tapestri

Tapestri® Single-Cell DNA + Protein Sequencing v3

User Guide

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Introduction

The *Mission Bio Tapestri® Platform* uses microfluidic droplet technology to combine single-cell lysates with barcoding beads and 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.

Tapestri® Platform Overview

The *Mission Bio Tapestri® Platform* consists of the instrument itself, the DNA cartridge, which represents the microfluidics device, and the 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 reagents from the reservoirs through the microfluidic device and out to PCR collection tubes that are mounted below the cartridge. The cartridge and tubes can be loaded and unloaded from the instrument and disposed of after the completion of the workflow. 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.

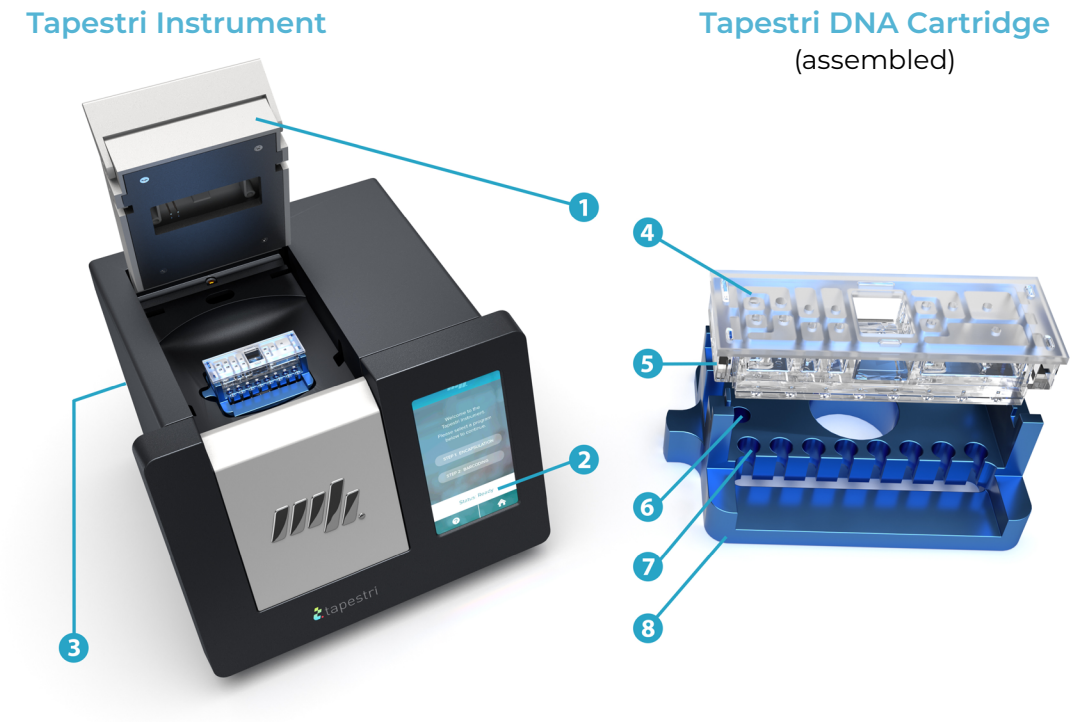


Figure 1. Tapestri Platform: Instrument and Assembled DNA Cartridge.

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 catridge.

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

6. **Encapsulation Collection Tube Slot**
To collect encapsulation emulsions.

7. **Barcoding Collection Tube Slots**
To collect barcoding emulsions.

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

Materials

Tapestri Single-Cell DNA + Protein Core Kit v3 Configuration

Component Name	Part Number	Storage
Tapestri Single-Cell DNA Core +4 Kit v3	MB03-0092	4°C
Tapestri Single-Cell DNA Core -20 Kit v3	MB03-0091	-20°C
Tapestri Single-Cell DNA Bead Kit v3	MB03-0093	-20°C
Tapestri Protein Staining Kit v3	MB03-0094	4°C

Tapestri Single-Cell DNA Core Kit v3 Components

Component Name	Kit	Storage
Cell Buffer	Tapestri Single-Cell DNA Core +4 Kit v3	4°C
Encapsulation Oil		4°C
Electrode Solution		4°C
Barcoding Oil		4°C
Extraction Agent (green cap)		4°C
Lysis Buffer (brown cap)	Tapestri Single-Cell DNA Core -20 Kit v3	-20°C
Barcoding Mix		-20°C
Library Mix		-20°C
Library Indices 1 – 8 (purple cap)		-20°C
Barcoding Beads (blue cap)	Tapestri Single-Cell DNA Bead Kit v3	-20°C

Tapestri Protein Staining Kit Reagents

Component Name	Kit	Storage
Blocking Buffer (orange cap)	Tapestri Protein Staining Kit v3	4°C
Antibody Tag Primer (red cap)		4°C
Biotin Oligo (blue cap)		4°C
Streptavidin Beads (brown cap)		4°C
2x Wash Buffer		4°C
Protein Library Indices 1-8 (yellow cap)		4°C

