tapestri

Tapestri® Single-Cell DNA Sequencing V2

User Guide

AML | CLL | Myeloid

Tumor Hotspot

Custom

Moving precision medicine FORWARD.

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Introduction

The *Mission Bio Tapestri® Platform* uses microfluidic droplet technology to combine cell lysate with barcoding beads anchored to gene specific primers to deliver a high-throughput single-cell genomics workflow for targeted DNA sequencing. Users can produce a sequencing-ready library starting from a single cell suspension in as few as 2 days. This User Guide describes the experimental procedure in detail.

About This Guide

This User Guide describes the experimental procedure when using the *Mission Bio Tapestri Platform* in detail.

Tapestri Platform Overview

The *Tapestri Platform* consists of the instrument itself, the DNA cartridge, which represents the microfluidics device, and reagents. The cartridge is equipped with reservoirs that are used to load reagents required for automated cell processing. Pressure supplied by the instrument drives the fluidics from the reservoirs through the microfluidic device out to PCR collection tubes that are mounted below the cartridge. The assembled cartridge and tubes can be loaded and unloaded from the instrument and disposed after the completion of the workflow.

The Tapestri Instrument is designed to receive the loaded cartridge and drive the fluidics with programmed, pressurized air. The instrument seals the cartridge using a lid over the top of a loaded cartridge via a rubber gasket and levered handle. The user interacts with the instrument via a touch screen interface, which can be used to select programs, monitor the status of running programs, and more.

Tapestri Instrument **Tapestri DNA Cartridge (assembled)**

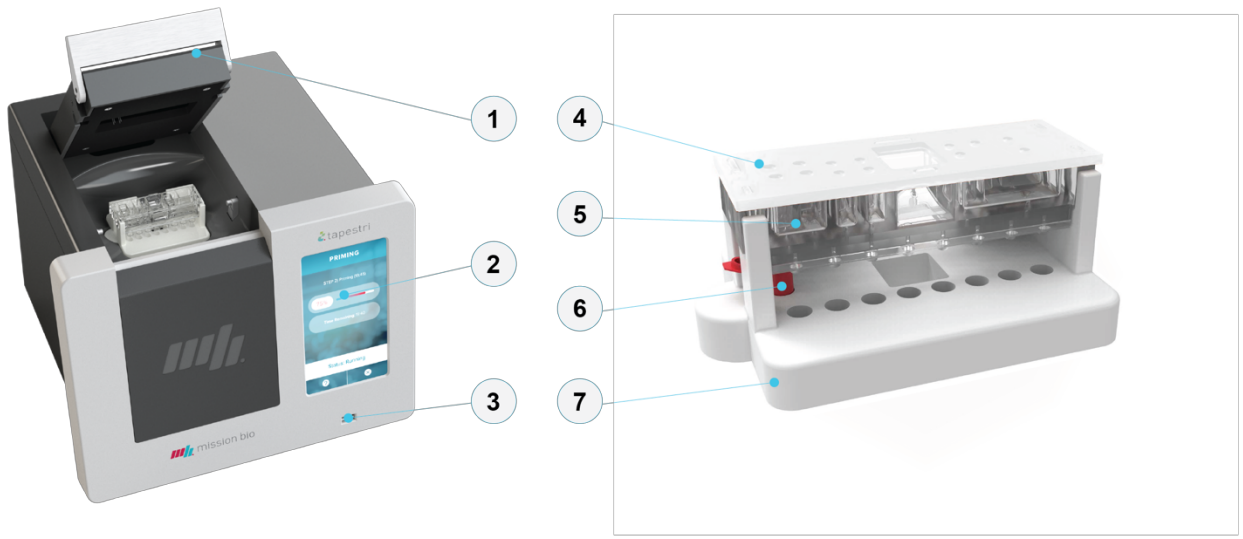


Figure 1. Tapestri Platform: Instrument and Assembled DNA Cartridge (Tapestri Single-Cell DNA AML Kit not shown)

1. **Lid**
   Levered lid to open and close the instrument and install the DNA Cartridge.

2. **Touchscreen**
   To interface with the instrument's software and select programs.

3. **USB Port**
   To export diagnostics data.

4. **Tapestri DNA Gasket**
   To seal the instrument lid.

5. **Tapestri DNA Cartridge**
   Microfluidics device to load with reagents and cells.

6. **Collection Tubes**
   To collect emulsions.

7. **Base Plate**
   Foundation to mount DNA Cartridge and collection tubes.

Materials

Tapestri Single-Cell DNA Kit Product Numbers

Tapestri Single-Cell DNA Custom Kit: **PN 145936***
Tapestri Single-Cell DNA Myeloid Kit: **PN 156528**
Tapestri Single-Cell DNA Tumor Hot Spot Kit: **PN 165919**
Tapestri Single-Cell AML Kit: **PN 132202**
Tapestri Single-Cell DNA Cartridge Kit: **PN 046459**

***For custom-designed gene panels only. Contact your local Sales Representative for additional information**

Tapestri Single-Cell DNA Core/Custom Kit Configuration

	Part Number				
Component Name	**AML**	**Myeloid**	**Tumor Hotspot**	**Custom**	**Storage**
Tapestri Single-Cell DNA Core Ambient Kit v2	MB51-0007				RT
Tapestri Single-Cell DNA Core -20 Kit v2	MB51-0010				-20°C
Tapestri Single-Cell DNA Bead Kit	MB51-0009				4°C

*Kits come either as Core configuration (fixed gene panels including AML, CLL, Myeloid, THP) or Custom configuration (custom-designed gene panels).

Tapestri Single-Cell DNA Core/Custom Kit Components

Component Name	Kit	Storage
Cell Buffer	Tapestri Single-Cell DNA Core Ambient Kit	RT
Encapsulation Oil		RT
Electrode Solution		RT
Barcoding Oil		RT
Extraction Agent (green cap)		RT
Lysis Buffer (brown cap)	Tapestri Single-Cell DNA Core -20 Kit	-20°C
Barcoding MM V2		-20°C

Library MM V2		-20°C
● V2 Index Primer 1 – 8 (purple cap)		-20°C
● Barcoding Beads (blue cap)	Tapestri Single-Cell DNA Bead Kit	4°C
● Fwd Primer Pool (black cap)	**AML, MYE, THP, CLL, Custom**	-20°C
● Rev Primer Pool (white cap)		
Tapestri DNA Cartridge (2 x 4x)	Tapestri Single-Cell DNA Cartridge Kit	RT
Tapestri DNA Gasket (2 x 4x)		RT

NOTE ***Make sure to use non-frost free freezers for all -20°C reagent storage.***

NOTE ***Please contact Mission Bio Support (support@missionbio.com) when interested in Custom Kit Reagents.***

Required Third Party Consumable Reagents

Component Name	Suggested Supplier (Part Number)	Protocol Step
AMPure XP Reagent	Beckman Coulter (A63880)	Targeted PCR, Library PCR
DPBS (1X)	Gibco (14190-144)	Encapsulation
Qubit® dsDNA HS Assay Kit	Qubit® (Q32851)	Targeted PCR
Ethanol, Molecular Biology Grade	Sigma (E7023)	AMPURE purification
Agilent DNA 1000 Kit Agilent DNA High Sensitivity Kit	Agilent Technologies (5067-1504) Agilent Technologies (5067-4626)	Library PCR
Dilution buffer (10 mM Tris-HCl with 0.05% Tween 20, pH 8.0) (OPTIONAL)	Teknova, T1485	Sequencing
Trypan Blue	Thermo Fisher (15250061)	Dead cell staining
RNase-free Filter Pipette Tips	Approved Supplier	Liquid handling
Nuclease free Microcentrifuge Tubes, 1.5 mL	Eppendorf (0030108035)	Cell/Reagent handling
0.2 mL PCR Tubes	USA Scientific (1402-8120) or Approved Supplier	Non-emulsion PCR
* 0.2 mL Axygen MAXYmum Recovery PCR Tubes	Axygen (PCR-02-L-C)	Emulsion handling
* Axygen Gel Tips	Axygen (TGL200RD57R)	Emulsion handling
Qubit Assay Tubes	Thermo Fisher (Q32856)	Post PCR quantitation
Reagent Troughs (OPTIONAL)	Thermo Fisher (E1032-10) or Approved Supplier	AMPURE purification
Narrow Stem Transfer Pipettes	Thermo Fisher (241) or Approved Supplier	Sequencing
KAPA Library Quantification Kit Illumina Platforms (OPTIONAL)	KAPA (KK4873)	Sequencing
Sequencing Reagent Kit 300 cycles (150bp PE) (MiSeq, HiSeq 2500, HiSeq 4000, NextSeq 550, NovaSeq 6000)	Illumina	Sequencing

NOTE **** These consumables are used for handling emulsion samples and must not be substituted. Only listed consumables have been validated by Mission Bio.***

Required Benchtop Equipment

Required Equipment	Suggested Supplier (Part Number)
MB Tapestri Instrument	Mission Bio (191335)
Countess® II Automated Cell Counter or equivalent	Thermo Fisher (AMQAX1000)
Agilent 2100 Bioanalyzer or Tapestation	Bioanalyzer: Agilent (G2939BA)
Qubit Fluorometer	Qubit: Thermo Fisher (Q33216)
Pipettes, 1 µl – 1000 µl	Mettler-Toledo, Rainin Pipettes
8 channel multi pipette	Mettler-Toledo, Rainin Pipettes
* UV light source	Analytik Jena Blak-Ray (XX-15L)
Microcentrifuge (1.5 mL, 0.2 mL PCR tubes)	Thermo Fisher (75004081)
Tube Vortexer	Thermo Fisher (88880017TS)
Thermal cycler with heated lid (100 µl volume, needs to support ramp rates between 1°C/s – 4°C/s)	Thermo Fisher (A24811) or Approved Supplier
0.2 mL 8-strip PCR tube Magnetic Separation Stand	Seqmatic (TM-700) or Approved Supplier
6-Tube Magnetic Separation Rack	New England Biolabs (S1506S)
MiSeq Sequencing Instrument [Optional]	Illumina
HiSeq 2500 Sequencing Instrument [Optional]	Illumina
HiSeq 4000 Sequencing Instrument [Optional]	Illumina
NextSeq 550 Sequencing Instrument [Optional]	Illumina
NovaSeq 6000 Sequencing Instrument [Optional]	Illumina

NOTE **** Wattage and wavelength of UV light are critical. Only the listed instrument has been validated by Mission Bio. Do not substitute.***

Protocol Overview

Single cells are individually partitioned into sub-nanoliter droplets. Barcoding Beads and PCR reagents are introduced using the Mission Bio Tapestri Instrument and DNA Cartridge. Cell lysis, protease digestion, cell barcoding and targeted amplification using multiplexed PCR occur within the droplets. Droplets are then disrupted, and barcoded DNA is extracted for Library Amplification. Final libraries are purified and can be sequenced on one of the supported Illumina Sequencer instruments.

