mission bio

Targeted Single-cell DNA + Protein Sequencing Using the Tapestri® Platform

NOTE
- ***Vortex all reagents unless directed otherwise.***
- ***Thaw -20 °C reagents on ice.***
- ***Avoid sources of static and pipette slowly and carefully when handling emulsions.***

Prepare Single-cell Suspension and Reconstitute Lyophilized Antibody Pool

Reconstitue Lyophilized Pool

1. Equilibrate BioLegened lyophilized Heme panel at room temperature for **5 minutes**.
2. Spin down at 10,000 x g for **30 seconds at RT**.
3. Resuspend lyophilized panel in **60 µL of Cell Staining Buffer (CSB)**. Vortex for **10 seconds**.
4. Incubate for **5 minutes at RT**.
5. Vortex again and spin down at 10,000 x g for **30 seconds at RT**.
6. Transfer entire **60 µL** to a Protein LoBind Eppendorf tube.
7. Centrifuge at 14,000 x g for **15 minutes at 4 °C** and store on ice.

Block Cells

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

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

10. Mix by pipetting up and down and incubate on ice for **15 minutes**.

IMPORTANT ***Provide debris-free cell suspension with >90% viability. Starting cell concentration needs to be 25,000 cells/µL.***

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.***

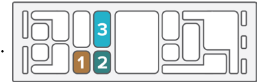
12. Mix by pipetting up and down and incubate on ice for 30 minutes

Wash Cells

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 serelogical pipette. Leave **500 µL** of supernatant behind. Do not disturb the pellet.
16. Repeat **Steps 13 - 15** for two additional washes.
17. Remove **13 mL** of supernatant using a serelogical 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. Filter the cells with a **40 um Flowmi cell strainer** and transfer to a 1.5 mL 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 upand down and count cells.
24. If necessary, use the Cell Buffer to dilute cells to **~3,000 cells/µL**.

Encapsulate Cells

1. Mount the Base Plate onto the Tapestri Instrument.
2. Place a 0.2 mL Axygen MAXYmum Recovery 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 **7.3 µL of Reverse Primer Pool** (●) into **92.7 µL** of **Lysis Buffer** (●).
5. Pipette **90 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 up to 100 µL of the oil layer** at the bottom of the sample, leaving no more than 5 µL of oil.

Lyse and Digest Cells

1. Place a sample tube into the thermal cycler and run the Lysis/Digest protocol.

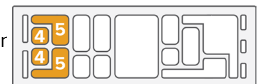
Step	Temperature	Time
1	50 °C	60 min
2	80 °C	10 min
3	4 °C	HOLD

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. Label eight 0.2 mL Axygen MAXYmum Recovery PCR tubes with the sample number and load them into the eight bottom slots of the Base Plate.



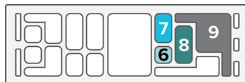
2. Mount the DNA Cartridge again onto the Base Plate.
3. Pipette **200 µL of** Electrode Solution into two **reservoirs 4** each and **500 µL of** Electrode Solution into the two **reservoirs 5** each.
4. **Apply DNA Gasket** on top of the cartridge and close the instrument lid.
5. Run the Priming program by pressing **Step 2: Barcoding** on the Tapestri Instrument touch screen.
6. Once the program completes, press **DONE** and proceed to **Step 9**.
7. In the meantime, retrieve **Barcoding Beads** (●) and **leave at room temperature (protect from light)** and prepare **300 µL Barcode Mix**:

Reagent	Volume (µL)
Barcoding MM V2	293
Forward Primer Pool (○)	5
Antibody Tag Primer (●)	2
Total Volume	**300**

8. Briefly **vortex the Barcode Mix**, centrifuge to collect the contents, and **store on ice**.

Load the DNA Cartridge

9. Retrieve the lysed and digested sample from the thermal cycler.
10. Slowly pipette the entire cell encapsulation sample (~80 µL), including any oil at the bottom, into **reservoir 6**.
11. Vortex **Barcoding Beads V2** (●) for 1 minute at high speed and carefully pipette **200 µL** of **Barcoding Beads V2** (●) into **reservoir 7**.



12. Pipette **250 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 slowly **remove up to 120 µL of oil** from the bottom layer of each sample tube.

UV Treatment (not applicable to MBT-2020 or higher)

IMPORTANT ***Make sure to use the Analytik Jena Blak-Ray XX-15L UV light source. Tapestri Instruments with serial numbers MBT-2020 and higher are equipped with a UV lamp that is turned on at the end of the Barcoding program (program runs 45 minutes).***

1. Place entire Base Plate with tubes (closed) containing emulsions on ice.
2. Place a UV light on top of the ice bucket and expose the samples to non-irradiating **UV light for 8 minutes**.