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

Required Third Party Consumables and Reagents

Component Name	Suggested Supplier (Part Number)	Protocol Step
TotalSeq™-D Heme Oncology Cocktail	BioLegend (399906)	Cell Staining
Human TruStain FcX (Fc Receptor Blocking Solution)	BioLegend (422301)	Cell Staining
Cell Staining Buffer	BioLegend (420201)	Cell Staining
AMPure XP Reagent	Beckman Coulter (A63880)	Targeted PCR, Library PCR
Qubit® dsDNA HS Assay Kit	Qubit® (Q32851)	Targeted PCR
Ethanol, Molecular Biology Grade	Sigma (E7023) or Approved Supplier	AMPure purification
Agilent DNA 1000 Kit or Agilent DNA High Sensitivity Kit	Agilent Technologies (5067-1504) Agilent Technologies (5067-4626)	Post-PCR quantitation
Trypan Blue	Thermo Fisher (15250061) or Approved Supplier	Dead cell staining
Propidium Iodide (OPTIONAL)	Thermo Fisher (P3566) or Approved Supplier	Dead cell staining
TipOne RPT ultra low retention filter tip	USA Scientific (1180-8810) or Approved Supplier	Liquid handling
200 µL Wide bore tip, rack, sterile 1000 µL Wide bore tip, rack, sterile	USA Scientific (1011-8410) USA Scientific (1011-9410)	Cell handling
Flowmi™ Cell Strainers for 1000 µL pipette tips, 40 µm	Fisher Scientific (14-100-150)	Cell handling
1.5 mL DNA LoBind Microcentrifuge Tubes	Eppendorf (0030108035) or Approved Supplier	Cell/Reagent handling
* 0.2 mL Emulsion safe PCR tubes	USA Scientific (1402-8120) or Axygen (PCR-02-L-C) or Axygen (PCR-02D-L-C)	Emulsion handling
200 uL Gel Loading Pipette Tips	Axygen (TGL200RD57R) or Approved Supplier	Emulsion handling
0.2 mL PCR Tubes	USA Scientific (1402-4708) or Approved Supplier	Non-emulsion PCR
Qubit Assay Tubes	Thermo Fisher (Q32856)	Post-PCR quantitation
15 mL DNA LoBind conical tubes	Eppendorf (30122208) or Approved Supplier	Protein
1.5 mL Protein LoBind tubes	Eppendorf (22431081) or Approved Supplier	Protein
KAPA Library Quantification Kit Illumina Platforms (OPTIONAL)	KAPA (KK4873)	Sequencing
Sequencing Reagent Kit 300 cycles (150bp PE) (MiSeq, HiSeq 2500, HiSeq 4000, NextSeq 550/1000/2000, NovaSeq 6000, NovaSeq X, NextSeq 2000)	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)
Fluorescence microscope (optional)	Thermo Fisher or Approved Supplier
Centrifuge with temperature control and swinging bucket (needs to support 15 mL conical tubes)	Eppendorf (5810 R) or Approved Supplier
Agilent 2100 Bioanalyzer or Tapestation	Agilent (G2939BA), (G2992AA), (G2991BA)
Qubit Fluorometer	Qubit: Thermo Fisher (Q33216)
Pipettes, 1 µL – 1000 µL	Mettler-Toledo, Rainin Pipettes, or Approved Supplier
Microcentrifuge (1.5 mL PCR tubes) with temperature control	Thermo Fisher (5406000240) or Approved Supplier
Microcentrifuge (1.5 mL, 0.2 mL PCR tubes)	Thermo Fisher (75004081) or Approved Supplier
Tube Vortexer	Thermo Fisher (88880017TS) or Approved Supplier
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
Thermo Mixer	Eppendorf (5382000023) or Approved Supplier
Rotating Shaker	Thermo Fisher (15920D) or Approved Supplier
0.2 mL 8-strip PCR tube Magnetic Separation Stand	Seqmatic (TM-700) or Approved Supplier
1.5 mL tube Magnetic Separation Rack	New England Biolabs (S1506S) or Approved Supplier

Protocol Overview

Single cells stained with oligo-tagged antibodies are individually partitioned into 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. The Protein library is separated from the DNA library by size selection. Protein and DNA libraries are indexed and amplified separately. Final libraries are purified and can be sequenced on one of the supported Illumina Sequencer instruments.

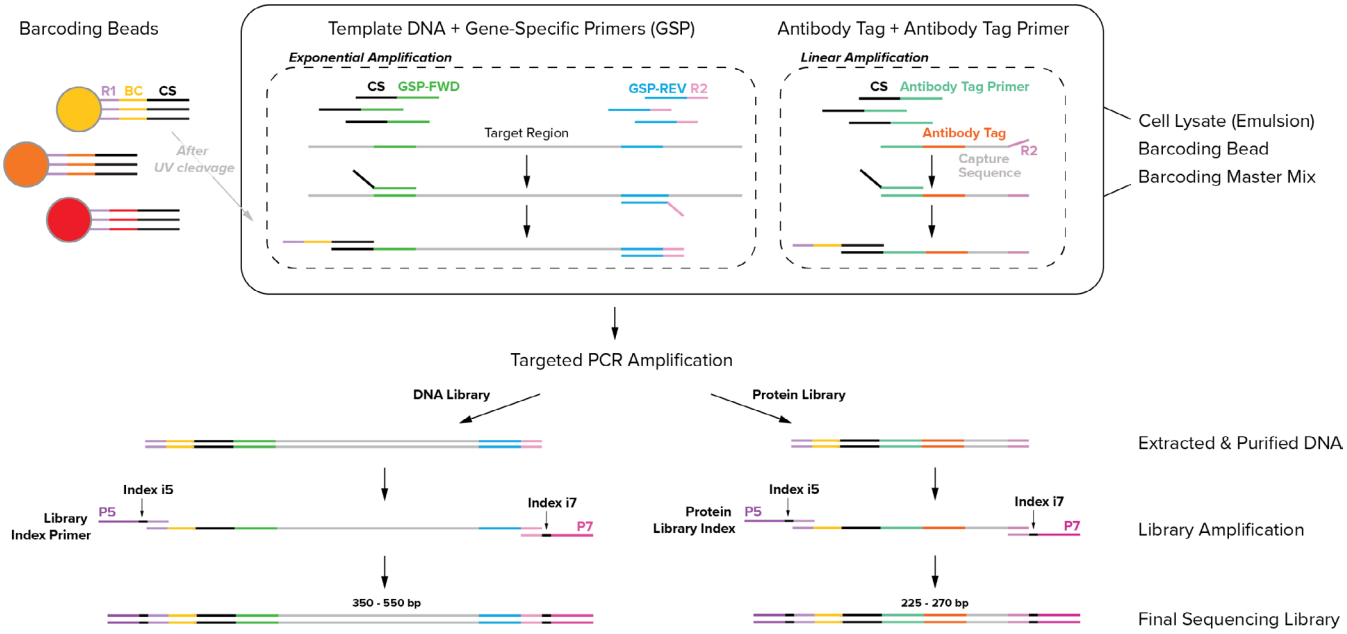


Figure 2. Overview of library construction. R1: Read 1, BC: barcode, CS: common sequence, GSP-FWD: gene-specific forward primer, GSP-REV: gene-specific reverse primer, P5: P5 Illumina adapter, P7: P7 Illumina adapter.

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 (targeted PCR, 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 direct contact with the sidewall of the tube, where emulsions directly interface, and hold tubes on the lid instead.

Cell Recovery

- Always pipette slowly and carefully when removing the supernatant and leave at least 0.5 mL of residual volume in the tube between the washing steps.
- Centrifugation times can be increased from 5 to 10 minutes to increase cell recovery.

Suggestions for working with the Tapestri Instrument and DNA Cartridge

- Avoid introduction of particles, fibers or clumped cells into the cartridge that may potentially clog the cartridge.
- Minimize exposure of the instrument, reagents, cartridges, and 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 their 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)*.

Thermal Cycling Programs

Always use a properly calibrated thermal cycler suited for 0.2 mL tubes with a minimum reaction volume of 100 µL for all incubations. Program all three thermal cycling protocols from Table 1 into the instrument. For all protocols, use a heated lid set to 105 °C and always use an adapter tray. 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

			2. Targeted PCR				
		Amplicon Number	**20 – 100**	**100 – 200**	**200 – 300**	**> 300**	
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	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	

3. Library PCR			
Step	**Temperature**	**Time**	**Cycle**
1	95 °C	3 min	
2	98 °C	20 sec	DNA | 10 PROT | 20
3	62 °C	20 sec	
4	72 °C	45 sec	
5	72 °C	2 min	
6	4 °C	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 using at least two fields of view. Concentrations found must agree within 10%.
- Cell suspensions must have > 90% viability. Mission Bio recommends Propidium Iodide, rather than Trypan Blue, for measuring viability (see below).
- 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.