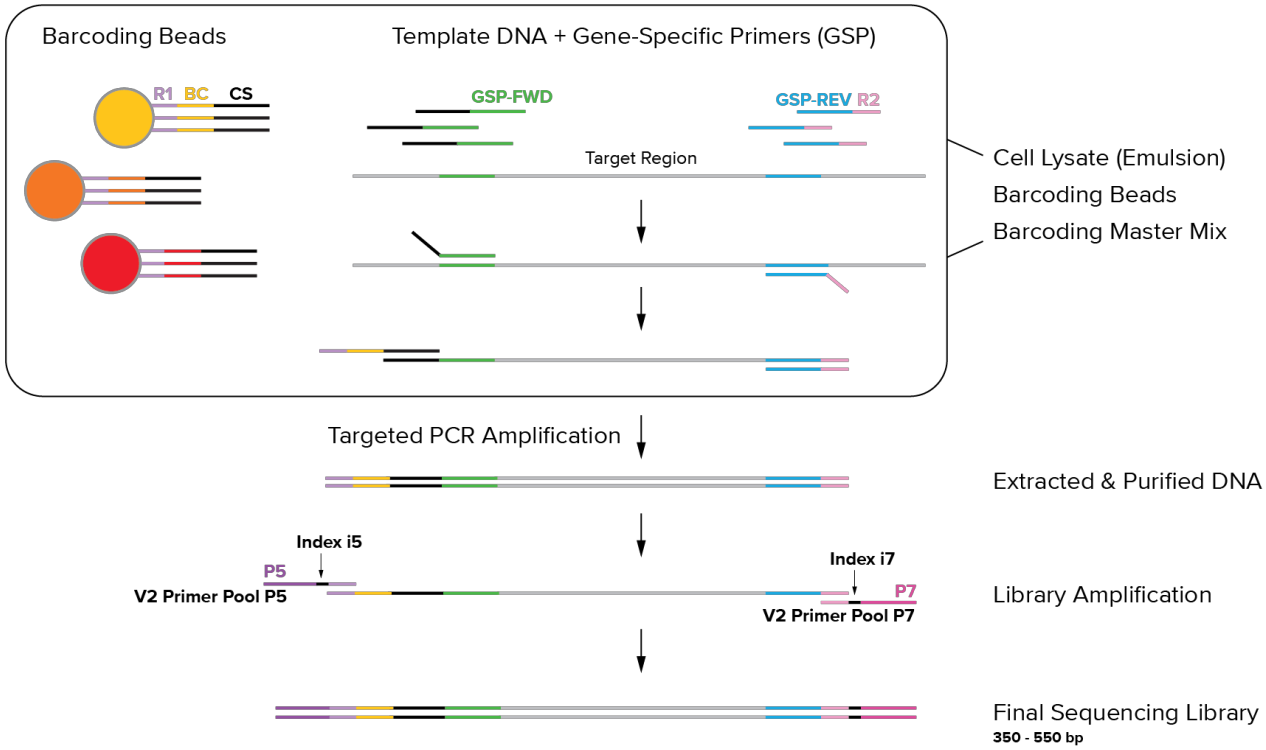


Figure 2. Overview of library construction. R1: Read 1, BC: barcode, CS: common sequence GSP-FWD: gene-specific forward primer, GSP-REV = gene-specific reverse primer, P5: P5 Illumina adapter, P7: P7 Illumina adapter.

Protocol Timeline

NOTE ***We strongly recommend proceeding through Cell Barcoding on day 1. If necessary, the emulsions after Cell Encapsulation may be stored at 4 °C overnight.***

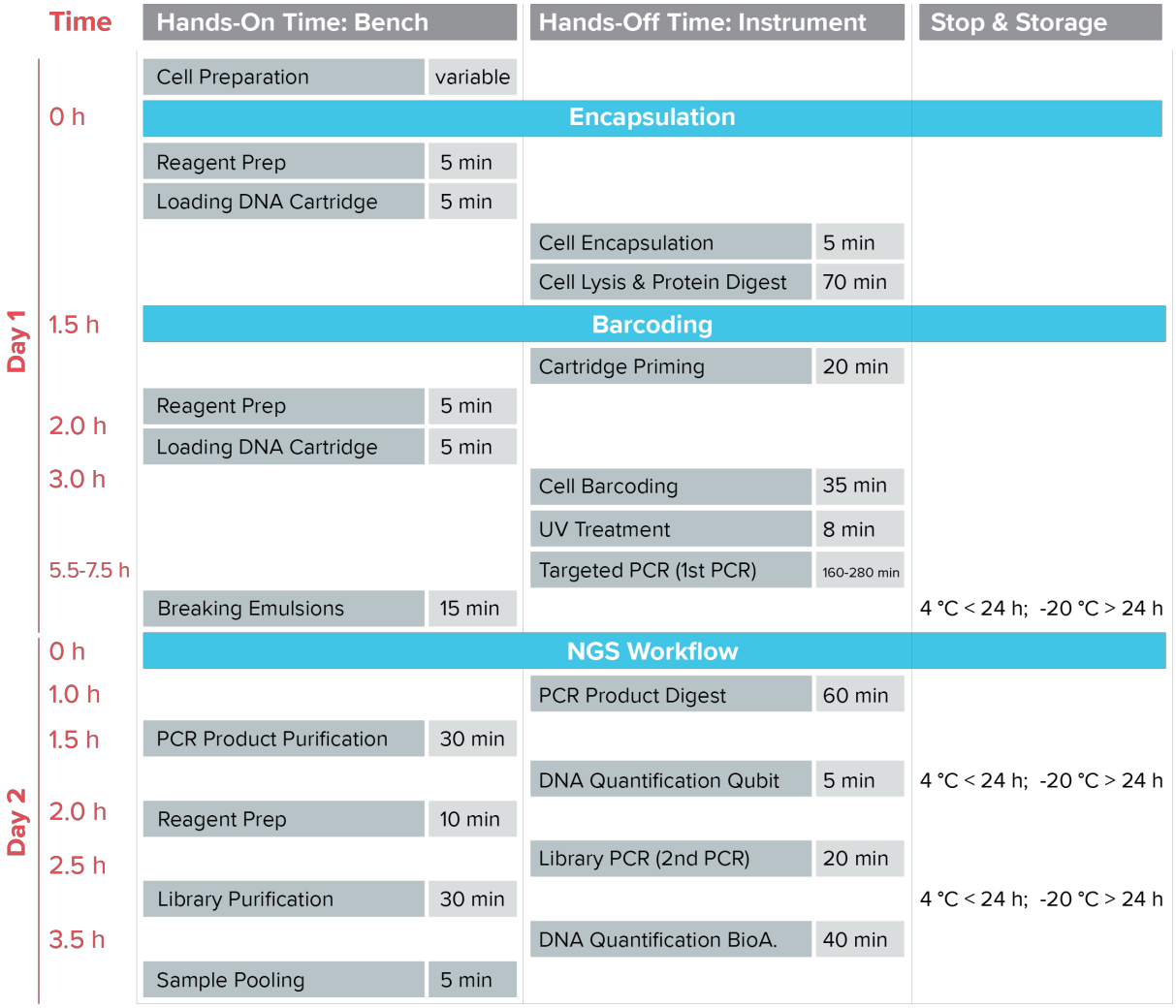


Figure 3. Projected durations for Encapsulation, Barcoding, Targeted Amplification and DNA Quantification.

Best Practices: Emulsion & DNA Cartridge

Cell Culture, Pre- and Post-PCR areas

- All cell sample preparation must be conducted in a designated area that is restricted to cell culture work.
- All Pre-PCR steps (encapsulation, barcoding, PCR master mix preparation) must be conducted in a lab space that is physically separated from amplified genetic material.
- All Post-PCR (amplified material) steps (library PCR, library purification, DNA quantification, sample pooling) must be conducted in a lab space that is physically separated from the unamplified genetic material.
- Do not transfer material (gloves, pipettes, tubes) or equipment from the Post-PCR area to the Pre-PCR area.
- Carefully clean bench areas and pipettes with 5% bleach before starting any protocol.

Cross-contamination

- When pipetting samples, change tips between samples.
- Use aerosol-resistant (filtered) pipette tips to reduce the risk of reagent carryover and sample-to-sample cross-contamination.

Suggestions for working with emulsions

- Consumables (gel tips, emulsion safe PCR tubes) have been carefully tested and specified. Do not substitute.
- Pipette emulsions very slowly and carefully and only when necessary.
- Avoid sources of static and any excess handling of emulsion samples
- Handle emulsion sample tubes carefully, avoiding contact with the top rim area of the tube, where emulsions directly interface with the tube.

Suggestions for working with the Tapestri Instrument and DNA Cartridge

The DNA cartridge is equipped with microfluidics channels that are as small as 40 µm and are used to transport reagents and cells. Care should be taken to avoid introduction of particles, fibers or clumped cells into cartridge that may potentially clog the cartridge. Minimize exposure of the instrument, reagents, cartridges, gaskets to sources of particles and fibers, such as open reagent reservoirs, laboratory wipes, clothing that easily sheds fibers, and dusty surfaces. Place DNA cartridges into original packaging after Encapsulation or Barcoding is completed. Lower the instrument lid when DNA cartridges are mounted on the instrument and are not in use.

Pay attention to the timing of loading the DNA cartridge and running the Encapsulation or Barcoding programs. Experimental steps should be executed successively as outlined in the protocol without delays.
Ensure that the instrument is not placed near a ventilation system or similar sources of high airflow. For additional information about requirements of the instrument's placement consult the *Tapestri Instrument Site Requirements Guide (PN 65307)*.

