Targeted Single-cell DNA + Protein 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 >90% viability. Starting cell concentration needs to be 25,000 cells/µL.*
- *Always use a PCR skirt.*

Stain Cells With Antibody Panel

IMPORTANT *See full protocol if using custom antibodies*

RECONSTITUTE LYOPHILIZED ANTIBODY PANEL

1. Equilibrate BioLegend lyophilized Heme panel at room temperature for **5 minutes**.
2. Spin down at 10,000 x g for **30 seconds at room temperature**.
3. Resuspend lyophilized panel in **60 µL of Cell Staining Buffer (CSB)**. Vortex for **10 seconds**.
4. Incubate for **5 minutes at room temperature**.
5. Vortex for **10 seconds** and spin down at 10,000 x g for **30 seconds at room temperature**.
6. Transfer entire **60 µL** to a 1.5 mL Protein LoBind Eppendorf tube.
7. Centrifuge at 14,000 x g for **15 minutes at 4°C**. In the meantime, proceed to the next step.

BLOCK CELLS

8. **Using CSB**, dilute cells to **25,000 cells/µL** in a total volume of **40 µL**.
9. In a 15 mL DNA LoBind conical tube, gently add the following reagents:

Reagent	Volume (µL)
Cell Suspension (25,000 cells/µL)	40
Blocking Buffer (●)	5
Human TruStain FcX™	5
Total Volume	**50**

10. Mix by pipetting up and down with a wide-bore P-200 pipette and incubate on ice for **15 minutes**.

STAIN CELLS

11. Using a P-200 pipette, add **50 µL** of the resuspended panel (**Step 7**) to the blocked cell suspension for a total volume of **100 µL**.

IMPORTANT *Avoid touching the bottom or sides of the tube with the pipette tip to avoid pelleted protein aggregates.*

12. Mix by pipetting up and down and incubate on ice for 30 minutes.

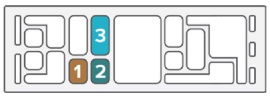
WASH CELLS

IMPORTANT *Equilibrate Encapsulation Oil for 30 minutes prior to use.*

13. Add **14 mL of pre-chilled CSB** to the cell staining solution.
14. Centrifuge at **400 x g** for **10 minutes at 4 °C** in a swinging bucket.
15. Carefully aspirate **13.5 mL** of supernatant using a serological pipette. Leave **500 µL** of supernatant behind. Do not disturb the pellet.
16. Repeat **Steps 13 - 15** for two additional washes (centrifuge for **5 minutes** instead of 10 minutes).
17. Remove **13 mL** of supernatant using a serological pipette.
18. Remove **900 µL** of supernatant using a P-1000 pipette, leaving **100 µL**.
19. Add **900 µL of CSB** to the cell pellet. Gently resuspend by pipetting up and down with a wide-bore P-1000 pipette.
20. **OPTIONAL**: Filter the cells with a **40 µM Flowmi cell strainer** and transfer to a 1.5 mL DNA LoBind Eppendorf tube.
21. Centrifuge at **400 x g** for **5 minutes at 4 °C**.
22. Carefully remove all supernatant. Do not disturb the pellet.
23. Resuspend the pellet in **60 µL of Cell Buffer (Mission Bio)** by pipetting up and down and count cells.
24. If necessary, use Cell Buffer to dilute cells to **2,800 - 3,200 cells/µL**. Cell concentrations as low as 1,000 cells/µL may be acceptable.

Encapsulate Cells

1. Mount the Base Plate onto the Tapestri Instrument.
2. Place a 0.2 mL emulsion-safe PCR tube into the middle 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).

Lyse and Digest Cells

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

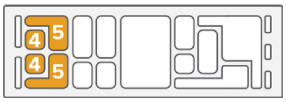
Step	Temperature	Time
1	50 °C	60 min
2	80 °C	10 min
3	4 °C	HOLD

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.



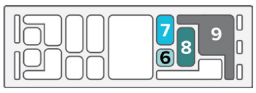
2. Mount the DNA Cartridge back onto the Base Plate.
3. Pipette **200 µL** of **Electrode Solution** into each **reservoir 4** and **500 µL** of **Electrode Solution** into each **reservoir 5**.
4. **Apply DNA Gasket** on top of the cartridge and close the instrument lid.
5. Run the Priming program by pressing **Step 2: Barcoding** on the Tapestri Instrument touch screen.
6. In the meantime, 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	293
Forward Primer Pool (●)	5
Antibody Tag Primer (●)	2
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:

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	11
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	13
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 cycle programs (11 and 13 cycles) 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, Streptavidin Beads, and 2X Wash Buffer 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. Thoroughly vortex AMPure XP reagent for 45 seconds at high speed immediately prior to usage.
3. **Add 280 µL (0.70X) of AMPure XP reagent** to tube.**Vortex for 5** seconds and quick-spin to collect the contents.
4. Incubate tube at room temperature for **5 minutes**.
5. Place on magnet, wait **5 minutes** for the beads to separate from the solution.

IMPORTANT *Do not discard the supernatant from the tube as it contains the protein library.*

6. Without removing the tube from the magnet, transfer the supernatant (**~680 µL**) from tube to a new 1.5 mL DNA LoBind Eppendorf tube and set aside at room temperature for **Protein Library Cleanup**.
7. Proceed with **DNA Library Cleanup I** followed by **Protein Library Cleanup I**.