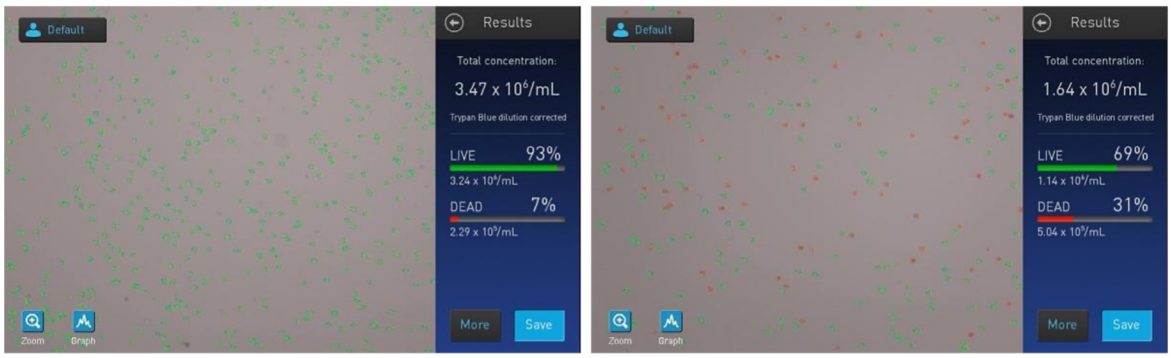


Figure 3. Representative images of high-quality cell suspension (left) and low-quality cell suspension (right).

Cell death assessment using Propidium Iodide (PI)

Mission Bio strongly recommends the use of fluorescent exclusion reagents such as Propidium Iodide (PI) to determine cell death/viability. PI-based assays compared to Trypan Blue-based assays may be more robust in accurately determining the percentage of dead/viable cells. Please follow the manufacturer's instructions when using PI-based viability assays.

DNA + Protein Protocol

1. Prepare Cell Suspension

DNA + Protein Protocol

1. Prepare Cell Suspension

This section describes the steps required to prepare a single-cell suspension, count cells, assess cell viability and cell suspension quality, and stain cells with oligo-tagged antibodies. The workflow is optimized for a starting cell number of 0.6 x 10^6 to 1 x 10^6 at greater than 90% viability in DPBS (w/o Ca^{2+}/Mg^{2+}) with a minimum volume of 40 µL. Some cell loss is to be expected throughout the antibody staining and washing procedure and therefore a recommended 15,000 – 25,000 cells/µL ensures a minimum cell concentration of 2,800 – 3,200 cells/µL in 35 µL needed for encapsulation.

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 cell lines, PBMCs, or BMMCs to be cryopreserved in 2 mL cryovials in a total volume of 0.5 mL and stored in liquid nitrogen or -80°C.***

Thaw Cells

1.1 Retrieve all reagents required for preparing the cell suspension:

- Cell Buffer (4 °C, +4 Kit) → place on ice
- Human TruStain FcX (Biolegend) → place on ice
- Blocking Buffer (●) (4 °C, Protein Staining Kit) → place on ice
- Cell Staining Buffer (BioLegend) → place on ice
- Reconstituted TotalSeq™-D Heme Oncology Cocktail → keep at RT
- Flowmi Cell Strainer

1.2 **Warm thawing media** (for instance 40% FBS + 60% base media) to 37 °C.

1.3 Remove cryovial of cells from liquid nitrogen or the -80 °C freezer, **immediately transfer** to a biosafety hood, twist the cap a quarter to relieve pressure, and immediately retighten.

1.4 **Immediately transfer to a 37° C water bath**, quickly thaw the vial by gently swirling the tube until a small amount of ice remains (< 1 minute). Be sure to avoid submerging the tube completely.

1.5 Remove tube and clean with 70% ethanol.

1.6 Using aseptic techniques, add **1 mL of pre-warmed thawing media drop-wise** to the cryovial. Transfer the entire contents of the vial to a 15 mL conical tube.

1.7 Using a wide bore P-1000 tip, **rinse** the vial with **1 mL of pre-warmed thawing media.** Transfer to the 15 mL conical tube containing the cells, drop by drop, making sure to pipette against the wall. Gently shake tube while adding.

1.8 Add **2 mL of thawing media** to 15 mL tube, drop by drop, making sure to pipette against the wall, and gently shake the tube while adding.

1.9 **Add 0.5 mL of thawing media** to 15 mL tube **every few seconds until 12 mL total volume** is reached. Gently mix the tube by hand after each addition.

1.10 Centrifuge at **400 x g for 5 minutes** at room temperature.

1.11 Immediately aspirate supernatant, leaving 0.5 mL to 1 mL of thawing media behind. **Do not disturb the cell pellet.**

1.12 Using a wide bore tip, gently resuspend the cell pellet in remaining thawing media by pipetting up and down ~5x.

1.13 Add 10 mL of thawing media to the tube.

1.14 Centrifuge at **400 x g for 5 minutes** at room temperature.

1.15 Immediately aspirate all supernatant. **Do not disturb the pellet**.

1.16 Using a wide bore P-1000 tip, resuspend the cells in 1 mL of Cell Staining Buffer (BioLegend, 420201) (CSB).

1.17 Centrifuge at **400 x g for 5 minutes** at room temperature.

1.18 Immediately aspirate all supernatant. **Do not disturb the pellet**.

1.19 Resuspend the cells in **50 µL of CSB**.

1.20 Quantify the cells using an automated cell counter or hemocytometer following best practices and the manufacturer's instructions.

1.21 Dilute cell suspension to **25,000 cells/µL using CSB** in a **minimum volume of 40 µL**.

1.22 **Store the cells on ice** until used for staining the cells (***Step 1.30***) and proceed **immediately** to ***Step 1.23***.

IMPORTANT ***Cells must not be stored longer than 30 minutes as a subset of cells (e.g., monocytes) are prone to stick to the tube plastic and may be unrecoverable.***

Reconstitute Antibody-Oligo Conjugate (AOC) Panel

The TotalSeq™-D Heme Oncology Cocktail (BioLegend) is supplied lyophilized in single reaction vials. The panel needs to be reconstituted prior to staining the cells.

1.23 Retrieve a vial of the room-temperature equilibrated lyophilized TotalSeq™-D Heme Oncology Cocktail.

1.24 Centrifuge the tube at **10,000 x g for 30 seconds** at room temperature.

1.25 Add **60 µL of CSB** (BioLegend) to the lyophilized panel. Close the tube with the original cap and vortex for 10 seconds.

1.26 Incubate at **room temperature for 5 minutes**.

1.27 **Vortex** the tube for **10 seconds** and centrifuge at **10,000 x g for 30 seconds** at room temperature.

1.28 **Transfer the entire volume (60 µL)** of reconstituted panel to a **Protein LoBind** Eppendorf tube.