Gene Panels

AML Panel (19 Genes, 50 Amplicons)

ASXL1	GATA2	KIT	PTPN11	TP53
DNMT3A	IDH1	KRAS	RUNX1	U2AF1
EZH2	IDH2	NPM1	SF3B1	WT1
FLT3	JAK2	NRAS	SRSF2	

CLL Panel (33 Genes, 272 Amplicons)

ATM	CD79B	EZH2	MED12	POT1	TP53
BCOR	CHD2	FAT1	MYD88	PLCG2	XPO1
BIRC3	CREBBP	FBXW7	NFKBIE	RPS15	ZMYM3
BRAF	CXCR4	KRAS	NOTCH1	SETD2	
BTK	DDX3X	LRP1B	NRAS	SF3B1	
CARD11	EGR2	MAP2K1	PAX5	SPEN	

Myeloid Panel (47 Genes, 330 Amplicons)

ASXL1	DNMT3A	IDH1	MYC	PTPN11	STAG2
ATM	ERG	IDH2	MYD88	RAD21	STAT3
BCOR	ETV6	JAK2	NF1	RUNX1	TET2
BRAF	EZH2	KDM6A	NPM1	SETBP1	TP53
CALR	FLT3	KIT	NRAS	SF3B1	U2AF1
CBL	GATA2	KMT2A	PHF6	SMC1A	WT1
CHEK2	GNAS	KRAS	PPM1D	SMC3	ZRSR2
CSF3R	HRAS	MPL	PTEN	SRSF2	

Tumor Hotspot Panel (59 Genes, 244 Amplicons)

ABL1	CSF1R	FGFR1	IDH2	MLH1	RB1
AKT1	CTNNB1	FGFR2	JAK1	MPL	RET
ALK	DDR2	FGFR3	JAK2	MTOR	SMAD4
APC	EGFR	FLT3	JAK3	NOTCH1	SMARCB1
AR	ERBB2	GNA11	KDR	NRAS	SMO
ATM	ERBB3	GNAQ	KIT	PDGFRA	SRC
BRAF	ERBB4	GNAS	KRAS	PI3KCA	STK11
CDH1	ESR1	HNF1A	MAP2K1	PTEN	TP53
CDK4	EZH2	HRAS	MAP2K2	PTPN11	VHL
CDKN2A	FBXW7	IDH1	MET	RAF1	

Thermal Cycling Programs

Always use a properly calibrated thermal cycler suited for 0.2 mL tubes with a maximum reaction volume of 100 µL for all incubations. Program all four thermal cycling protocols from *Table 1* into the instrument. For all protocols, use a heated lid set to 100 °C – 105 °C. For specific instrument operation, follow the instructions provided by the manufacturer.

1. Cell Lysis and Protein Digest

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

3. Library PCR

Step	Temperature	Time	Cycle
1	95 °C	3 min	
2	98 °C	20 sec	9
3	62 °C	20 sec	
4	72 °C	45 sec	
5	72 °C	2 min	
6	4 °C	HOLD	

2. Targeted PCR

			Amplicon Number	20 – 100	100 – 200	200 – 300	> 300	
			Panel	AML	Custom	THP/CLL	Myeloid	
Step	Ramp Rate	Temperature		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	

Table 1. Thermal cycling programs.

Cell Handling Guidelines

The steps provided in this protocol are applicable to non-adherent cells from culture, bone marrow aspirates and buffy coat fractions. If other cell types will be used, contact ***support@missionbio.com*** for additional support. Different cell types may require revised procedures including cell dissociation, washing, re-suspension or quantitation.

Cell counting

- Mission Bio strongly recommends the use of an automated cell counter, such as the Countess II Automated Cell Counter (Thermo Fisher).
- Optimal concentration range for cell counting with the Countess II ranges from 1 x 10^5 to 4 x 10^6 cells/mL.
- Final cell suspensions are measured at least twice. Concentrations found must agree within 10%.
- Cell suspensions must have > 80% viability.
- Final cell concentration values are based on the **total (live + dead)** cell counts.
- Avoid the use of samples containing significant debris, dead cells, or fragments of lysed cells.
- Example images of a well-prepared single cell suspension (left) and low-viability cell suspension (right) are shown below.

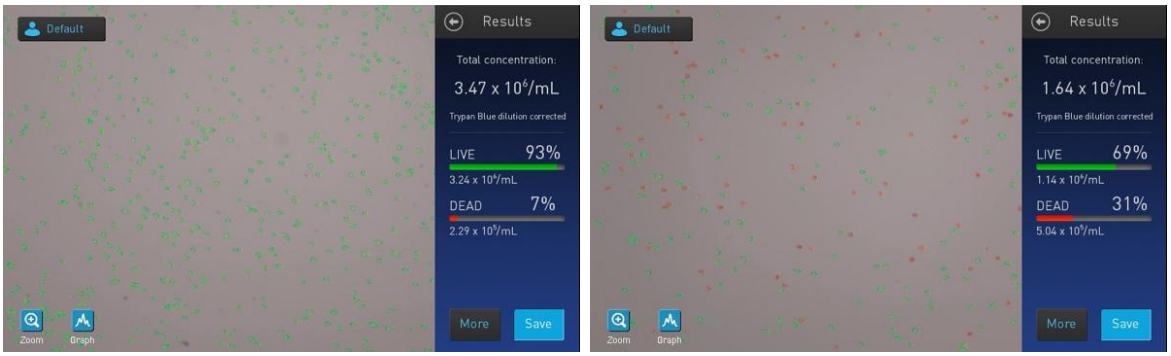


Figure 4. Representative images of high-quality cell suspension (left) and low-quality cell suspension (right).

Genomic Protocol

1 Prepare Cell Suspension

Genomic Protocol

1 Prepare Cell Suspension

This section describes the steps required to prepare a single-cell suspension, count cells, and assess cell viability and cell suspension quality. The Mission Bio Tapestri workflow is optimized for a single-cell suspension input of 3,000 – 4,000 cells/µl at greater than 80% viability in a total volume of 50 µl.

NOTE

- ***Thaw reagents at room temperature unless directed to thaw them on ice.***
- ***Store reagents according to manufacturer's storage recommendations as soon as they are received. Vortex and then centrifuge reagents as directed.***
- ***The following procedure assumes the cells to be cryopreserved in 2 mL cryovials in a total volume of 0.5 mL and stored in liquid nitrogen or -80°C.***

1.1 Retrieve all reagents required for preparing the cell suspension:
- 1x DPBS w/o Ca^{2+}/Mg^{2+}
- 70% ethanol
- Cell Buffer (Ambient Kit)

1.2 Remove the cryovial containing the frozen cells from liquid nitrogen storage or -80 °C freezer and **immediately place it into a 37 °C water bath**.

1.3 **Quickly thaw the cells** (< 1 minute) by gently swirling the vial in the 37 °C water bath until there is just a small bit of ice left in the vial.

1.4 **Transfer 1 mL of room temperature 1x DPBS** dropwise into the vial.

1.5 Carefully resuspend the cells by pipetting 1 time up and down and transfer the cells into a new 1.5 mL Eppendorf tube.

1.6 **Centrifuge** at appropriate speed – we suggest **400 x g – for 5 minutes**. The actual centrifugation speed and duration vary from cell type to cell type. Centrifugation is complete when the pellet is visible, and the supernatant is clear.

1.7 Aspirate the supernatant without disturbing the cell pellet.

1.8 **Add 1 mL of 1x DPBS to the cell pellet** to wash the cells, then **centrifuge at 400 x g for 5 minutes**.

1.9 Aspirate the supernatant without disturbing the cell pellet.

1.10 **Add 50 µl of Cell Buffer** and carefully resuspend cells by gently pipetting up and down to disaggregate the cell pellet until no cell clumps are visible.

IMPORTANT ***Mission Bio's Cell Buffer contains density gradient medium. Cells that are resuspended in Cell Buffer are difficult to pellet via centrifugation.***

1.11 **Count the cells** using an automated cell counter and dead-cell exclusion dye (e.g., Trypan Blue) according to the manufacturer's instructions. Assess both single cell suspension quality and cell viability.

1.12 **Dilute cell suspension to ~3,500 cells/µl** using Cell Buffer in a total volume of at least 50 µl.

IMPORTANT ***Use of cell concentrations outside the range of 3,000 – 4,000 cells/µl or viability 80% may adversely affect results. If the minimum concentration of 3,000 cells/µl cannot be met in a total volume of 50 µl, the total volume of Cell Suspension may be reduced to as low as 35 µl.***

1.13 **Place cell suspension on ice** until required in ***Section 2 – Encapsulate Cells***. Do not keep cell suspensions on ice for longer than 30 minutes before proceeding to encapsulation.

Genomic Protocol

2 Encapsulate Cells

2 Encapsulate Cells

In this step, cells are encapsulated with Lysis Buffer and Protease to create a cell emulsion. For input cell concentrations of 3,000 – 4,000 cells/µl, approximately 5% of all emulsion droplets will contain a cell.

IMPORTANT

- ***Handle emulsions with caution, avoiding sources of static and pipetting slowly and carefully.***
- ***Use only the consumables (sample tubes and pipette tips) validated by Mission Bio (see Tapestri Instrument and DNA Cartridge and list of Required Third Party Consumable Reagents).***

2.1 Turn on the Tapestri Instrument at least 5 minutes prior to use.

2.2 Retrieve all reagents required for cell encapsulation:

- Tapestri DNA Cartridge
- Tapestri DNA Gasket
- Lysis Buffer (●) (-20 °C Kit)
- Reverse Primer Pool (●) (-20 °C Kit)
- Encapsulation Oil (Ambient Kit)
- Cell Suspension (prepared in ***Section 1 – Prepare Cell Suspension***)

Thaw Lysis Buffer and Reverse Primer Pool on ice.

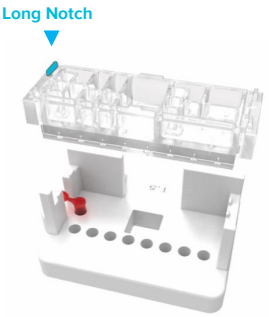
2.3 In the Pre-PCR area, carefully open a new Tapestri DNA Cartridge.

IMPORTANT

- ***Avoid dust and debris at all times when handling the DNA cartridge.***
- ***Each DNA cartridge is packaged with one DNA Gasket to be used throughout the run. Store both DNA cartridge and DNA Gasket in protective packaging when not in use during the experiment. Use within 24 hours after opening.***

2.4 Mount the Base Plate onto the Tapestri Instrument. **Place a 0.2 mL Axygen MAXYmum Recovery PCR tube into the middle of the slot at the left of the Base Plate** for collecting the encapsulation emulsion product. Position the tube with the open lid facing left.

2.5 **Place the DNA Cartridge onto the Base Plate** with the long notch on the side of the cartridge oriented on the top left, as shown, and place in instrument.

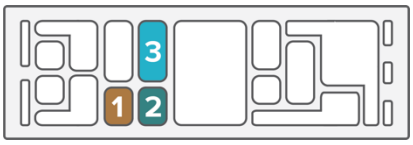


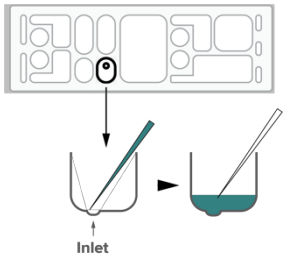
IMPORTANT ***Minimize electrostatic sources. Only the Axygen MAXYmum Recovery PCR tubes have been validated by Mission Bio as nuclease-free and emulsion-safe. Do not substitute with other PCR tubes.***

2.6 In a new tube, prepare **Lysis Mix** by adding **7.3 µl of Reverse Primer Pool** (●) into **92.7 µl of Lysis Buffer** (●).

