# Tapestri® Single-Cell DNA + Protein 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 >90% viability. Starting cell concentration needs to be 25,000 cells/µL.*
- *Always use a PCR skirt.*
- *Equilibrate Encapsulation Oil at RT for 30 minutes prior to use.*

## Stain Cells With Antibody Panel

**IMPORTANT** *See full protocol if using custom antibodies.*

### RECONSTITUTE LYOPHILIZED ANTIBODY PANEL

1. Equilibrate lyophilized TotalSeq™-D AOC cocktail (BioLegend) 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, 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 200 µL wide bore tip 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 with a 200 µL wide bore tip 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 remove **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** twice for two additional washes, centrifuging 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 1000 µL wide bore tip.
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 **50 µL of Cell Buffer (Mission Bio)** by pipetting up and down.
24. Count cells. If necessary, 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 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).

## Lyse and Digest Cells

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

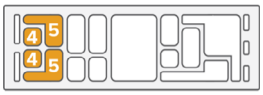
| Step | Temperature | Time |
|---|---|---|
| 1 | 50 °C | 60 min |
| 2 | 80 °C | 10 min |
| 3 | 4 °C | HOLD |

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.



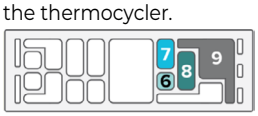

2. Mount the DNA Cartridge back onto the Base Plate.
3. Pipette **200 µL** of **Electrode Solution** into each **reservoir 4** and **500 µL** of **Electrode Solution** into each **reservoir 5**.
4. **Apply DNA Gasket** on top of the cartridge and close the instrument lid.
5. Run the Priming program by pressing **Step 2: Barcoding** on the Tapestri Instrument touch screen.
6. In the meantime, 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 | 288 |
| 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 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 | 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, 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, 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 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.

**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**) to a new 1.5 mL DNA LoBind Eppendorf tube and set aside at room temperature for **Protein Library Cleanup I**.
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**. With the tube on the magnet, remove the ethanol without disturbing the AMPure XP beads and discard.
9. Repeat **Step 8** 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.

### PROTEIN LIBRARY CLEANUP I
*PREPARE STREPTAVIDIN BEADS*

29. Thoroughly vortex Streptavidin beads at high speed immediately prior to usage.
30. Transfer **100 µL of Streptavidin Beads** to a new 1.5 mL DNA LoBind Eppendorf tube.
31. Place the tube onto the magnet and wait **2 minutes** for the beads to separate from the solution.
32. Without removing the tube from the magnet, remove the supernatant and discard.
33. Carefully add **1 mL** of **2X Wash Buffer**; wait **1 minute**. With the tube on the magnet, remove the 2X Wash Buffer without disturbing the Streptavidin Beads and discard.
34. Repeat **Step 33** once for a total of two washes.
35. esuspend the beads in **690 µL of 2X Wash Buffer** and set aside until **Step 40** below.

*ISOLATE ANTIBODY TAGS*

36. Retrieve the tube that contains the protein library (from **Step 6** above). Split the solution into **two 1.5 mL DNA LoBind Eppendorf tubes** (340 µL each).
37. To each tube, add **2 µL of Biotin Oligo** (●). Vortex and quick-spin.
38. Incubate at **96 °C for 5 minutes**.
39. Transfer the tubes immediately onto **ice** and incubate for **5 minutes**.
40. To each tube, add **342 µL of Streptavidin Beads** resuspended in 2X Wash Buffer (from **Step 35** above).
41. Incubate for **20 minutes on a shaker at room temperature**.
42. Quick-spin to collect the contents.
43. Place the tubes onto the magnet and wait **5 minutes** for the beads to separate from the solution.
44. 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.
45. Without removing the tubes from the magnet, remove the supernatant and discard.
46. Carefully add **1 mL** of the freshly prepared **1X Wash Buffer**; wait **1 minute**. With the tube on the magnet, remove the 1X Wash Buffer without disturbing the Streptavidin Beads and discard.
47. Remove the tubes from the magnet and wash a second time with **1 mL nuclease-free water** by pipetting up and down 5 times.
48. Place the tubes onto the magnet and wait **3 minutes** for the beads to separate from the solution.
49. Without removing the tubes from the magnet, remove the supernatant and discard.
50. 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 total). *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 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 Library<br>**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 and thoroughly vortex 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 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.

### PROTEIN LIBRARY CLEANUP II

**26.** Place the sample tube (Protein Library) onto the magnet and wait **2 minutes** for the **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 supernatant. **Vortex for 5 seconds** and quick-spin to collect the contents.

**29.** Incubate the tube at **room temperature for 5 minutes.**

**30.** Place the tube onto the magnet and wait **5 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 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.

**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 beads.

**35.** Dry AMPure XP beads in the tube on the magnet by incubating at **room temperature for 5 minutes**. *Avoid overdrying the beads.*

**36.** Remove the tube from the magnet. Add **17 µL** of nuclease-free water into the tube. 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 at least **2 minutes**, or until the solution is clear.

**39.** Transfer **15 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 Libraries

**1.** Quantify the libraries using Qubit or equivalent. Determine the size distribution of the libraries 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 DNA library is < 2.0 ng/µL or final Protein library is < 1.0 ng/µL.*

***IMPORTANT*** *Refer to the Tapestri Single-Cell DNA + Protein Sequencing v3 User Guide (MB05-0018) for additional information on how to accurately quantify Tapestri libraries that include significant 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 Sequencing v3 User Guide (MB05-0018) for sequencing recommendations.*