1.29 Centrifuge the tube at **14,000 x g for 15 minutes at 4° C**. Once completed, the reconstituted panel must be used immediately in ***Step 1.32***. In the meantime, proceed to the next step.

Stain Cells

1.30 In a 15 mL DNA **LoBind** conical tube add the following reagents:

Reagent	Volume (µL)
Cell Suspensions in CSB (25,000 cells/µL)	40.0
Blocking Buffer (●)	5.0
Human TruStain FcX	5.0
Total Volume	**50.0**

Table 2. Reagents to block cells.

1.31 **Gently mix** by pipetting up and down with a 200 µL wide bore tip and **incubate the solution for 15 minutes on ice**.

1.32 After 15 minute incubation, using a P-200, **aspirate 50 µL** of the reconstituted TotalSeq™-D Heme Oncology Cocktail and **add to the blocked cell suspension**. Cell staining solution total volume is 100 µL.

IMPORTANT ***Avoid touching the bottom or sides of the tube containing the reconstituted TotalSeq™-D Heme Oncology Cocktail with the pipette tip to avoid pelleted protein aggregates. Aggregates are not visible.***

1.33 **Gently mix** by pipetting up and down with a 200 µL wide bore tip.

1.34 **Incubate cell staining solution for 30 minutes on ice.**

1.35 **Add 14 mL of pre-chilled CSB** to the cell staining solution, pipetting against the tube wall.

1.36 Centrifuge at **400 x g for 10 minutes at 4° C** in a swinging bucket.

1.37 Carefully **aspirate and discard 13.5 mL** of supernatant using a serological pipette.

IMPORTANT ***Aspirate from the top of the solution and avoid touching the bottom and sides of the tube. Leave at least 0.5 mL of supernatant behind. Do not disturb or resuspend the cell pellet. Cell pellet may not be visible.***

1.38 **Repeat** steps ***1.35*** to ***1.37*** for two additional washes, centrifuging at **400 x g for 5 minutes each at 4° C**.

1.39 **Remove and discard supernatant, leaving ~100 µL:** Aspirate all but 1 mL of supernatant using a serological pipette, then switch to a P-1000 tip to remove the remaining supernatant (~900 µL).

1.40 **Add 900 µL of Cell Staining Buffer** to the cell pellet and resuspend by gently pipetting up and down several times using a 1 mL wide bore tip.

1.41 **Filter the cells with a 40 µm Flowmi cell strainer:** Aspirate 1 mL of the cell suspension, insert the filter onto a 1.5 mL DNA LoBind Eppendorf tube, and release the filtered suspension through the filter into the new tube

1.42 Centrifuge at **400 x g for 5 minutes at 4° C.**

1.43 Inspect the cell pellet and carefully remove all supernatant. **Do not disturb the cell pellet.** Use a P-200 or P-20 to remove all the supernatant.

1.44 **Resuspend the pellet in 50 µL of Cell Buffer (Mission Bio)** by pipetting up and down several times.

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

1.46 Dilute cell suspension to **2,800 – 3,200 cells/µL** using Cell Buffer. Confirm concentration using cell counter prior to loading.

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

IMPORTANT ***Use of cell concentrations outside the range of 2,800 – 3,200 cells/µL or viability below 90% may adversely affect results.***

1.47 **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.

DNA + Protein Protocol

2. Encapsulate Cells

2. Encapsulate Cells

In this step, cells are encapsulated with Lysis Buffer to create a cell emulsion. For input cell concentrations of 2,800 – 3,200 cells/µL, approximately 5% of all emulsion droplets will contain a cell, following a Poisson distribution.

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.

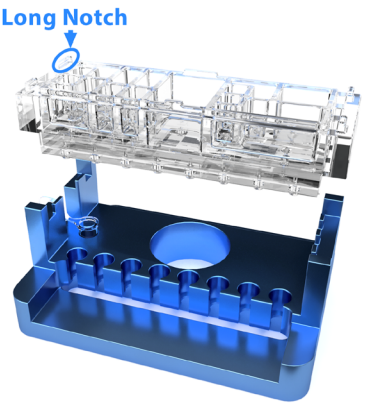
2.2 Retrieve all reagents required for cell encapsulation:

- Tapestri DNA Cartridge (Cartridge Kit) → keep at RT
- Tapestri DNA Gasket (Cartridge Kit) → keep at RT
- Lysis Buffer (●) (-20 °C, -20 Kit) → place on ice
- Reverse Primer Pool (●) (-20 °C, -20 °C Oligo Pool Kit) → place on ice
- Encapsulation Oil (4 °C, +4 Kit) → equilibrate to RT
- Cell Suspension (prepared in ***Section 1 – Prepare Cell Suspension***) → place on ice

2.3 In a 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. Pre-label and **place a 0.2 mL emulsion-safe 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.

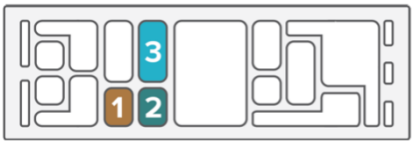
2.5 **Place the DNA Cartridge onto the Base Plate** with the long notch on the side of the cartridge oriented on the top left, as shown.

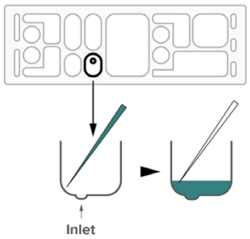
IMPORTANT ***Minimize electrostatic sources. Only Axygen MAXYmum Recovery PCR tubes (PCR-02-L-C) or (PCR-02D-L-C) and USA Scientific (1402-8120) have been validated by Mission Bio as emulsion-safe. Do not substitute with other PCR tubes.***

2.6 In a new tube, prepare **Lysis Mix** by adding **5.1 µL of Reverse Primer Pool** (●) into **65 µL of Lysis Buffer** (●), vortex and briefly centrifuge.

2.7 Pipette 60 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.

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. Be careful not to spill oil into surrounding reservoirs while loading the cartridge

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 5 minutes.

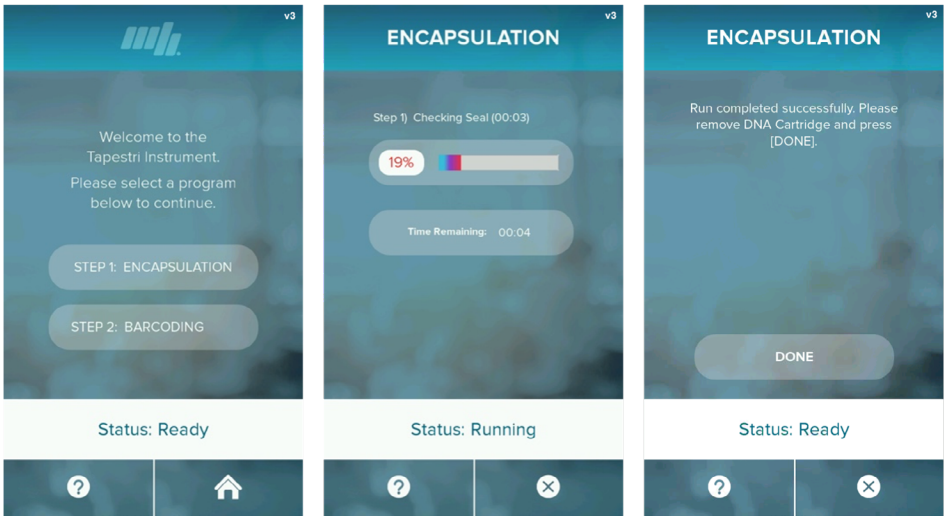


Figure 4. Touchscreen displays show main menu (left), screen after selecting 'Step 1: Encapsulation' program (middle), and final screen after Encapsulation is completed (right).