2.7 Pipette 100 µl of **Lysis Mix** into **reservoir 1**.

2.8 Pipette 35 µl of **Cell Suspension** into **reservoir 2**.





Pipette slowly into the bottom of the reservoir where the inlet is located. Raise the pipette tip as the liquid level in the reservoir is rising, keeping the tip slightly submerged.

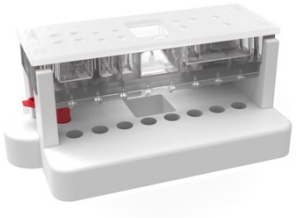
The total volume of **Cell Suspension** may be as low as 35 µl. Ensure that the inlet is fully covered with **Cell Suspension** before starting the Cell Encapsulation program.

2.9 Pipette 200 µl of **Encapsulation Oil** into **reservoir 3**.

IMPORTANT ***Make sure to apply the DNA Gasket and start the program within 1 minute after loading the Encapsulation Oil.***

2.10 **Apply the Tapestri DNA Gasket** to the top of the cartridge. Ensure that it is oriented correctly.

2.11 Firmly **close the instrument lid**, until the lid handle is level and flush with the top of the lid and instrument.



2.12 Run the **Encapsulation** program by pressing **Step 1: Encapsulation** on the Tapestri Instrument touchscreen. Press **NEXT** and confirm to start the run. The program runs for about 5 minutes.

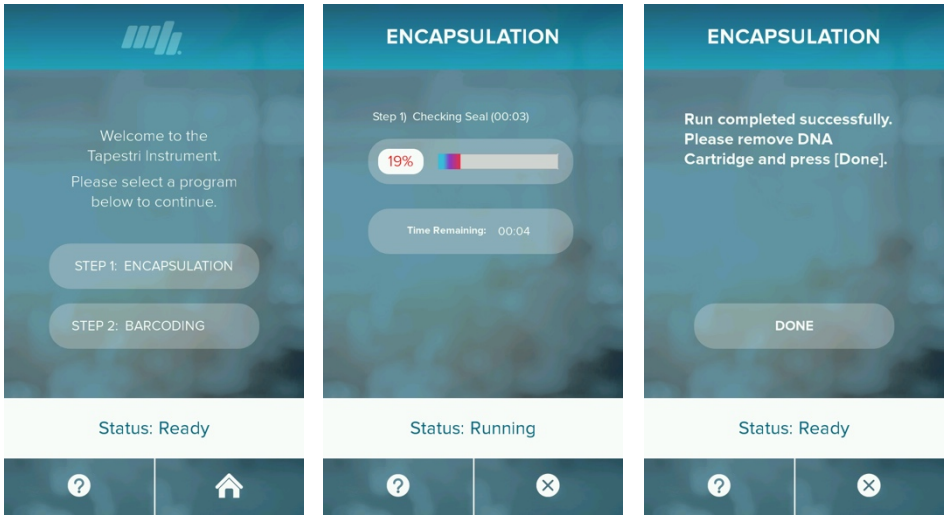
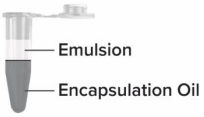


Figure 5. Touchscreen displays show main menu (left), screen after selecting 'Step 1: Encapsulation' program (middle), and final screen after Encapsulation is completed (right).

2.13 When the touchscreen displays **DONE**, carefully open the lid and **remove the cartridge from the Base Plate**. **Remove the gasket from the lid and set aside.**

2.14 **Carefully transfer the emulsion sample tube** to a 96-well plate holder and assess emulsion quality**.** Encapsulated cells appear as a white layer.



2.15 **Return the cartridge back onto the Base Plate** seated inside the instrument and close the lid to protect it from environmental debris.

2.16 The sample tube contains 50 – 80 µl of cell emulsion (top layer) and 80 – 120 µl encapsulation oil (bottom layer) for a total volume of 130 – 200 µl.

2.17 Use a ***gel loading tip*** to carefully **remove up to 100 µl of oil** from the bottom layer of the sample.

IMPORTANT ***Hold the tube by the lid. Remove oil only. Make sure the gel loading tip is at the very bottom of the sample tube and wait ~5 seconds before removing oil. This will minimize removal of cell emulsion.***
After removal, ~70 µl of cell emulsion and ~10 µl of oil remain at the bottom of the tube. Make sure the entire tube volume does not exceed the maximum volume specified in the thermal cycler manufacturer's instructions (typically 100 µl).

Genomic Protocol

3 Lyse and Digest Cells

3 Lyse and Digest Cells

In this step, cells are lysed and DNA binding proteins are enzymatically digested to make DNA accessible for downstream target amplification.

3.1 **Run the "Lysis/Digest" protocol** on the thermal cycler according to the manufacturer's instructions, using the following parameters:

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

Table 2. Thermal cycling protocol for 'Lysis/Digest'.

3.2 When the run completes, **store the lysed and digested samples at 4 °C** until required in ***Section 4 – Barcode Cells***. The volume of oil at the bottom of the tube is expected to increase slightly after thermal cycling.

NOTE ***We strongly recommend proceeding through Section 4 – Barcode Cells on day 1. If necessary, the encapsulation emulsion products may be stored at 4 °C overnight, upright in a sealed container to avoid condensation.***

Genomic Protocol

4 Barcode Cells

4 Barcode Cells

In this step, the drops containing encapsulated cell lysate are combined with drops containing both Barcoding Master Mix and Barcoding Beads. These newly generated drops are then distributed into 8 PCR collection tubes, to create 8 cell-barcoding emulsion samples.

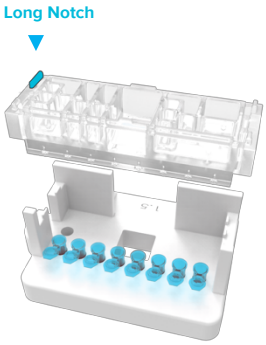
4.1 Retrieve all reagents required for Cell Barcoding:

- Barcoding MM V2 (-20 °C Kit)
- Barcoding Beads (●) (4 °C Barcoding Bead Kit)
- Forward Primer Pool (●) (-20 °C Primer Pools Kit)
- Barcoding Oil (Ambient Kit)
- Electrode Solution (Ambient Kit)

Thaw all -20 °C reagents on ice, except for the Barcoding Beads.

IMPORTANT ***Protect Barcoding Beads from light and thaw at room temperature.***

Prime the DNA Cartridge for Barcoding

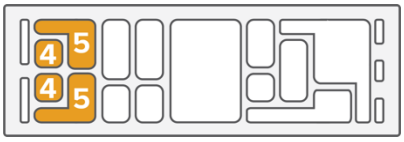


IMPORTANT ***Use emulsion-safe PCR tubes (Axygen MAXYmum Recovery).***

4.2 Label eight 0.2 mL emulsion-safe PCR tubes with the sample number and **load them into the eight bottom slots of the Tapestri Base Plate** with the open lids toward you.

4.3 Retrieve Tapestri DNA Cartridge from its packaging and mount onto Base Plate (used during Cell Encapsulation).

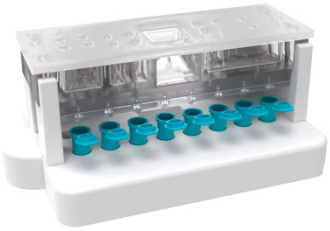
4.4 Pipette **200 µl** of **Electrode Solution** into each **reservoir 4** of the cartridge.



4.5 Pipette **500 µl** of **Electrode Solution** into each **reservoir 5** of the cartridge.

4.6 **Apply the DNA Gasket** and firmly close the instrument lid, until the lid handle is level and flush with the top of the lid and instrument.

4.7 Run the **Priming** program by pressing **Step 2: Barcoding** on the Tapestri Instrument touchscreen. Press **NEXT** and confirm to start the program. The **program runs for about 20 minutes** before automatically pausing to allow for loading of the remaining reagents.



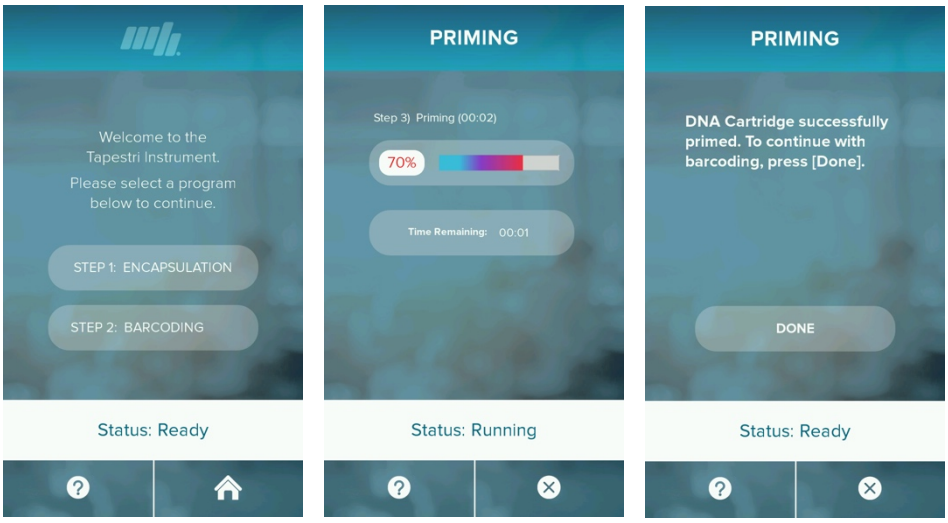


Figure 6 Touchscreen displays show main menu (left), screen after selecting 'Step 2: Barcoding'. program (middle), and final screen after Priming is completed (right).

Prepare Barcoding Master Mix

4.8 **Prepare 300 µl Barcoding Master Mix Solution** as shown in the following table. A total of 250 µl will be used for Barcoding.

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

Table 3. Reagents for Barcoding Master Mix.

4.9 **Briefly vortex the Barcoding Master Mix** and centrifuge to collect the contents and store on ice.

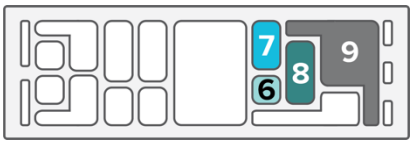
IMPORTANT ***After the Priming program has completed the Barcoding program must be started within 30 minutes.***

Load the DNA Cartridge

4.10 When the touchscreen displays DONE, **retrieve the emulsion containing the encapsulated cell lysate** from the thermal cycler at 4 °C (see ***Section 2 – Encapsulate Cells***).

4.11 Open the instrument lid and **pipette all of the content of the encapsulated emulsion** (~80 µl) – including 5 – 10 µl Encapsulation Oil at the bottom of the tube – into **reservoir 6**.