Targeted PCR Amplification

1. After UV exposure, remove the Base Plate from the ice, transfer the sample tubes to the thermal cycler, and run the Targeted PCR protocol:

			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. 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 down for **10 seconds** in a mini centrifuge to separate the aqueous and oil layers.
6. Pipette **42 µL of the aqueous top layer** in each tube into two new 1.5 mL low-bind Eppendorf tubes. **Pool** contents from **tubes 1 - 4** and contents from **tubes 5 - 8** into two new tubes for a **total of 168 µL each**. Do not transfer any oil.

Digest PCR Product

1. For each of the two pooled samples, prepare a **200 µL digestion mix** by adding **20 µL** DNA Clean up Buffer (●) & **12 µL** Clean up Enzyme (●).
2. Mix by pipetting up and down and quick-spin the tubes.
3. **Split** each sample tube into two new tubes, each containing 100 µL.
4. Transfer all four tubes to a thermal cycler, and run the following protocol:

Step	Temperature	Time
1	37 °C	60 min
2	4 °C	HOLD

Clean Up PCR Product

NOTE ***Equilibrate Ampure XP reagent, Streptavidin Beads, and Wash Buffer to room temperature. Prepare 5 mL fresh 80% ethanol using nuclease-free water.***

1. Thoroughly vortex Ampure XP reagent for **45 seconds** at high speed immediately prior to usage.
2. **Recombine contents** into two new 1.5 mL Eppendorf tubes.
3. **Add 140 µL (0.7 X) of Ampure XP reagent** to each of the two tubes. **Vortex for 10 seconds** and quick-spin to collect the contents.
4. Incubate tubes 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 tubes as it contains the protein library.***

6. Without removing the tubes from the magnet, transfer the supernatant (**~340 µL**) from each tube to two new 1.5 mL low-bind Eppendorf tubes 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. To each tube on the magnet, carefully add **800 µL** of the freshly prepared **80% ethanol**, wait **30 seconds**, and remove **800 µL** of ethanol without disturbing the Ampure beads.
9. Repeat **Step 8** once, for a total of two wash cycles.
10. Keeping the tubes on the magnet, remove all residual ethanol from each tube without disturbing the beads.
11. Dry Ampure bead pellets in the tubes on the magnet by incubating at **room temperature for 5 minutes**. Avoid overdrying the beads.
12. Remove the tubes from the magnet. Add **60 µL** of nuclease-free water into each tube. Vortex and quick-spin to collect the contents.
13. Incubate the tubes at **room temperature for 2 minutes**.
14. Place the tubes onto the magnet and wait for at least **2 minutes** or until the solutions are clear.
15. Transfer and combine **50 µL of purified PCR product** from each tube to a single new 0.2 mL PCR tube. Avoid overdrying the beads.
16. Store the tube with **100 µL purified PCR product** at **room temperature** until further usage.
17. Quantify **1 µL of purified PCR product** from each sample, using the High Sensitivity Qubit Kit (or equivalent assay) according to the manufacturer's instructions.

NOTE ***The DNA quantity in each sample tube may vary between 0.2 - 4.0 ng/µL. If yields are outside this range, contact support@missionbio.com for additional support.***

18. The PCR product sample can be stored at **-20 °C long term**.

Protein Library Cleanup I

Preparing Streptavidin Beads

1. Transfer **100 µL of Streptavidin Beads** to a new 1.5 mL low-bind Eppendorf tube.
2. Place on a magnet and wait **2 minutes** for the beads to separate from the solution.
3. Remove the supernatant and discard. Wash the beads with **1 mL of 2X Wash Buffer**.
4. Repeat **Step 3** for a second time.
5. Remove the supernatant and resuspend the beads in **690 µL of 2X Wash Buffer** and set aside until later usage.

Isolate Antibody Tags

6. Retrieve the two tubes with the supernatant from **Step 6** of the Clean Up PCR Product section.
7. To each tube, add **2 µL of Biotin Oligo** (●) to the supernatant.
8. Incubate at **96 °C for 5 minutes**.
9. Transfer the tubes immediately onto ice and incubate for **5 minutes**.
10. Add and mix **342 µL of Streptavidin Beads** resuspended in 2X Wash Buffer - from **Step 6** above - to each Biotin Oligo-treated sample tube.
11. Incubate for **20 minutes on a shaker at room temperature**.
12. Place on magnet, wait **5 minutes** for the beads to separate from the solution.
13. 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.
14. Remove the supernatant and wash the Streptavidin Beads with **1 mL of 1X Wash Buffer**.
15. Discard the supernatant, remove tubes from the magnet & wash a second time with **1 mL nuclease-free water**.
16. Place on the magnet, wait **3 minutes** for beads to separate from the solution.
17. Remove the supernatant. In each tube, resuspend the beads in **25 µL of nuclease-free water**. Transfer & combine into a new 0.2 mL PCR tube (50 µL). The Protein library is bound to the Streptavidin Beads (brown).