2.13 When the touchscreen displays **DONE**, carefully open the lid and **remove the cartridge and gasket from the Tapestri instrument.**

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 **Mount 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 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).

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 emulsion.***
- ***After removal, 100 µL of oil + emulsion will remain at the bottom of the tube. Make sure the entire tube volume does not exceed 100 µL.***

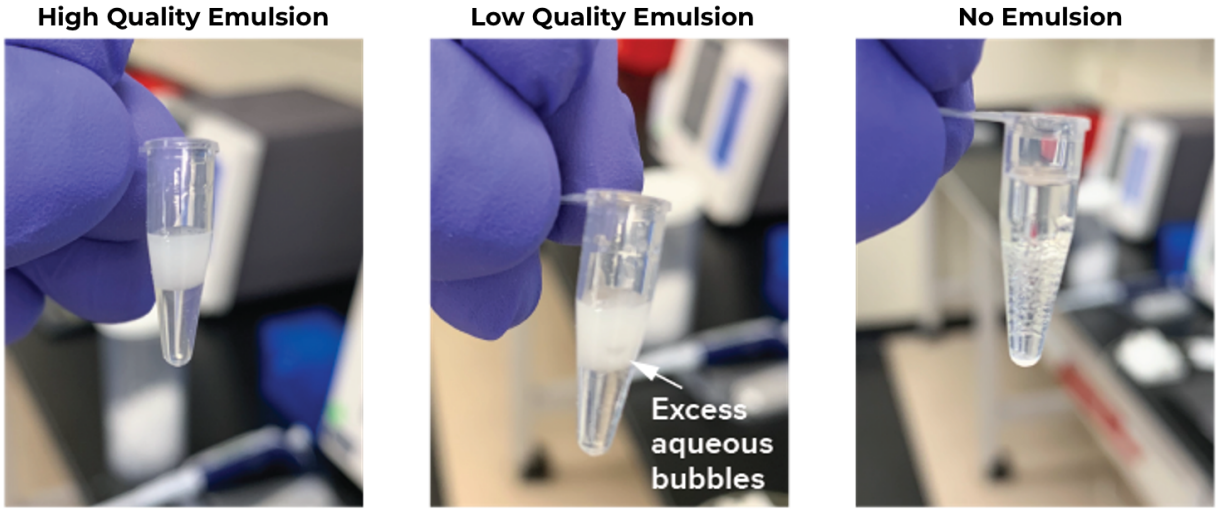


Figure 5. Emulsion Quality

If low-quality or no emulsions are detectable, please contact support@missionbio.com.

DNA + Protein 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 "Cell Lysis and Protein 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 3. 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.***

DNA + Protein Protocol

4. Barcode Cells

4. Barcode Cells

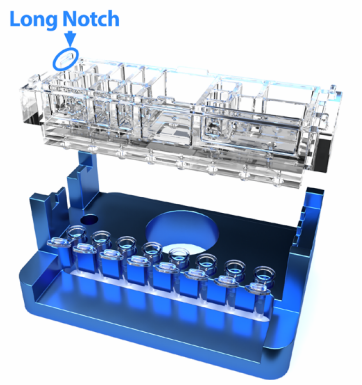
In this step, the droplets containing encapsulated cell lysate are combined with drops containing both Barcoding Mix and Barcoding Beads. These newly generated drops are then distributed into 8 PCR collection tubes, to create 8 cell-barcoding emulsion samples.

4.1 Retrieve all reagents required for Cell Barcoding:

- Barcoding Mix (-20 °C, -20 Kit) → place on ice
- Barcoding Beads (●) (-20 °C, -20 Barcoding Bead Kit) → *thaw at room temperature and protect from light.*
- Forward Primer Pool (●) (-20 °C, -20 °C Oligo Pool Kit) → place on ice
- Barcoding Oil (4 °C, +4 Kit) → equilibrate to RT
- Electrode Solution (4 °C, +4 Kit) → equilibrate to RT
- Antibody Primer Tag (●) (4 °C, Protein Staining Kit) → place on ice

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

Prime the DNA Cartridge for Barcoding

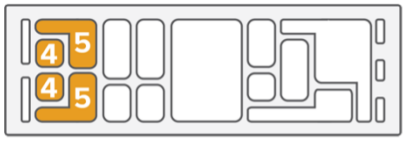


IMPORTANT ***Use emulsion-safe PCR tubes.***

4.2 Place eight 0.2 mL emulsion-safe PCR tubes **into the eight slots at the bottom of the Tapestri Base Plate** with the open lids toward you.

4.3 Mount the Tapestri DNA Cartridge (used during Cell Encapsulation) onto the Base Plate.

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 20 minutes** before automatically pausing to allow for loading of the remaining reagents.

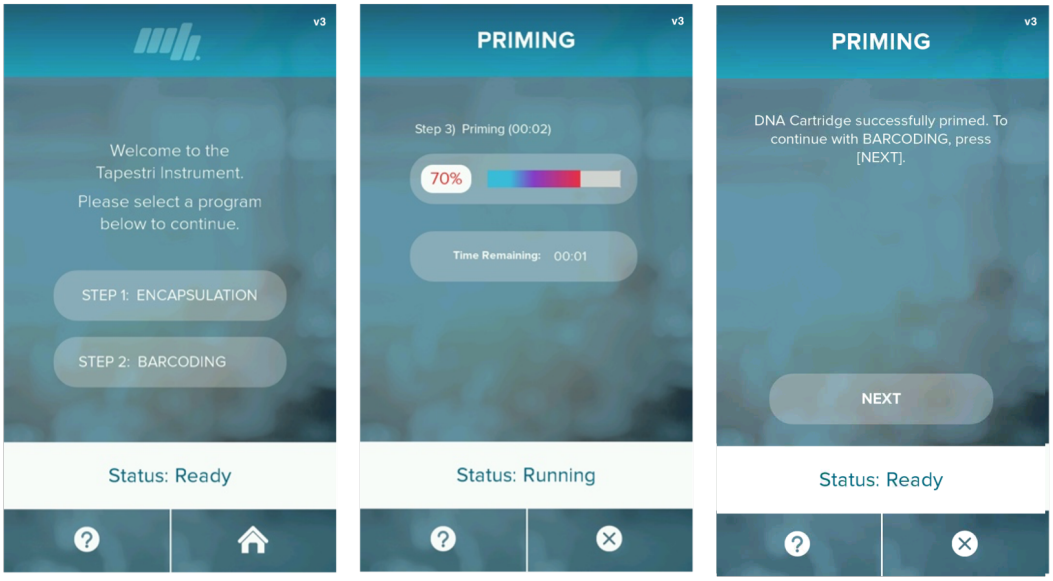


Figure 6. Touchscreen displays show main menu (left), screen after selecting 'Step 2: Barcoding'. program (middle), and final screen after Priming is completed (right).