IMPORTANT ***Remember to avoid sources of static and pipette slowly and carefully when handling emulsions.***



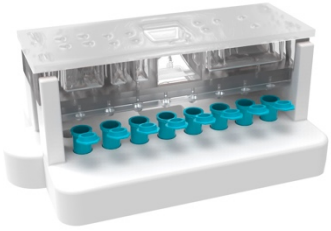
4.12 Retrieve the Barcoding Beads (●) and **vortex tube for 1 minute at high speed**. *Protect Barcoding Beads from light and thaw at room temperature.*

4.13 Carefully pipette **200 µl of Barcoding Beads** (●) into **reservoir 7**. *Pipette slowly and do not introduce bubbles.*

4.14 Pipette **250 µl of Barcoding Master Mix** into **reservoir 8**.

4.15 Pipette **1.25 mL of Barcoding Oil** into the **reservoir 9**. Be careful not to spill oil into surrounding reservoirs while loading the cartridge.

IMPORTANT ***Make sure to apply the DNA Gasket and start the Cell Barcoding program within 1 minute of loading the oil.***



4.16 **Apply the DNA Gasket** and firmly close the instrument lid, until the lid handle is level and flush with the top of the lid and instrument.

4.17 **Run the Barcoding program** by pressing NEXT on the Tapestri Instrument touchscreen in the following figure. This program will **complete in 35 minutes**.

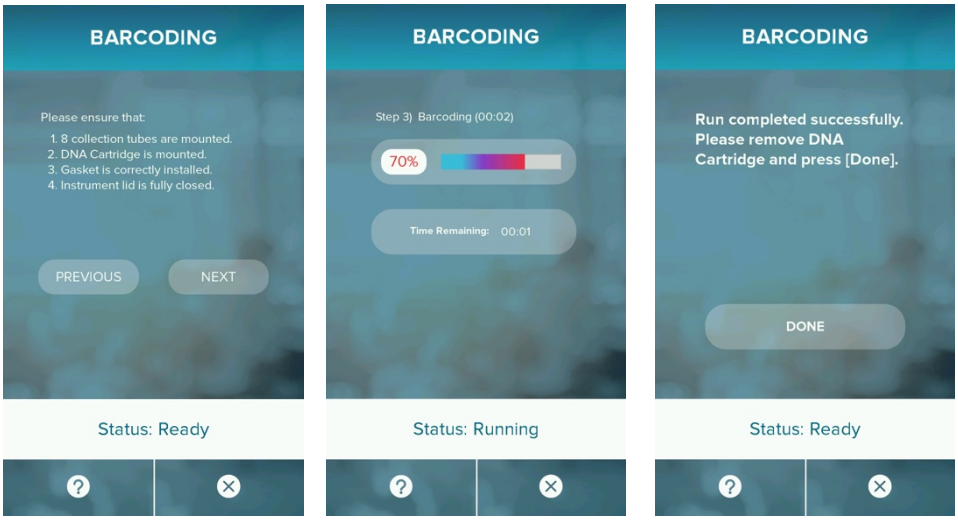


Figure 7. Touchscreen displays before the second part of Barcoding (left), the status during Barcoding (middle), and final screen after Barcoding is completed (right).

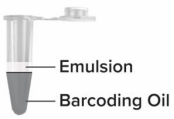
4.18 When the screen displays Run completed successfully, press DONE, carefully open the lid and **remove the Base Plate together with the cartridge to collect the eight tubes** containing the barcoded emulsion. Place the cartridge on the bench.

4.19 Remove the cartridge from the Base Plate.

NOTE ***The volumes of oil and emulsion may vary across all 8 tubes. If more than 100 µl of Barcoding Beads remains in reservoir 7 contact support@missionbio.com proceed with the workflow.***

4.20 Visually evaluate the emulsion quality. The barcoded DNA **emulsions are visible as a white solid layer** on top of the oil layer (~20 µl).

4.21 Using a **gel loading pipette tip carefully remove up to 90 µl of Barcoding Oil** from the bottom of each tube. The volume of the oil must be ~35 µl per tube and the total volume in each tube must be no more than 100 µl. Place sample tubes back onto the Base Plate.



IMPORTANT ***Hold tubes by the lid. Insert pipette tip only once when removing oil.***

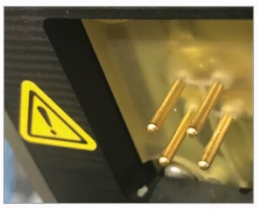
Clean Electrode Pins

NOTE ***The electrode pins on the bottom of the instrument lid are in direct contact with the Electrode Solution during Priming and Cell Barcoding. Gradual buildup of salt deposits might eventually hinder instrument function. Electrodes are disabled when the instrument lid is open.***

4.22 With a dust-free cloth and deionized water **clean all four electrode pins** on the bottom of the instrument lid.

4.23 **Dry the electrode pins** using a dry dust-free cloth.

Dirty (salt deposits)



Clean

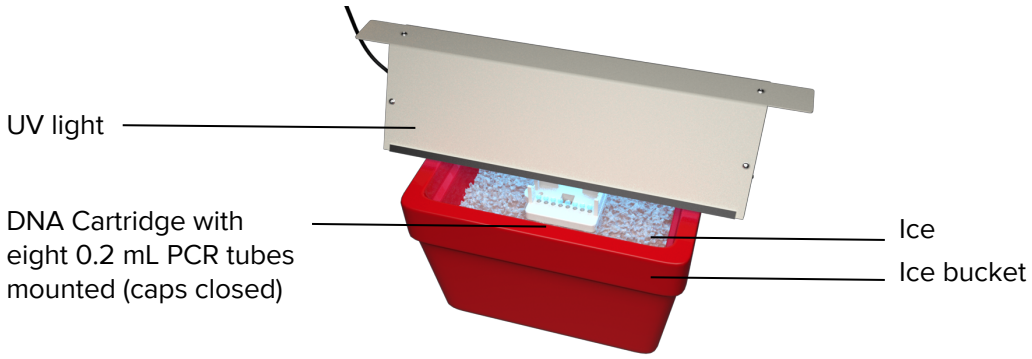
Genomic Protocol

5 UV Treatment and Targeted PCR Amplification

5 UV Treatment and Targeted PCR Amplification

In this step, emulsions containing cell lysate, barcoding beads, and PCR reagents are exposed to UV light to cleave off barcode-containing forward primers from the barcoding beads prior to targeted PCR amplification.

5.1 Place the entire Base Plate with tubes containing barcoded DNA on ice.



5.2 Place a UV light on top of the ice bucket and **expose the samples to non-irradiating UV light for 8 minutes.** The distance between sample and UV light must not exceed 5 inches.

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

5.3 After UV exposure, remove the Base Plate from the ice, **transfer the samples to the thermal cycler, and run the "Targeted PCR" protocol** according to the manufacturer's instructions.

Make sure to select the correct thermal cycling program with the **correct annealing/extension times (Steps 4 and 8, see table below)** that are compatible with the targeted gene panel you processed your samples with.

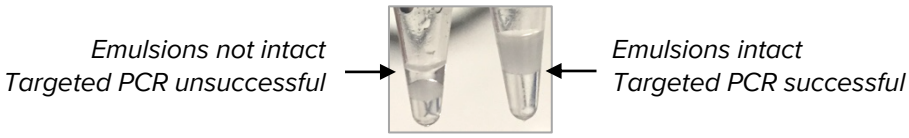
IMPORTANT ***Ensure that the emulsions in all eight tubes (white top layer) sit within the height of the block of the thermal cycler that is temperature controlled. Use a skirt or empty PCR tubes placed at the corners of the thermal cycling block to maximize even heat transfer.***

			2. Targeted PCR				
		Amplicon Number	20 – 100	100 – 200	200 – 300	> 300	
		Panel	AML	Custom*	THP/CLL	Myeloid	
Step	Ramp	Temperature	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	

Table 4. Thermal cycling programs for Targeted PCR.

*Please contact ***support@missionbio.com*** for additional information.

IMPORTANT ***If you observe an aqueous layer on top of the white-appearing emulsion layer, please contact support@missionbio.com.***



Break Emulsions and Pool Tubes

5.4 Retrieve the following reagents needed for PCR product purification:

- Extraction Agent (🟢)
- Nuclease-free water

5.5 **Pool the entire contents** of tubes 1 – 4 and tubes 5 – 8 into two new 1.5 mL Eppendorf tubes.

5.6 Add **40 µl of Extraction Agent (🟢) to each sample tube**. Vortex and spin for 10 seconds.

5.7 **Incubate at room temperature for 3 minutes** until the entire emulsion changes from white to clear in color. *If emulsions don't lose their white appearance, add 20 µl of additional Extraction Agent, vortex and spin for 10 more seconds.*

5.8 Add **180 µl of nuclease-free water to each of the two tubes**. Mix by briefly vortexing and then spinning for 10 seconds in a benchtop centrifuge to separate the aqueous and oil layers.

5.9 Pipette **168 µl of the aqueous top layer** in each tube into a **new 1.5 mL Eppendorf tube each.** *Do not transfer any oil.*

5.10 Store samples at 4 °C or proceed to ***Section 6 – Cleanup PCR Products***.

STOPPING POINT ***This is a good place to stop in the protocol if there is not adequate time to continue to clean up the libraries in one day (~ 1 hr). The amplified PCR products can be stored at 4 °C for < 24 hours or -20 °C for > 24 hours.***

Genomic Protocol

6 Cleanup PCR Products

6 Cleanup PCR Products

Digest PCR Product

6.1 For each of the two pooled samples prepare a 200 **µl Digestion Mix** by adding **20 µl** DNA Clean up Buffer (●) and **12 µl** Clean up Enzyme (●).

Reagent	Volume (µl)
Pooled sample	168
DNA Clean up Buffer (●)	20
Clean up Enzyme (●)	12
Total	**200**

Table 4. Reagents for Digestion Mix.

6.2 Mix by pipetting up and down and quick spin the tubes.
6.3 Transfer both tubes to a heat block and run the **digest at 37 °C for 60 minutes**.

Ampure XP Library Cleanup

6.4 Thoroughly **vortex Ampure XP reagent for 15 seconds** at high-speed. Equilibrate the **Ampure XP reagent to room temperature**.
6.5 Prepare **5 mL fresh 80% ethanol** using nuclease-free water.

NOTE ***Measure volumes for 100% ethanol and nuclease-free water separately. Make sure to tightly close all ethanol containers when not in use, since ethanol can absorb water over time, leading to lower concentrations.***

6.6 Thoroughly **vortex Ampure XP reagent** at high speed immediately prior to usage.
6.7 **Add 200 µl of nuclease-free water** to each tube.
6.8 For each 400 µl sample tube, **add 288 µl of Ampure XP reagent**.
6.9 **Vortex for 5 seconds** and quick-spin to collect contents.
6.10 Incubate the tubes at **room temperature for 5 minutes**, and then place the tubes on the magnet.
6.11 Allow at least **2 minutes** for the Ampure beads to separate from solution.
6.12 Without removing the tubes from the magnet, **remove the clear liquid from each tube** and discard. *The DNA is adhered to the beads.*

6.13 In each tube, **wash Ampure bead pellets** while keeping the tubes on the magnet:
   a. Carefully **add 800 µl** of the freshly prepared **80% ethanol**.
   b. Wait **30 seconds**.
   c. **Remove ethanol** without disturbing the Ampure beads.
   d. **Repeat** these steps once, for a total of two wash cycles.

