Targeted Single-Cell DNA Sequencing using the Tapestri® Platform V2

For more information, consult the Tapestri Single-Cell User Guide (PN 3354)

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

Prepare Single Cell Suspension

IMPORTANT ***Provide debris-free cell suspension with > 80% viability. Final cell concentration is 3,000 - 4,000 cells/µl.***

1. Using **Cell Buffer** dilute cells to ~ 3,500 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 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 (3,500 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 instrument lid.
9. Run the Cell Encapsulation program by pressing **Step 1: Encapsulation** on the Tapestri instrument touch screen.
10. Once program is completed, press **DONE** and remove cartridge and collection tube from 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.

Lysis + Protease Digest

1. Place sample tube into 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.

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 **reservoirs 4** and **500 µl of Electrode Solution** into **reservoirs 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. Once program is completed, press **DONE** and proceed to step 9.
7. In the meantime retrieve **Barcoding Beads V2** (●) and **leave at room temperature (protect from light)** and prepare **300 µl Barcode Mix** as shown in the table on the right top side of this page.
8. Briefly **vortex the Barcode Mix** and centrifuge to collect the contents and **store on ice**. *Continued on second column of this page.*

Reagent	Volume (µl)
Barcoding MM V2	295
Forward Primer Pool (◯)	5
Total Volume	**300**

Load the DNA Cartridge

9. Retrieve lysed and digested sample from thermal cycler.
10. 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 instrument lid.
15. Run the Cell Barcoding program by pressing **NEXT** on the Tapestri Instrument touch screen.
16. Once the program is completed, 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 up to 120 µl of oil** from the bottom layer of each sample tube.

NOTE ***Volumes may vary. The final volume of oil must be 5-35 µl per tube & the total volume in each tube must not exceed 100 µl.***

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 45min).***

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:

			Custom	AML	CLL/THP	Myeloid	
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. 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. **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** (○) and **12 ul 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, **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 to room temperature. Prepare 5 mL fresh 80% ethanol using nuclease-free water.***

1. **Recombine contents** into two new 1.5 mL Eppendorf tubes.
2. **Add 200 µl of nuclease-free water** to each tube (total volume = 400 µl).
3. Thoroughly vortex Ampure XP reagent at high speed immediately prior to usage.
4. **Add 288 µl of Ampure XP reagent** to each of the two tubes. **Vortex for 5 seconds** and quick-spin to collect contents.
5. Incubate tubes at room temperature for **5 minutes**.
6. Place on magnet and wait 5 min for the beads to separate from solution.
7. Without removing the tubes from the magnet, remove the clear liquid from each tube and discard.
8. To each tube 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 beads.**
12. Remove the tubes from the magnet. **Add 65 µl of nuclease-free water** into each tube. Vortex and quick-spin to collect the contents.
13. Incubate tubes at room temperature for **2 minutes**.
14. Place the tubes onto the magnet and wait for at least **2 minutes** or until 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 transfer of beads.**
16. Store the tube with 100 µl purified PCR product on ice.
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 ng/µl to 4.0 ng/µl. If yields are outside this range, contact support@missionbio.com for additional support.***

18. Store purified PCR product sample at -20 °C until proceeding to the next step.

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 18**, **Clean Up PCR Product**).
2. Add Library MM V2 and V2 Index Primer (●) as follows:

Reagent	Volume
Library MM V2	25
V2 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 thermal cycler 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 5 mL fresh 80% ethanol using nuclease-free water.***

1. **Add 50 µl of nuclease-free water** to the tube (total volume = 100 µl).
2. **Add 69 µl of Ampure XP reagent** to the 100 µl sample tube. **Vortex for 5 seconds** and quick-spin to collect contents.
3. **Follow Steps 5 - 18 of Section *Clean Up PCR Product*** with the following modifications: **Wash with 200 µl ethanol**, **elute DNA in 12 µl of nuclease-free water** and **transfer 1 x 10 µl of purified product** to a new 0.2 mL PCR tube.

Quantify and Pool Library

1. Quantify the library using a Agilent Bioanalyzer. DNA 1000 chips may be used with 1 µl of undiluted samples or DNA HS chips may be used with 10x diluted samples.
2. Use the **Tapestri Sample Quantification Tool (PN 40676)** to pool 5 nM of each of the sample libraries.
3. Verify the concentration of the pooled library using a Qubit Fluorometer or equivalent instrument.

IMPORTANT ***Refer to the Tapestri Single-Cell DNA Sequencing User Guide (PN 3354) for additional information on how to accurately quantify Tapestri libraries that include large-size off-target products.***

Sequence Tapestri Single-Cell DNA Library

IMPORTANT ***The final library consists of target-specific amplicons ranging from 350 - 550 bp.***

- Illumina MiSeq, HiSeq 2500, HiSeq 4000, NextSeq 550, and NovaSeq 6000 are currently supported.
- Paired-end Illumina sequencing chemistry is required (2x150 bp).
- Each sample is split into two dual-indexed libraries.
- When multiplexing more than 8 samples use Illumina's Nextera dual indices.
- 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.