Prepare Barcoding Mix

4.8 **Prepare 300 µL Barcode Mix** as shown in the following table. A total of 267 µL will be used for Barcoding.

Reagent	Volume (µL)
Barcoding Mix	293.0
Forward Primer Pool (⚪)	5.0
Antibody Tag Primer (🔴)	2.0
Total	**300.0**

Table 4. Reagents for Barcode Mix.

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

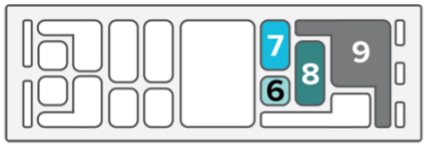
IMPORTANT ***After the Priming program has completed the Barcoding program must be started within 30 minutes.***

Load the DNA Cartridge

4.10 Once priming is complete and instrument screen displays "NEXT", quick-spin Barcoding Beads (🔵) to collect contents.

4.11 Take **67 µL** of the prepared Barcode Mix and add it to the bead tube.

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



4.12 **Retrieve the emulsion containing the encapsulated cell lysate** from the thermal cycler at 4 °C (see ***Section 3 – Lyse and Digest Cells***).

4.13 Open the instrument lid and **slowly pipette all of the contents of the encapsulated emulsion** into **reservoir 6**.

4.14 *Vortex the Barcoding Beads at full speed for 1 min*. Carefully pipette **250 µL of Barcoding Beads** (●) **into reservoir 7**. *Pipette slowly and do not introduce bubbles.*

4.15 Pipette **200 µL of Barcoding Mix** into **reservoir 8**

4.16 Pipette **1.25 mL of Barcoding Oil** into **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 Barcoding program within 1 minute of loading the Barcoding Oil.***

4.17 **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.

4.18 **Run the Barcoding program** by pressing NEXT on the Tapestri Instrument touchscreen in the following figure. This program will **complete in 45 minutes**.

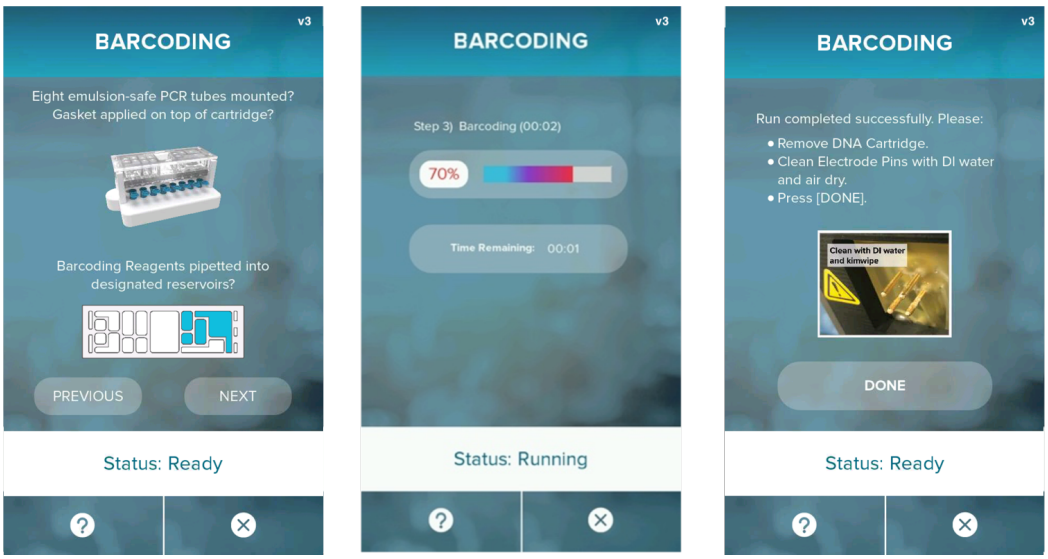


Figure 7. Touchscreen displays before the second part of Barcoding (left), the status during Barcoding (middle), and final screen after Barcoding is completed (right).

4.19 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.

NOTE ***The volumes of oil and emulsion may vary across all 8 tubes. Occasionally, a tube may be empty; this is not cause for concern as all 8 channels are connected. If more than 190 µL of Barcoding Beads or more than 15 µL of emulsions remain in reservoirs proceed with the workflow and contact support@missionbio.com.***

4.20 Visually evaluate the emulsion quality. The barcoded **emulsions are visible as a white layer** on top of the oil layer.

4.21 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.

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

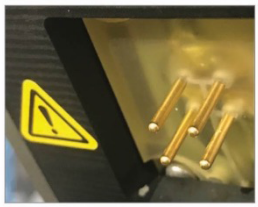
Clean Electrode Pins

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

DNA + Protein Protocol

5. Targeted PCR Amplification

5. Targeted PCR Amplification

5.1 **Transfer the samples to a 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 4 below)** that are compatible with the targeted DNA panel you processed your samples with.

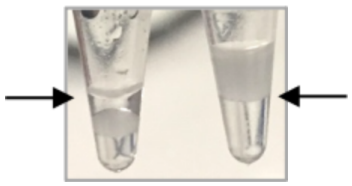
IMPORTANT ***Ensure that the emulsions in all eight tubes (white top layer) sit within the height of the block of the thermal cycler that is temperature controlled. Use a PCR skirt to ensure even heat transfer.***

		Amplicon Number	20 – 100	101 – 200	201 – 300	> 300	
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	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	

Table 5. Thermal cycling programs for Targeted PCR.

IMPORTANT ***Ensure ramp rate is set to 1 °C/s for emulsion stability. If you observe an aqueous layer on top of the white-appearing emulsion layer, please contact support@missionbio.com.***

Emulsions not intact
Targeted PCR unsuccessful



Emulsions intact
Targeted PCR successful

NOTE ***STOPPING POINT: Emulsions can be left on thermocycler at 4 °C overnight.***

Break Emulsions and Pool Tubes

5.2 Retrieve the following reagents needed for PCR product purification:

- Extraction Agent (●) (+4 °C, +4 Kit) → equilibrate to RT
- Nuclease-free water

5.3 **Add 10 µL of Extraction Agent** (●) **to each sample tube.** Vortex briefly and spin for 20 seconds.