6.14 Keeping the tubes on the magnet, **remove all residual ethanol** from each tube without disturbing the Ampure beads.

6.15 **Dry Ampure bead pellets** in the tubes on the magnet by incubating at room temperature for **4 – 6 minutes**. *Over-dried beads may be more difficult to suspend.*

6.16 Remove the tubes from the magnet.

6.17 **Add 65 µl** of nuclease-free water into each tube.

6.18 **Vortex each tube for 10 seconds**, quick-spin to collect the contents, and incubate the tubes at room temperature for **2 minutes**.

6.19 Place the tubes onto the magnet and **wait for at least 2 minutes** or until solutions are clear.

6.20 **Transfer and combine 50 µl** of purified PCR product from each tube to a **single** new 0.2 mL PCR tube each for a total of 100 µl. *Avoid transfer of Ampure beads*.

6.21 [OPTIONAL] If Ampure beads persist in the supernatant, place the 0.2 mL PCR tubes onto a 96-well magnet stand, wait 5 minutes and transfer 50 µl of purified PCR products to a new 0.2 mL PCR tubes.

6.22 **Quantify 1 µl of purified PCR product**, using the **High Sensitivity Qubit Kit** (or equivalent assay) according to the manufacturer's instructions.

NOTE ***The DNA 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.***

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

STOPPING POINT ***This is a good place to stop in the protocol if there is not adequate time to continue to Library PCR (~ 1 hr). The purified PCR products must be stored at 4 °C or -20 °C and will be stable for up to six months.***

Genomic Protocol

7 PCR Target Library

7 PCR Target Library

During Target Library PCR the P5 and P7 adapter (Illumina) sequences are added to the amplicons required for sequencing. Each V2 Index Primer includes a two unique index sequence

Use the following index combination when indexing your samples.

# of Samples	Option 1	Option 2	Option 3	Option 4
1	Any index			
2	1 + 4	2 + 3	5 + 7	6 + 8
3	1 + 2 + 3	4 + 6 + 8		
4	1 + 2 + 3 + 4	5 + 6 + 7 + 8		
5	1 + 2 + 3 + 4 + one from (6, 7, 8)	5 + 6 + 7 + 8 + one from (1, 3, 4)		
6	1 + 2 + 3 + 4 + two from (6, 7, 8)	5 + 6 + 7 + 8 + two from (1, 3, 4)		
7	1 + 2 + 3 + 4 + 6 + 7 + 8			
8	1 + 2 + 3 + 4 + 5 + 6 + 7 + 8			
> 8	Contact Mission Bio			

Table 5. Index combinations for different sample multiplexing schemes.

7.1 Retrieve the following reagents required for Library PCR

- Purified PCR products (from the previous step)
- V2 Index Primer 1 – 8 (●) (-20 °C Kit)
- Library MM V2 (-20 °C Kit)
- Nuclease-free water (Ambient Kit)

7.2 In a Pre-PCR area **label one new 0.2 mL PCR tube** with the index number of the V2 Index Primer and **dilute the sample to 0.2 ng/µl in a total of 15 µl** using the *V2 Tapestri Sample Quantification Tool (PN40676)*. *Use nuclease-free water to prepare each dilution and store the remaining stock solutions of purified PCR products at -20 °C.*

7.3 **Add 25 µl of Library MM V2** and **10 µl of V2 Index Primer** (●) to the tube containing 15 µl of diluted sample for a total 50 µl.

IMPORTANT ***Make sure to avoid cross-contamination when handling the P7 Indices.***

7.4 **Vortex and quick-spin** the tubes to collect contents.

7.5 Transfer the samples to the thermal cycler, then **run the Library PCR protocol** according to the manufacturer's instructions, using the following parameters:

Step	Temperature	Time	Cycle
1	95 °C	3 min	
2	98 °C	20 sec	9
3	62 °C	20 sec	
4	72 °C	45 sec	
5	72 °C	2 min	
6	4 °C	HOLD	

Table 6. Thermal cycling program for Library PCR.

7.6 Remove the samples from thermal cycler and store at room temperature.

Library cleanup

7.7 Thoroughly **vortex Ampure XP reagent for 15 seconds** at high-speed. Equilibrate the **Ampure XP reagent to room temperature**.

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

NOTE ***Measure volumes for 100% ethanol and nuclease-free water separately. Make sure to tightly close all ethanol containers when not in use, since ethanol can absorb water over time, leading to lower concentrations.***

7.9 Thoroughly **vortex Ampure XP reagent** at high-speed immediately prior to usage.

7.10 **Add 34.5 µl of Ampure XP reagent** to the 50 µl sample.

7.11 **Vortex for 5 seconds** and quick-spin to collect contents.

7.12 Incubate the tube at **room temperature for 5 minutes**, and then place the tube on the magnet.

7.13 Allow at least **2 minutes** for the Ampure beads to separate from solution.

7.14 Without removing the tube from the magnet, **remove the clear liquid** and discard.
The DNA is adhered to the beads.

7.15 **Wash Ampure bead pellets** while keeping the tube on the magnet:

a. Carefully **add 200 µl** of the freshly prepared **80% ethanol**.
b. Wait **30 seconds**.
c. **Remove ethanol** without disturbing the Ampure beads.
d. **Repeat** these steps once, for a total of two wash cycles.

7.16 Keeping the tube on the magnet, **remove all residual ethanol** without disturbing the Ampure beads.

7.17 **Dry Ampure bead pellets** in the tube on the magnet by incubating at room temperature for at least **2 minutes**. *Over-dried beads may be more difficult to suspend.*

7.18 Remove the tube from the magnet.

7.19 **Add 12 µl** of nuclease-free water into the tube.

7.20 **Vortex tube for 5 seconds**, quick-spin to collect the contents, and incubate at room temperature for **2 minutes**.

7.21 Place the tube onto the magnet and **wait for at least 2 minutes** or until solutions are clear.

7.22 **Transfer 10 µl** of purified PCR product from the tube to a new 0.2 mL PCR tube.
Avoid transfer of Ampure beads.

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

STOPPING POINT ***This is a good place to stop in the protocol if there is not adequate time to finish in one day (~ 1 hr). The purified Library PCR products can be stored at -20 °C.***

Genomic Protocol

8 Quantify and Normalize Sequencing Library

8 Quantify and Normalize Sequencing Library

8.1 Retrieve the following for library quantitation:

- Purified sample libraries
- Agilent DNA 1000 kit

Quantify Using Agilent Bioanalyzer

NOTE ***Agilent TapeStation 2200/4200 or Fragment Analyzer (Advanced Analytical) may be used if an Agilent Bioanalyzer 2100 is not available.***

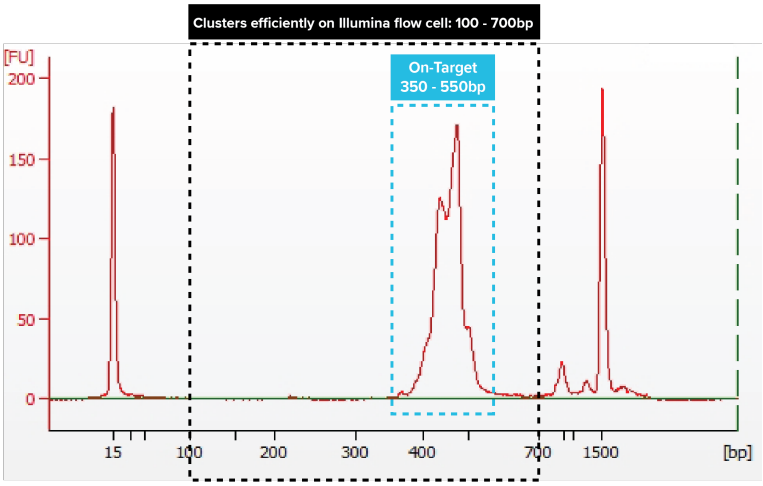
8.2 Use **1 µl of DNA** and follow Agilent's protocol instructions to prime, load, and run DNA samples from all tubes on a DNA 1000 chip.

8.3 Verify the DNA Library product size and purity and quantify following manufacturer's instructions.

NOTE **A final concentration of on-target product between 2 – 20 ng/µl can be expected.**

AML Libraries

Libraries generated with the AML panel in general produce high-quality on-target amplicons (**blue rectangle**) with only a few off-target fragments (e.g., primer dimers).

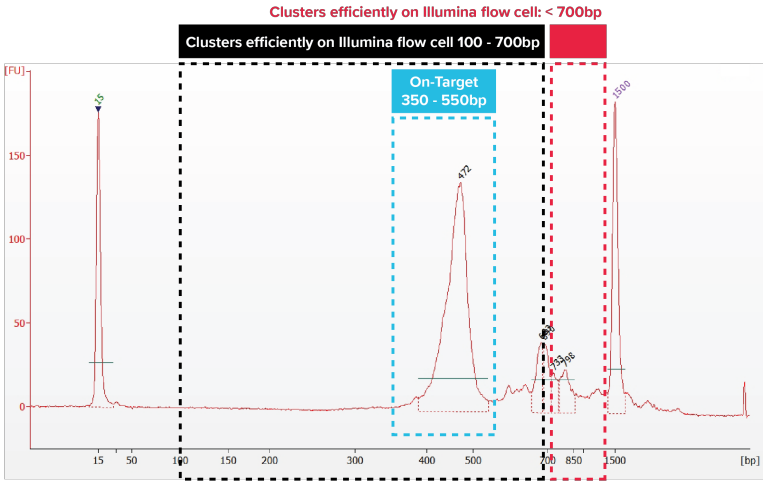


8.4 Quantify the concentration of the libraries based on a range of 100 – 700bp (**black rectangle**) to include products that may efficiently cluster on the Illumina flow cell.
This minimizes the potential to over-cluster when sequencing the libraries.
*Use this value in **Step 8.7**.*

NOTE ***If significant quantities (> 30%) of smaller products are seen (e.g., primer dimers), contact support@missionbio.com for additional support. An additional Ampure cleanup step may be required.***

Myeloid, Tumor Hot Spot, and CLL Libraries

Libraries generated with 250+ amplicon panels (e.g., Myeloid, Tumor Hot Spot, CLL) may produce large-size off-target fragments that need to be taken into account when quantifying the concentration of the libraries.



