Targeted Single-Cell DNA Sequencing using the Tapestri System

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.***

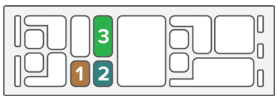
Prepare Single Cell Suspension

IMPORTANT ***Provide debris-free cell suspension with > 80% viability. Final cell concentration is 2,000 - 4,000 cells/µl.***

1. Using ***Cell Buffer*** dilute cells to ~ 3,000 cells/µl in a total volume of at least 100 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. Pipette **100 µl of** Lysis Buffer (●) into reservoir 1.
5. Pipette **100 µl of** Cell Suspension **(3,000 cells/µl)** into reservoir 2.
6. Pipette **200 µl of** Encapsulation Oil into reservoir 3.
7. **Apply DNA Gasket** on top of the cartridge and close instrument lid.
8. Run the Cell Encapsulation program by pressing **Step 1: Encapsulation** on the Tapestri instrument touch screen.
9. Once program is completed, press **DONE** and remove cartridge and collection tube from base plate.
10. 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

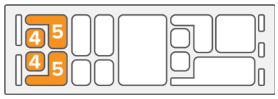
2. Once complete, store the lysed and digested sample at 4 °C until required in the next step.

Barcode Cells

IMPORTANT ***Protect Barcoding Beads from light.***

Prime the DNA Cartridge

1. Label eight 0.2 mL Axygen MAXYmum Recovery PCR tubes with numbers 1 to 8 and load them into the eight bottom slots of the Base Plate.

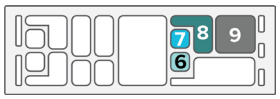


2. Mount the DNA Cartridge again onto the Base Plate.
3. Pipette **200 µl of** Electrode Solution into reservoirs 4 and **500 µl of** Electrode Solution into reservoirs 5.
4. **Apply DNA Gasket** on top of the cartridge and close instrument lid.
5. Run the Priming program by pressing **Step 2: Barcoding** on the Tapestri Instrument touch screen.
6. Once program is completed, press **DONE** and proceed to step 9.
7. In the meantime prepare 300 µl Barcoding Master Mix as shown in the table on the right top side of this page.
8. Briefly vortex the Barcoding Master Mix and centrifuge to collect the contents and store on ice. *Continued on second column of this page.*

	AML	Myeloid
Reagent	**Volume (µl)**	**Volume (µl)**
Barcoding Mix	240	235
Barcoding Mix Additive	60	50
Rv Primer Pool Custom	-	15
Total Volume	**300**	**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. Retrieve Barcoding Beads and vortex tube for 10 seconds at high speed.



12. Carefully pipette **200 µl of** Barcoding Beads into reservoir 7.
13. Pipette **250 µl of** Barcoding Master 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 instrument lid.
16. Run the Cell Barcoding program by pressing **NEXT** on the Tapestri Instrument touch screen.
17. Once the program is completed, 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 up to 90 µl of oil** from the bottom layer of each sample tube.

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

UV Treatment

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**.

IMPORTANT ***Make sure to use the Analytik Jena Blak-Ray XX-15L UV light source.***

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:

		AML	Myeloid	
Step	**Temperature**	**Time**	**Time**	**Cycle**
1	95 °C	10 min	10 min	
2	95 °C	30 sec	30 sec	20
3	72 °C	10 sec	10 sec	
4	61 °C	4 min	12 min	
5	72 °C	30 sec	30 sec	
6	72 °C	2 min	2 min	
7	4 °C	HOLD	HOLD	

Break Emulsions

2. Add **5 µl of Extraction Agent** (●) to each tube. Vortex and quick spin.
3. Incubate at room temperature for **3 minutes**.
4. **Add 100 µl of nuclease-free water** to each sample.
5. Briefly vortex and spin down for 10 seconds in a mini centrifuge to separate the aqueous and oil layers.
6. Pipette 100 µl of the aqueous top layer in each tube into a new 0.2 mL PCR tube. **Do not transfer any oil**.

Clean Up PCR Product

NOTE ***Equilibrate Ampure XP reagent to room temperature. Prepare 5 mL fresh 80% ethanol using nuclease-free water.***