5.4 **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 5 µL of additional Extraction Agent, vortex briefly and spin for 30 more seconds.*

5.5 **Add 45 µL of nuclease-free water to each of the tubes.** Mix by briefly vortexing and then spinning for 10 seconds in a benchtop centrifuge to separate the aqueous and oil layers.

5.6 Pipette **42 µL of the aqueous top layer** from each tube into one **new 1.5 mL DNA LoBind Eppendorf tube. Pool contents from tubes 1 – 8**. Total volume will be 336 µL. *Do not transfer any oil or Barcoding Beads.*

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

NOTE ***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 (~ 2.5 hr). The amplified PCR products can be stored at 4 °C for < 24 hours or -20 °C for > 24 hours.***

DNA + Protein Protocol

6. Cleanup PCR Products

6. Cleanup PCR Products

Digest PCR Product

6.1 Retrieve all reagents required for digesting the PCR product:

- DNA Clean up Buffer (●) (-20 °C, -20 Kit) → place on ice
- Clean up Enzyme (●) (-20 °C, -20 Kit) → place on ice

6.2 To the pooled sample (336 µL), add **40 µL DNA Clean up Buffer** (●) and **24 µL Clean up Enzyme** (●). Total volume will now be 400 µL.

6.3 Briefly vortex and quick-spin the tube.

6.4 Transfer the tube to a thermo mixer and **digest at 37 °C for 60 minutes**.

6.5 While sample is digesting, equilibrate AMPure XP to room temperature.

6.6 Remove the tube from the thermo mixer, store at room temperature and continue with Separate DNA and Protein Libraries.

Separate DNA and Protein Libraries

6.7 **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.**

6.8 Thoroughly **vortex AMPure XP reagent for 45 seconds** at high-speed. Equilibrate the **AMPure XP reagent to room temperature.**

6.9 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.***

6.10 **Add 280 µL (0.70X) of AMPure XP reagent to digested sample.**

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

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

6.13 Allow at least **5 minutes** for the AMPure XP beads to separate from solution.

IMPORTANT ***Do not discard the supernatant from the tube as it contains the Protein Library.***

6.14 Without removing the tube from the magnet, **transfer the supernatant (~680 µL) from the tube** to a new 1.5 mL DNA LoBind Eppendorf tube and set aside at room temperature for **Protein Library Cleanup I** in Step 6.44. *The DNA library is bound to the beads.*

6.15 Proceed with **DNA Library Cleanup I** followed by **Protein Library Cleanup I**.

DNA Library Cleanup I

6.16 **Wash AMPure XP bead pellet** while keeping the tube on the magnet:

- a. Carefully **add 1 mL** of the freshly prepared **80% ethanol.**
- b. Wait **30 seconds.**
- c. **Remove ethanol** without disturbing the AMPure XP beads.
- d. **Repeat** steps a - c once, for a total of two wash cycles.

6.17 Keeping the tube on the magnet, using a P-10 pipette, **remove all residual ethanol** from the tube without disturbing the AMPure XP beads.

6.18 **Dry AMPure XP bead pellet** in the tube on the magnet by incubating at room temperature for **4 – 6 minutes**. *Over-dried beads may be more difficult to resuspend.*

6.19 Remove the tube from the magnet.

6.20 **Add 110 µL** of nuclease-free water into the tube.

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

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

6.23 **Transfer 100 µL** of purified PCR product from the tube to a new 0.2 mL PCR tube. *Avoid transfer of AMPure XP beads.*

6.24 Thoroughly **vortex AMPure XP reagent.** 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.

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

6.26 Place the tube onto the magnet, wait **5 minutes** for the beads to separate from the solution.

6.27 Without removing the tube from the magnet, remove the supernatant and discard.

6.28 **Wash AMPure XP bead pellet** 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 XP beads.
- d. **Repeat** steps a - c once, for a total of two wash cycles.

6.29 Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the beads.

6.30 **Dry AMPure XP bead pellet** in the tube on the magnet by incubating at **room temperature for 5 minutes**. *Over-dried beads may be more difficult to resuspend.*

6.31 Remove the tube from the magnet. Add **110 µL** of nuclease-free water into the tube. Vortex and quick-spin to collect the contents.

6.32 Incubate the tube at **room temperature for 2 minutes**.

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

6.34 Transfer **100 µL of purified PCR product** to a new 0.2 mL PCR tube.

6.35 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

6.36 Retrieve all reagents required for cleaning up the protein library:

- Streptavidin Beads (●) (+4 °C, Protein Staining Kit) → equilibrate to RT
- 2X Wash Buffer (+4 °C, Protein Staining Kit) → equilibrate to RT
- Biotin Oligos (●) (+4 °C, Protein Staining Kit) → place on ice
- Ensure heat block set to 96°C

6.37 Thoroughly **vortex Streptavidin Beads** (●) at high speed immediately prior to usage.

6.38 **Transfer 100 µL of Streptavidin Beads** (●) to a new 1.5 mL DNA LoBind Eppendorf tube.

6.39 Place the tube on magnet and wait for 2 minutes for beads to separate from solution.

6.40 Remove the supernatant and discard.

6.41 Wash the beads while keeping the tube on the magnet:

- a. Carefully **add 1 mL of 2X Wash Buffer**.
- b. **Wait 1 minute** for solution to clear.
- c. **Remove 2X Wash Buffer** without disturbing the beads.
- d. **Repeat** steps a – c once, for a total of two wash cycles.

6.42 Resuspend the beads **690 µL of 2X Wash Buffer** and set aside until later usage in Step 6.46.

Isolate Antibody Tags

6.43 **Retrieve the tube** with the supernatant from **Step 6.13** of the Clean Up PCR Products section. **Split the solution into two 1.5 mL DNA LoBind Eppendorf tubes (340 µL each)**.

6.44 To each tube**, add 2 µL of Biotin Oligo** (●) to the supernatant, vortex briefly and quick-spin.

6.45 Incubate at 96 °C for 5 minutes.

6.46 Transfer the tubes immediately onto ice and incubate for 5 minutes.

6.47 **Add and mix 342 µl of Streptavidin Beads** resuspended in 2X Wash Buffer – from Step 6.43 above – to each Biotin Oligo-treated sample tube.

6.48 **Incubate tubes for 20 minutes** on a shaker at room temperature.

6.49 Quick-spin to collect contents.

6.50 Place the tubes on magnet and **wait 5 minutes** for the beads to separate from solution.

6.51 While waiting, **prepare 3 mL of 1X Wash Buffer** by mixing 1.5 mL of 2X Wash Buffer with 1.5 mL of nuclease-free water.

6.52 Remove the supernatant.

6.53 Wash the Streptavidin Beads while keeping the tube on the magnet:

- a. Carefully **add 1 mL of 1X Wash Buffer**.
- b. **Wait 1 minute** for solution to clear.
- c. **Remove 1X Wash Buffer** without disturbing the beads.

6.54 Remove the tubes from the magnet and **wash a second time with 1 mL nuclease-free water** by pipetting up and down five times.