8.5 Quantify the concentration of the libraries based on a range of 100 – 700 bp (**black rectangle**) to include products that may efficiently cluster on the Illumina flow cell.
*Use this value in **Step 8.7**.*

8.6 Quantify the concentration of the libraries based on a range of 700 – 1,200 bp (**red rectangle**) to record the fraction of products that will impact the final concentration of the pooled library when quantifying with a Qubit Fluorometer.

Normalize and Pool Libraries

8.7 Use the *Tapestri Sample Quantification Tool (PN 40676)* to dilute each tube library.

8.8 **Pool 5 nM of each of the libraries equimolar.** The final concentration of DNA in the pooled library will be ~ 5 nM (0.9 – 1.3 ng/µl).

8.9 **Re-quantify the pooled library** with a Qubit Fluorometer.

AML Libraries

The final concentration of the library will be between **0.9 – 1.3 ng/µl**.

Myeloid, Tumor Hot Spot, and CLL Libraries

The final concentration of the library will be between **0.9 – 1.6 ng/µl**. The products quantified between 700 – 1,200 bp account for the increased concentration > 1.3 ng/µl. Ensure that the relative fraction of large-size off-target products as measured in **Steps 8.5 – 8.6** is consistent with the Qubit measurement.

NOTE ***Alternatively pooled libraries may be quantified using quantitative PCR (KAPA Library Quantification Kit Illumina Platforms, PN KK4873).***

Genomic Protocol

9 Sequence Library

9 Sequence Library

Parameter	Specification
Final library size	**350 bp – 550 bp** with peak at ~460 bp
Supported sequencers	MiSeq, HiSeq 2500, NextSeq 550, HiSeq 4000, NovaSeq 6000
Supported sequencing chemistries	See Table 7 on next page.
Index 1 (i7)	**Yes** (8nt). Index 1 – 8 sequences are different from Illumina's Indices. *Illumina Indices may be used (4 µM).*
Index 2 (i5)	**Yes** (8nt). Index 1 – 8 sequences are different from Illumina's Indices. *Illumina Indices may be used (4 µM).*
Number of unique i7/i5 index pair per sample	**1**
Custom sequencing primer?	**No**
Sequencing chemistry	**2 x 150 bp** (in some cases 500 cycle kits may be used with 300 cycle runs programmed)
PhiX %	**5 % – 20 %** see Table 7 on next page
Compatible with non-Tapestri libraries?	**Yes,** if libraries are of similar size.
Number of expected FASTQ files per sample	**2: one Read 1/Read 2 pair representing one unique i7/i5 combination** If the library is distributed across more than one flow cell lane, please merge lane-specific FASTQ files that belong to one sample.

Please refer to the Illumina User Guides listed in **Table 8**. A detailed overview of how to set up a sequencing run for MiSeq Systems can be found in the **Appendix - MiSeq Sequencing Reaction Setup**.

					Samples / Sequencing Run / Flow Cell						
					Panel (# of amplicons)						
Sequencer	**Final Library Input [pM]**	**PhiX %**	**Cluster Density [K/mm2]**	**# of Lanes**	**AML 50**	**MYE 330**	**THP 244**	**CLL 286**	**Custom variable**	**Sequencing Chemistry**	**# of Read Pairs*** [10^6]**
MiSeq V3*	20 – 22	5 %	1,200 – 1,500	1	1	0	0	0	Varies	V3 Paired End	25 – 30
HiSeq 2500**	8 – 10	10 %	750 – 900	2	8	1	2	1	Varies	V2 SBS Rapid Mode	300 – 350
NextSeq 550 Mid-Output	1.8 – 2	20 %	150 – 175	4	3	0	1^	1^	Varies	V2.5 Paired End	120 – 140
NextSeq 550 High-Output	1.8 – 2	20 %	150 – 175	4	10	2^	2	2^	Varies	V2.5 Paired End	360 – 420
HiSeq 4000	250 – 300	15 %	1,350 – 1,550	8	118	18	24	20	Varies	Standard SBS	4,000 – 5,000
NovaSeq 6000 SP	300 – 400	15 %	600 – 800	1	18	3	4	3	Varies	SP Reagent 300 cycles	650 – 800
NovaSeq 6000 S1	300 – 400	15 %	> 80% occupancy	2	36	6	7	5	Varies	S1 Reagent 300 cycles	1,300 – 1,600
NovaSeq 6000 S2	300 – 400	15 %	> 80% occupancy	2	90	14	19	16	Varies	S2 Reagent 300 cycles	3,300 – 4,000
NovaSeq 6000 S4	300 – 400	15 %	> 80% occupancy	4	250	34	46	39	Varies	S4 Reagent 300 cycles	8,000 – 10,000

Table 7. Recommended sequencing specifications and sample multiplexing.

*MiSeq V3 kit is only available as 600 cycle kit which needs to be run with 2x150bp paired end sequencing
**HiSeq 2500 V2 SBS Rapid Mode kit is only available as 500 cycle kit which needs to be run with 2x150bp paired end sequencing
***Paired-end sequencing required ^With 10,000 cell output, coverage may be below recommendations.

User Guide	**MiSeq**	**HiSeq 2500**
System Guide	MiSeq System Guide (PN 1000000061014)	HiSeq 2500 System Guide (PN 15035786)
Denaturing and Diluting Libraries Reference Guide	MiSeq System – Denature and Dilute Libraries Guide (PN 15039740)	HiSeq Systems – Denature and Dilute Libraries Guide (PN 15050107)
Custom Primer Guide	MiSeq System – Custom Primers Guide (PN 15041638)	HiSeq System – Custom Primers Guide (PN 15061846)
cBot System	-	cBot System Guide (PN 15006165)
User Guide	**HiSeq 4000**	**NovaSeq 6000**
System Guide	HiSeq 4000 System Guide (PN 15066496)	NovaSeq 6000 System Guide (PN 1000000019358)
Denaturing and Diluting Libraries Reference Guide	HiSeq Systems – Denature and Dilute Libraries Guide (PN 15050107)	NovaSeq 6000 System Guide (PN 1000000019358)
Custom Primer Guide	HiSeq System – Custom Primers Guide (PN 15061846)	NovaSeq Series – Custom Primers Guide (PN 10000000222266)

Table 8. Illumina User Guides

Troubleshooting

Step	Problem	Potential Cause	Recommended Action
Instrument	Instrument lid does not close.	DNA cartridge and/or Gasket not properly installed.	Check correct orientation of DNA cartridge and ensure that the Gasket is properly seated on DNA Cartridge.
		Multiple Gaskets installed.	Make sure no second Gasket is still attached under the lid before closing.
		One or both pins on the side of the chip door missing.	Ensure that both pins are on either side. Contact Support.
	Instrument reports sealing error message.	Gasket and/or manifold not clean.	Check that the Gasket and manifold are clean and free of dust.
		DNA cartridge and/or Gasket not properly installed.	Check correct orientation of DNA cartridge and ensure that the Gasket is properly seated on DNA cartridge.
		Multiple Gaskets installed.	Make sure no second Gasket is still attached under the lid before closing.
	Touch screen becomes unresponsive.	Instrument operating system under-powered.	Power cycle instrument by turning it off, wait 20 seconds, and turning it back on.
Encapsulation	Volumes of cell emulsion and/or oil are too low.	Clogged channel on the DNA cartridge.	Contact Support.
		Reagents loaded incorrectly on DNA cartridge.	Ensure proper cartridge reagent loading according to the instructions.
		Instrument lid broken.	Ensure that both pins are on either side. Contact Support.
Barcoding	Volumes of cell emulsion and/or oil are too low in all or a subset of eight tubes.	Subset of channels clogged on the DNA cartridge.	Contact Support.
		DNA cartridge and/or Gasket not properly installed.	Check correct orientation of DNA cartridge and ensure that the Gasket is properly seated on DNA cartridge.

	If only a subset is affected, you may proceed the workflow with the unaffected tubes.	Incorrect reagent loading on DNA cartridge.	Ensure proper cartridge reagent loading following the instructions.
		Instrument lid broken.	Ensure that both pins are on either side. Contact Support.
Targeted PCR	Low DNA yield < 0.2 ng/µl.	UV cleave step omitted.	Repeat the protocol with a fresh aliquot of sample. If the problem persists, contact Support.
		Sample lost during AMPURE cleanup.	Ensure to use fresh 80% EtOH and follow protocol instructions.
		Incorrectly prepared Barcoding Master Mix.	Ensure to add 60 µl of Barcoding Mix Additive to 240 µl of Barcoding Mix.
Library PCR	Low DNA yield < 1.0 ng/µl.	Lost sample during AMPURE cleanup.	Ensure to use fresh 80% EtOH and follow protocol instructions.
		Template DNA concentration too low.	Ensure to accurately quantify the input concentration of Targeted PCR product using the Tapestri Sample Quantification Tool (PN 40676).
		Incorrectly prepared Library PCR Master Mix.	Ensure to correctly prepare the Library PCR Master Mix with 5 µl of Library Prep Primer and 5 µl of one of the eight Library P7 Indices 1 - 8 per sample.
Sequencing	No sequencing data generated/only PhiX sequencing data generated.	Forgot Mission Bio Custom Seq Primer.	Add 4 µl of Mission Bio Custom Seq Primer into the designated well of the Illumina Sequencer cartridge and mix well. Refer to Illumina's *Custom Primer's Guide* document.

Appendices

Frequently Asked Questions (FAQs)

Can I use Tapestri to measure mRNA transcripts in single cells?

No. Tapestri is currently available only for genomic (DNA) single cell analysis.

Can I use my own Library and Targeted PCR reagents?

No. Only the reagents supplied with the Tapestri Single Cell DNA AML, CLL, Myeloid, THP or Custom Bead Kit are fully validated and supported.

Can I analyze more cells by increasing my cell sample concentration?

The Tapestri workflow and instrumentation have been optimized for cell concentrations between 3,000 and 4,000 cells/µl. Working with cell concentrations outside of this range is not recommended.

Can I store my emulsions after Cell Encapsulation or Cell Barcoding for a few days before continuing with the protocol?

The Cell Encapsulation emulsions (1 tube) may be stored at 4 °C overnight. The Cell Barcoding emulsions (8 tubes) must be processed immediately to ensure efficient PCR amplification of targets.

Can I use the Nanodrop for both Targeted PCR and Library PCR quantification?

For targeted PCR product quantification we strongly suggest using the Qubit Fluorometer and/or Agilent Bioanalyzer. Quantification of the products after Library PCR must be performed with a method that measures the quantity and size of PCR products (i.e., Bioanalyzer, Tapestation). We do not recommend using the Nanodrop to quantify PCR products as concentration measurements may be inaccurate.

How should I pool my samples for sequencing?