DNA LIBRARY CLEANUP I

8. 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.
9. Repeat **Step 8** once, for a total of two washes.
10. Keeping the tube on the magnet, remove all residual ethanol from tube without disturbing the beads.
11. Dry AMPure bead pellets 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 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 solutions are 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 tube at room temperature for **5 minutes**.
18. Place on magnet, 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**. Without removing the tube from the magnet, remove the ethanol without disturbing the AMPure XP beads.
21. Repeat **Step 20** once, for a total of two washes.
22. Keeping the tube on the magnet, remove all residual ethanol from tube without disturbing the beads.
23. Dry AMPure bead pellets 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 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 solutions are 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.

PROTEIN LIBRARY CLEANUP I

PREPARE STREPTAVIDIN BEADS

29. Thoroughly vortex Streptavidin beads. Transfer **100 µL of Streptavidin Beads** to a new 1.5 mL DNA LoBind Eppendorf tube.
30. Place on a magnet and wait **2 minutes** for the beads to separate from the solution.
31. Remove the supernatant and discard. Wash the beads with **1 mL of 2X Wash Buffer**. Wait **1 minute** for solution to clear.
32. Repeat **Step 31** once for a total of two washes.
33. Remove the supernatant and resuspend the beads in **690 µL** of 2X Wash Buffer. Set aside until **Step 38** below.

ISOLATE ANTIBODY TAGS

34. Retrieve the tube with the supernatant from the **Clean Up PCR Product** section that contains the protein library. **Split the solution into two 1.5 mL DNA LoBind Eppendorf tubes (340 µL each)**.
35. To each tube, add **2 µL of Biotin Oligo** (●) to the supernatant. Briefly vortex and quick-spin.
36. Incubate at **96 °C for 5 minutes**.
37. Transfer the tubes immediately onto ice and incubate for **5 minutes**.
38. Add and mix **342 µL of Streptavidin Beads** resuspended in 2X Wash Buffer - from **Step 6** above - to each Biotin Oligo-treated sample tube.
39. Incubate for **20 minutes on a shaker at room temperature**.
40. Place on magnet, wait **5 minutes** for the beads to separate from the solution.
41. Prepare **3 mL of 1X Wash Buffer** by mixing 1.5 mL of 2X Wash Buffer with 1.5 mL of nuclease-free water and set aside.
42. Remove the supernatant and wash the Streptavidin Beads with **1 mL of 1X Wash Buffer**.
43. Discard the supernatant, remove tubes from the magnet and wash a second time with **1 mL nuclease-free water**. Mix by pipetting up and down 5 times.
44. Place on the magnet, wait **3 minutes** for beads to separate from the solution.
45. Remove the supernatant. In each tube, resuspend the beads in **25 µL of nuclease-free water**. Transfer and combine into a new 0.2 mL PCR tube (50 µL). The Protein library is bound to the Streptavidin Beads (brown). Proceed to Library PCR or store at 4 °C.

Library PCR

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

IMPORTANT *Ensure DNA Library Index Primers (●) are used for DNA, and Protein Library Index Primers (●) are used for protein.*

	DNA	Protein
Reagent	**Volume [µL]**	
Library Mix (●)	25	25
DNA Library Index Primer (●)	10	-
Protein Library Index Primer (●)	-	10
Targeted DNA PCR product	15	
Resuspended Streptavidin Beads containing Antibody Tags	-	15
Total Volume	**50**	**50**

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

Step	Temperature	Time	Cycle
1	95 °C	3 min	
2	98 °C	20 sec	10 for DNA Library 20 for Protein Library
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. Thoroughly vortex AMPure XP reagent for 15 seconds at high speed.*

DNA LIBRARY CLEANUP II

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.

PROTEIN LIBRARY CLEANUP II

26. Place the tube (Protein Library) on the magnet and wait **2 minutes for Streptavidin Beads** to separate from the solution.
27. Without removing the tube from the magnet, transfer **50 µL** of supernatant to a new 0.2 mL PCR tube.
28. Add **45 µL (0.90X)** of AMPure XP reagent to the 50 µL sample tube. Vortex for 10 seconds and quick-spin to collect the contents.
29. Incubate the tube at room temperature for **5 minutes**.
30. Place on the magnet and wait **2 minutes** for the beads to separate from the solution.
31. Without removing the tube from the magnet, remove the supernatant and discard.
32. Carefully add **200 µL** of freshly prepared **80% ethanol**, wait **30 seconds**, and remove the ethanol without disturbing the AMPure XP beads.
33. Repeat **Step 32** once for a total of two washes.
34. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the AMPure XP beads.
35. Dry AMPure bead pellets in the tube on the magnet by incubating at room temperature for **2 minutes**. Avoid overdrying the beads.
36. Remove the tube from the magnet and add **17 µL of nuclease-free water**. Vortex and quick-spin to collect the contents.
37. Incubate the tube at room temperature for **2 minutes**.
38. Place the tube onto the magnet and wait for **2 minutes** or until the solution is clear.
39. Transfer **15 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 + Protein v3 User Guide (MB05-0018) 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 + Protein v3 User Guide (MB05-0018) for sequencing recommendations.*

Firmware Update

IMPORTANT

- *Firmware update MUST be completed prior to beginning Tapestri Single-Cell DNA+Protein 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.