Library PCR

1. In two 0.2 mL PCR tubes, add **15 µL** of the Targeted DNA PCR product (**Step 16**, DNA Library Cleanup I) and **15 µL** of the resuspended Streptavidin Beads containing Antibody Tags (**Step 17**, Protein Library Cleanup I), respectively.
2. Set up two different Library PCR reactions, one for the DNA Library and one for the Protein Library as follows:

IMPORTANT ***Ensure DNA V2 index primers (●) are used for DNA, and Protein Library Indicies (●) are used for protein.***

	DNA	Protein
Reagent	**Volume [µL]**	
Library MM V2	25	25
V2 Index Primer (●)	10	-
Protein Library Indices 1-8 (●)	-	10
Targeted DNA PCR product	15	
Resuspended Streptavidin Beads containing Antibody Tags	-	15
Total Volume	**50**	**50**

3. Vortex and quick-spin tubes to collect the contents.
4. Transfer the sample tubes to a thermal cycler and run the Library PCR protocol (see the top of the second column of this page).

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. Prepare 5 mL fresh 80% ethanol using nuclease-free water.***

DNA Library Cleanup II

1. Add **50 µL of nuclease-free water** to the sample tube (DNA Library).
2. Add **69 µL 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 beads.
7. Repeat **Step 6** once for a total of two wash cycles.
8. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the Ampure 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 **100 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 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 beads.
19. Repeat **Step 18** once for a total of two wash cycles.
20. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the Ampure 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 **15 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 **2 minutes** or until the solution is clear.
25. Transfer **12 µL** of purified PCR product from the tube to a new 0.2 mL PCR tube or 1.5 mL low-bind Eppendorf tube. Avoid overdrying the beads.

Protein Library Cleanup II

1. Place the tube on the magnet and wait **2 minutes for Streptavidin Beads** to separate from the solution.
2. Without removing the tube from the magnet, transfer **50 µL** of supernatant to a new 0.2 mL PCR tube.
3. Add **45 µL (0.9 X)** of Ampure XP reagent to the 50 µL sample tube. Vortex for **10 seconds** and quick-spin to collect the contents.
4. Incubate the tube at **room temperature for 5 minutes**.
5. Place on the magnet and wait **2 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 **200 µL** of freshly prepared **80% ethanol**, wait **30 seconds**, and remove the ethanol without disturbing the Ampure beads.
8. Repeat **Step 7** once for a total of two wash cycles.
9. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the Ampure beads.
10. Dry Ampure bead pellets in the tube on the magnet by incubating at room temperature for **2 minutes**. Avoid overdrying the beads.
11. Remove the tube from the magnet and add **17 µL of nuclease-free water**. Vortex and quick-spin to collect the contents.
12. Incubate the tube at room temperature for **2 minutes**.
13. Place the tube onto the the magnet and wait for **2 minutes** or until the solution is clear.
14. Transfer **15 µL** of purified PCR product from the tube to a new 0.2 mL PCR tube or 1.5 mL low-bind Eppendorf tube. Avoid overdrying the beads.

Quantify and Pool Library

1. Quantify both libraries using an Agilent Bioanalyzer. **Dilute the sample 10X** and run **1 µL** of the sample on a High-Sensitivity chip.
   Use the **Tapestri Sample Quantification Tool (PN 40678)** to pool a total of **5 nM library**. Relative percentage of DNA Libary and Protein library is sample-dependent. Please contact support@missionbio.com.
2. Verify the concentration of the pooled library using a Qubit Fluorometer or equivalent instrument.

IMPORTANT ***Refer to the Tapestri Single-cell DNA + Protein User Guide (PN 3360) 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 User Guide (PN 3360) for additional information.

- Illumina MiSeq, HiSeq 2500, HiSeq 3000/4000, NextSeq 550/1000/2000, and NovaSeq 5000/6000 are supported.
- Paired-end Illumina sequencing chemistry is required (2x150 bp).
- Please refer to the following Illumina User Guides.

1. Sequence the library following manufacturer's instructions.

For technical support visit www.missionbio.com/support or email support@missionbio.com.

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