Please follow the instructions in the *Tapestri Sample Quantification Tool (PN 40676)*. Individual sample-tube libraries are pooled equimolar yielding a 5 nM pooled library.

Can I pool my Tapestri samples with other Illumina Indexed samples for sequencing?

Yes. Please refer to the Appendix of this document for additional information.

Can I run my Tapestri Single-Cell DNA Library on a NextSeq or HiSeq or NovaSeq?

Yes, Mission Bio currently supports MiSeq, HiSeq 2500, HiSeq 4000, NextSeq 550 and NovaSeq 6000 platforms.

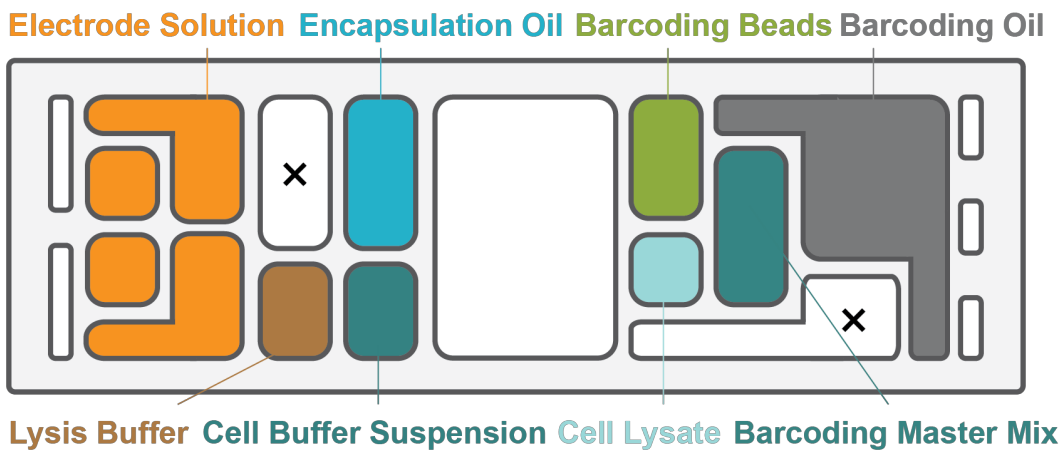
What sequencing depth is required, per cell, per amplicon?

Please refer to the *Table 8 - Recommended sequencing specifications and sample multiplexing* on page 44.

Can I design my own primers for the Tapestri Platform?

Yes. Please contact ***support@missionbio.com*** to learn more about our Custom Panel Program.

Cartridge Map



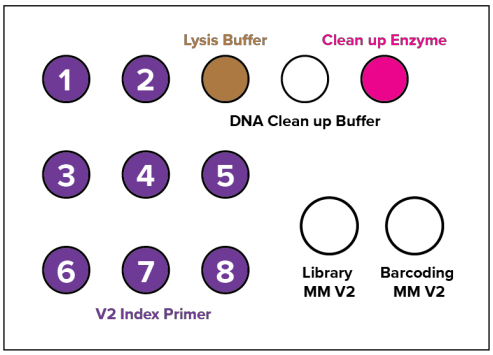
Sequence Information for V2 Index Primer 1 – 8

Primer	Sequence i7	Sequence i5
1	CTGATCGT	ATATGCGC
2	ACTCTCGA	TGGTACAG
3	TGAGCTAG	AACCGTTC
4	GAGACGAT	TAACCGGT
5	CTTGTCGA	GAACATCG
6	TTCCAAGG	CCTTGTAG
7	CGCATGAT	TCAGGCTT
8	ACGGAACA	GTTCTCGT

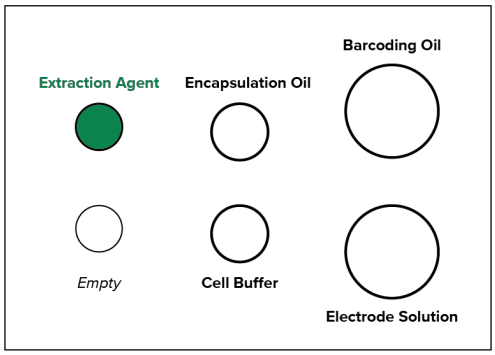
Table 9. Sequence nucleotide information for V2 Index Primer 1 – 8. Sequences are unique to Mission Bio and do not overlap with Illumina's i7 indices (N701 to N729).

Kit Contents

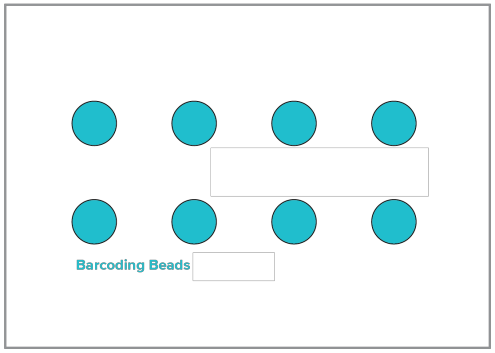
TAPESTRI SINGLE-CELL DNA CORE -20°C KIT



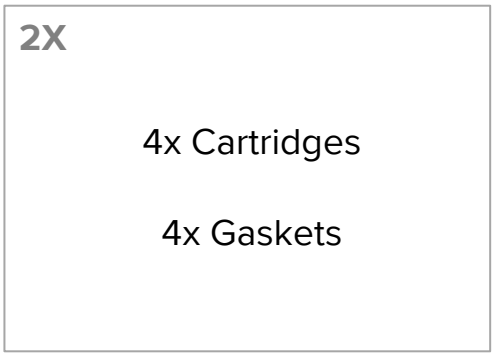
TAPESTRI SINGLE-CELL DNA CORE AMBIENT KIT



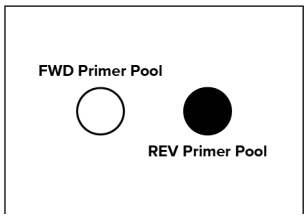
TAPESTRI SINGLE-CELL DNA BEAD KIT



CARTRIDGE KIT



PRIMER POOL KIT



MiSeq Sequencing Reaction Setup

Dilute Reagents

A.1 Dilute 1 µl of the 10 nM PhiX stock in 9 µl Library dilution buffer to prepare a 1 nM PhiX control sample.

Denature and neutralize library and PhiX DNA

A.2 Thaw the HT1 Buffer.

A.3 Prepare a fresh aliquot of 0.2 M NaOH by combining 200 µl 1 M NaOH and 800 µl nuclease-free water.

A.4 In a 1.5 mL low-bind Eppendorf tube Combine 5 µl of the 5 nM pooled library with 5 µl of 0.2 M NaOH. Vortex and spin down.

A.5 In a 1.5 mL low-bind Eppendorf tube Combine 5 µl of 1 nM PhiX control sample with 5 µl of 0.2 M NaOH. Vortex and spin down.

A.6 Incubate both the pooled sample library and the PhiX library for at least 10 minutes at room temperature.

A.7 Neutralize the pooled sample library by adding 990 µl of HT1 Buffer. The sample library now has a concentration of 25 pM.

A.8 Neutralize the PhiX library by adding 990 µl of HT1 Buffer. The PhiX library now has a concentration of 5 pM.

Load final sample and custom primers on MiSeq cartridge

A.9 Prepare a final library with a 5% PhiX spike-in by combining the following reagents. The amounts shown are for a standard run and provide a final library loading concentration of ~ 20 pM with 5% PhiX spike-in.

Reagent	Volume (µl)
Denatured Pooled Library (25 pM)	532
Denatured PhiX Sample (5 pM)	140
HT1 Buffer	28
Total	700

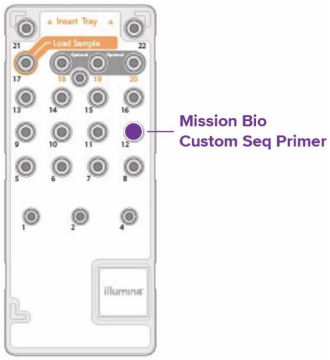
Table 10. Reagents for sequencing-ready library.

A.10 On the Cartridge Tray, use a pipette tip to puncture the foil seal on well 17 and then carefully load 650 µl of your sample into the well.

NOTE ***Well number 12 contains 680 µl Illumina's Read 1 primer mix, which needs to be replaced by Mission Bio's Custom Seq Primer.***

A.11 Use a Narrow Stem Transfer pipette (Thermo Fisher) to carefully transfer 400 µl of Illumina Read 1 primer mix from well 12 to a new low-bind 1.5 mL Eppendorf tube.

A.12 Add 4 µl of the Mission Bio Custom Seq Primer (●) to the mix and, vortex and spin down. Carefully reload the entire mix into well 12 of the Miseq cartridge.



NOTE ***The final concentration of the primer is ~ 0.5 µM.***

A.13 Sequence samples on a MiSeq instrument following manufacturer's instructions.

References

1. Lgr6 is a stem cell marker in mouse skin squamous cell carcinoma. P.Y. Huang et al., *Nature Genetics* 49(11):1624-1632 (2017).
2. RNA-Seq following PCR-based sorting reveals rare cell transcriptional signatures. M. Pellegrino, A. Sciambi, J.L. Yates, J. Mast, C. Silver, D.J. Eastburn, *BMC Genomics* 17:361 (2016).
3. Ultrahigh-Throughput Mammalian Single-Cell Reverse-Transcriptase Polymerase Chain Reaction in Microfluidic Drops. D.J. Eastburn, A. Sciambi, A.R. Abate, *Analytical Chemistry* 85, 8016 (2013).
4. Microfluidic droplet enrichment for targeted sequencing. D.J. Eastburn, Y. Huang, M. Pellegrino, A. Sciambi, L. Ptáček, A. Abate, *Nucleic Acids Research* Jul 27; 43(13):e86. (2015).
5. Picoinjection enables digital detection of RNA with droplet rt-PCR. D.J. Eastburn, A. Sciambi, A.R. Abate, *PLoS ONE* 8(4): e62961 (2013).
6. Identification and genetic analysis of cancer cells with PCR-activated cell sorting. D.J. Eastburn, A. Sciambi, A.R. Abate, *Nucleic Acids Research* 42, e128 (2014).

Tapestri Instrument Specifications

- Model: Tapestri Instrument
- Part Number (PN): 191335
- Mains Voltage: 115 VAC
- Frequency: 50/60 Hz
- Current: 2.0 A Max.
- Circuit Breaker: 16 Amp
- Ambient Temperature Range: 15 °C to 30 °C (59 °F – 86 °F)
- Relative Humidity (Non-Condensing): 5% to 85%
- Maximum Altitude: 6,562 ft (2,000 m)
- HV Cable Length: 59" (1500 mm)
- Overall Dimensions. H/W/D: 12.5"/31.75 cm x 11.75"/29.85 cm x 12.25"/31.10 cm

Visit www.missionbio.com for additional support.