1. For each 100 µl sample, **add 75 µl of Ampure XP reagent**. Vortex for 10 seconds and quick-spin to collect contents.
2. Incubate tubes at room temperature for **5 minues**.
3. Place on magnet and allow 2 minutes for the beads to separate from solution.
4. Without removing the tubes from the magnet, remove the clear liquid from each tube and discard.
5. To each tube carefully **add 200 µl of the freshly prepared 80% ethanol**, wait 30 seconds, and **remove 200 µl of ethanol** without disturbing the SPRI beads.
6. **Repeat step 5 once**, for a total of two wash cycles.
7. Keeping the tubes on the magnet, remove all residual ethanol from each tube without disturbing the beads.
8. Dry SPRI bead pellets in the tubes on the magnet by incubating at room temperature for **2 minutes**. **Avoid overdyring beads.**
9. Remove the tubes from the magnet. Add 20 µl of nuclease-free water into each tube. Thoroughly resuspend the beads by pipetting up and down several times until no bead clumps are visible.
10. Incubate tubes at room temperature for **2 minutes**.
11. Place the tubes onto the magnet and wait for **2 minutes** or until solutions are clear.
12. **Transfer 15 µl of purified PCR product** from each tube to a fresh 0.2 mL PCR tube. **Avoid transfer of beads.**
13. Store the extracted purified PCR product tubes on ice.
14. Quantify 2 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.***

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

Library PCR

1. Normalize all eight sample tubes to 0.2 ng/µl in 10 µl each using the **Tapestri Sample Quantification Tool (PN 40676)**.
2. In a Pre-PCR area prepare the Library PCR Master Mix as follows:

	Reagent	Volume (µl per 1 reaction	Volume (µl Pre-Mix)
Pre-Mix	Library Mix	25.0	225.0
	Library P5 Primer (🔴)	5.0	45.0
	Nuclease-free water	5.0	45.0
	Library P7 Index Primers 1-8 (🟡)	5.0 per tube	5.0 per tube
	Purified PCR product (0.2 ng/µl)	10.0 per tube	10.0 per tube
	Total Volume	**50.0**	**315.0 (Pre-Mix)**

3. **Aliquot 35.0 µl of Pre-Mix** to each of the eight 0.2 mL PCR tubes containing 10 µl of 0.2 ng/µl purified PCR product.
4. Add **5 µl of one P7 Index Primer** (🟡) to one sample tube separately. Label the tube with the ID number of the primer added.
5. Vortex and quick-spin all eight 50 µl PCR samples to collect contents.
6. Transfer samples to a thermal cycler and run the Library PCR protocol:

Library PCR (continued)

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. For each 50 µl sample, **add 31.5 µl of Ampure XP reagent**. Vortex for 10 seconds and quick-spin to collect contents.
2. Follow Steps 2 - 13 of Section ***Clean Up PCR Product*** with the following modifications: **Elude the DNA in 12 µl of nuclease-free water** (instead of 20 µl) and **transfer 10 µl of purified product** from each tube to a fresh 0.2 mL PCR tube (instead of 15 µl).

Quantify and Pool Library

1. Quantify all eight libraries 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 eight libraries in a total volume of up to 40 µl.
3. Verify the concentration of the pooled library using a Qubit Fluorometer or equivalent.

Sequence Tapestri Single-Cell DNA Library (MiSeq Setup)

IMPORTANT ***Illumina's Read 1 primer in well 12 of the Miseq cartridge needs to be replaced with Mission Bio's Custom Seq Primer (🟣).***

1. **Dilute 1 µl of 10 nM PhiX stock library** in 9 µl dilution buffer (10 mM Tris-HCl with 0.05% Tween 20, pH 8.0) to prepare a 1 nM PhiX library.
2. Prepare a fresh aliquot of 0.2 M NaOH by combining 200 µl 1 M NaOH and 800 µl nuclease-free water.
3. In two separate 1.5 mL low-bind Eppendorf tubes **combine 5 µl of the 5 nM sample library with 5 µl of 0.2 M NaOH** or **5 µl of the 1 nM PhiX library with 5 µl of 0.2 M NaOH**. Vortex, spin down to collect contents.
4. Incubate both libraries for **10 minutes** at room temperature.
5. **Add 990 µl of HT1 Buffer** to each library to neutralize the samples. The pooled Tapestri Single-Cell DNA library is now concentrated at 25 pM. The PhiX library is now concentrated at 5 pM.
6. Prepare the final sample library as follows:

Reagent	Volume (µl)
Denaturated Pooled Tapestri DNA Library (25 pM)	532
Denaturated PhiX Library (5 pM)	140
HT1 Buffer	28
Total	**700**

7. **Load 650 µl of the sample library into well 17** on the Miseq cartridge (150 PE chemistry).
8. Use a pipette to carefully **transfer 400 µl of Illumina Read 1 primer mix** from well 12 to an 1.5 mL Eppendorf tube.
9. Add 4 µl to **Mission Bio Custom Seq Primer** (🟣) to the mix, vortex and spin down to collect contents.
10. **Reload the entire mix** into well 12 of the Illumina Miseq cartridge.
11. Sequence the library following manufacturer's instructions.

For technical support visit www.missionbio.com/support
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