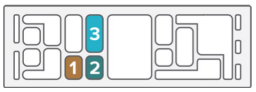
- *Ensure instrument is using the current firmware.*
- *Vortex all reagents unless directed otherwise.*
- *Thaw -20 °C reagents on ice.*
- *Avoid sources of static and pipette slowly and carefully when handling emulsions.*
- *Provide debris-free cell suspension with > 80% viability.*
- *Final cell concentration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

Prepare Single Cell Suspension

1. Use Cell Buffer to dilute cells to **2,800 - 3,200 cells/µL**. Minimum cell concentration is 1,000 cells/µL.

Encapsulate Cells

1. Mount the Base Plate onto the Tapestri Instrument.
2. Place a 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 the 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 and Digest Cells

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

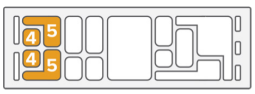
Step	Temperature	Time
1	50 °C	60 min
2	80 °C	10 min
3	4 °C	HOLD

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 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	290
HhaI Enzyme (150,000 U/mL)	5
Forward Primer Pool (●)	5
Total Volume	**300**

IMPORTANT *If running an undigested control sample, replace the 5 µL of HhaI Enzyme with 5 µL of Barcoding Mix.*

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	37 °C	30 min	30 min	30 min	30 min	
2	4 °C/s	98 °C	6 min	6 min	6 min	6 min	
3	1 °C/s	95 °C	30 sec	30 sec	30 sec	30 sec	10
4		72 °C	10 sec	10 sec	10 sec	10 sec	
5		61 °C	3 min	4.5 min	6 min	9 min	
6		72 °C	20 sec	20 sec	20 sec	20 sec	
7	1 °C/s	95 °C	30 sec	30 sec	30 sec	30 sec	10
8		72 °C	10 sec	10 sec	10 sec	10 sec	
9		48 °C	3 min	4.5 min	6 min	9 min	
10		72 °C	20 sec	20 sec	20 sec	20 sec	
11	4 °C/s	72 °C	2 min	2 min	2 min	2 min	
12		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. Incubate at room temperature for **3 minutes**.
4. **Add 45 µL of nuclease-free water** to each tube.
5. Briefly vortex, then spin down 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 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

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**.
2. Add **400 µL of nuclease-free water** to the tube. Quantify the final volume using a pipette, and add more water if necessary to adjust volume to 800 µL.
3. Thoroughly vortex AMPure XP reagent for 45 seconds at high speed immediately prior to usage.
4. Add **576 µL (0.72X) of AMPure XP reagent** to the tube. **Vortex for 5 seconds** and quick-spin to collect the contents.
5. Incubate the tube at **room temperature for 5 minutes**.
6. Place the tube onto the magnet and wait **5 minutes** for the beads to separate from the solution.
7. Without removing the tube from the magnet, remove the supernatant and discard.
8. 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.
9. Repeat **Step 7** once, for a total of two washes.
10. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the beads.
11. Dry AMPure XP beads in the tube on the magnet by incubating at **room temperature for 5 minutes**. *Avoid overdrying the beads.*
12. Remove the tube from the magnet. Add **110 µL** of nuclease-free water into the tube. Vortex and quick-spin to collect the contents.
13. Incubate the tube at **room temperature for 2 minutes**.
14. Place the tube onto the magnet and wait for at least **2 minutes**, or until the solution is clear.
15. Transfer **100 µL of purified PCR product** to a new 0.2 mL PCR tube.
16. 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.
17. Incubate the tube at **room temperature for 5 minutes**.
18. Place the tube onto the magnet and wait **5 minutes** for the beads to separate from the solution.
19. Without removing the tube from the magnet, remove the supernatant and discard.
20. Carefully add **200 µL** 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.
21. Repeat **Step 20** once, for a total of two washes.
22. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the beads.
23. Dry AMPure XP beads in the tube on the magnet by incubating at **room temperature for 5 minutes**. *Avoid overdrying the beads.*
24. Remove the tube from the magnet. Add **110 µL** of nuclease-free water into the tube. Vortex and quick-spin to collect the contents.
25. Incubate the tube at **room temperature for 2 minutes**.
26. Place the tube onto the magnet and wait for at least **2 minutes**, or until the solution is clear.
27. Transfer **100 µL of purified PCR product** to a new 0.2 mL PCR tube.
28. 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 the Library PCR reaction as follows:

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

2. Vortex and quick-spin tube to collect the contents.
3. 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 and thoroughly vortex for 15 seconds at high speed.*

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 tube. Vortex for **5 seconds** and quick-spin to collect the contents.
3. Incubate the tube at **room temperature for 5 minutes**.
4. Place the tube onto the magnet and 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 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.
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 beads.
9. Dry AMPure XP beads 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. Add **110 µL** of nuclease-free water into the tube. Vortex and quick-spin to collect the contents.
11. Incubate the tube at **room temperature for 2 minutes**.
12. Place the tube onto the magnet and wait for at least **2 minutes**, or until the solution is clear.
13. Transfer **100 µL of purified PCR product** to a new 0.2 mL PCR tube.
14. Add **72 µL (0.72X) of AMPure XP reagent** to the tube. Vortex for **5 seconds** and quick-spin to collect the contents.
15. Incubate the tube at **room temperature for 5 minutes**.

16. Place the tube onto the magnet and 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 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.
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 beads.
21. Dry AMPure XP beads 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. Add **15 µL** of nuclease-free water into the tube. 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 **12 µL of purified PCR product** to a new 0.2 mL PCR tube or 1.5 mL DNA LoBind Eppendorf tube. Store at -20 °C.

Quantify and Pool Library

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 library is < 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 significant off-target products*

Sequence Tapestri Single-cell DNA Libraries

IMPORTANT

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