6.55 Place the tubes onto the magnet and **wait 3 minutes** for the beads to separate from solution.

6.56 Remove the supernatant and in each tube **resuspend the beads in 25 µL of nuclease-free water**. Transfer and combine into a new 0.2 mL PCR tube for a total of 50 µL. *The Protein PCR products are bound to the streptavidin beads (brown).*

NOTE ***STOPPING POINT: This is a good place to stop in the protocol if there is not adequate time to continue to Library PCR (~ 2 hr).***

- ***The purified DNA PCR products can be stored at 4 °C for < 24 hours or -20 °C long-term and will be stable for up to six months.***
- ***The amplified Protein PCR products can be stored at 4 °C overnight.***
- ***Do not store the Protein PCR product at -20 °C.***

DNA + Protein Protocol

7. Library PCR

7. Library PCR

During Library PCR the P5 and P7 adapter sequences (Illumina) are added to the DNA and Protein PCR products for sequencing. Each Library Index and Protein Library Index includes both an i5 and i7 index adapter.

Use the following index combinations when indexing your DNA and Protein libraries.

# of Samples	Recommended Indices (*DNA* + Protein)
1	(***2*** ● + 3 ●)
2	(***2*** ● + 3 ●) + (***4*** ● + 7 ●)
3	(***2*** ● + 3 ●) + (***4*** ● + 7 ●) + (***5*** ● + 6 ●)
4	(***2*** ● + 3 ●) + (***4*** ● + 7 ●) + (***5*** ● + 6 ●) + (***1*** ● + 8 ●)
5+	Any combination can be used. Ensure indices are unique.

Table 6. v3 Index combinations for different sample multiplexing schemes.

7.1 Retrieve the following reagents required for Library PCR

- Purified PCR products, DNA and Protein (from Section 6)
- Library Indices 1 – 8 (●) (-20 °C, -20 Kit) → place on ice
- Protein Library Indices 1-8 (●) (+4°C, Protein Staining Kit) → place on ice
- Library Mix (-20 °C, -20 Kit) → place on ice
- Nuclease-free water

7.2 **In a Pre-PCR area, set up two different Library PCR reactions in two new 0.2 mL PCR tubes**, one for the DNA Library and one for the Protein Library as follows (table on following page):

IMPORTANT ***Ensure Library Indicies are used for DNA (●) and Protein Library Indices are used for protein (●). Record the index number used for each sample. Make sure to avoid cross-contamination when handling the Indices.***

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

Table 7. Reagents for Library PCR Mix

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

7.4 Transfer the samples to two separate thermal cyclers, 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	**10** for DNA Library **20** for Protein Library
3	62 °C	20 sec	
4	72 °C	45 sec	
5	72 °C	2 min	
6	4 °C	HOLD	

Table 8. Thermal cycling program for Library PCR.

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

Library cleanup

NOTE ***Always use freshly prepared 80% ethanol.***

7.6 Thoroughly **vortex AMPure XP reagent for 45 seconds** at high-speed. Equilibrate the **AMPure XP reagent to room temperature.**

DNA Library Cleanup II

7.7 Thoroughly **vortex AMPure XP reagent** at high-speed immediately prior to usage.

7.8 Add **50 µL of nuclease-free water to the sample tube.**

7.9 **Add 69 µL (0.69X) of AMPure XP reagent** to the 100 µL sample.

7.10 **Vortex for 10 seconds** and quick-spin to collect contents.

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

7.12 Allow at least **2 minutes** for the AMPure XP beads to separate from solution.

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

7.14 **Wash AMPure XP 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 XP beads.
- d. **Repeat** steps a – c once, for a total of two wash cycles.

7.15 Keeping the tube on the magnet, **remove all residual ethanol** without disturbing the AMPure XP beads.

7.16 **Dry AMPure XP 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.*

7.17 Remove the tube from the magnet.

7.18 **Add 110 µL** of nuclease-free water into the tube.

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

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

7.21 Transfer 100 µL of purified PCR product from the tube to a new 0.2 mL PCR tube. *Avoid transfer of AMPure XP beads.*

7.22 Thoroughly **vortex AMPure XP reagent. Add 72 µL (0.72 X) of AMPure XP reagent** to the 100 µL sample.

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

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

7.25 Allow at least **2 minutes** for the AMPure XP beads to separate from solution.

7.26 Without removing the tube from the magnet, **remove the supernatant** and discard. *The DNA is bound to the beads.*

7.27 **Wash AMPure XP 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 XP beads.

» d. **Repeat** steps a – c once, for a total of two wash cycles.

7.28 Keeping the tube on the magnet, **remove all residual ethanol** without disturbing the AMPure XP beads.

7.29 **Dry AMPure XP bead pellet** 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.*

7.30 Remove the tube from the magnet.

7.31 **Add 15 µL of nuclease-free water** into the tube.

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

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

7.34 **Transfer 12 µL of purified PCR product** from the tube to a new 0.2 mL PCR tube. *Avoid transfer of AMPure XP beads.*

7.35 Store purified DNA library at -20 °C until proceeding to the next step.

Protein Library Cleanup II

7.36 **Place the tube onto the magnet and wait** for 2 minutes for Streptavidin Beads to separate from the solution.

7.37 Without removing the tube from the magnet, **transfer 50 µL of supernatant** in to a new 0.2 mL PCR tube.

7.38 Thoroughly **vortex AMPure XP reagent. Add 45 µL (0.90X) of AMPure XP reagent** to the 50 µL sample.

7.39 **Vortex for 10 seconds** and quick-spin to collect contents.

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

7.41 **Allow at least 2 minutes** for the AMPure XP beads to separate from solution.

7.42 Without removing the tube from the magnet, **remove the supernatant** and discard.

7.43 **Wash AMPure XP 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 XP beads.
» d. **Repeat** steps a – c once, for a total of two wash cycles.

7.44 Keeping the tube on the magnet, **remove all residual ethanol** without disturbing the AMPure XP beads.

7.45 **Dry AMPure XP bead pellet** in the tube on the magnet by incubating at room temperature for at least 2 minutes. *Over-dried beads may be more difficult to resuspend.*

7.46 **Remove the tube** from the magnet.

7.47 **Add 17 µL of nuclease-free water** to the tube.

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

7.49 **Place the tube onto the magnet and wait** for at least 2 minutes or until the solution is clear.

7.50 **Transfer 15 µL of purified PCR product** from the tube to a new 0.2 mL PCR tube. *Avoid transfer of AMPure XP beads.*

NOTE ***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.***

DNA + Protein Protocol

8. Quantify and Normalize Sequencing Library

8. Quantify and Normalize Sequencing Library

8.1 Retrieve the following for library quantitation:

- Purified sample libraries (DNA library and protein library)
- Agilent DNA High Sensitivity Kit or Agilent DNA 1000 kit
- Qubit™ dsDNA HS Kit

Quantify Libraries

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

8.2 Follow Qubit protocol to verify library concentration of DNA and Protein libraries.

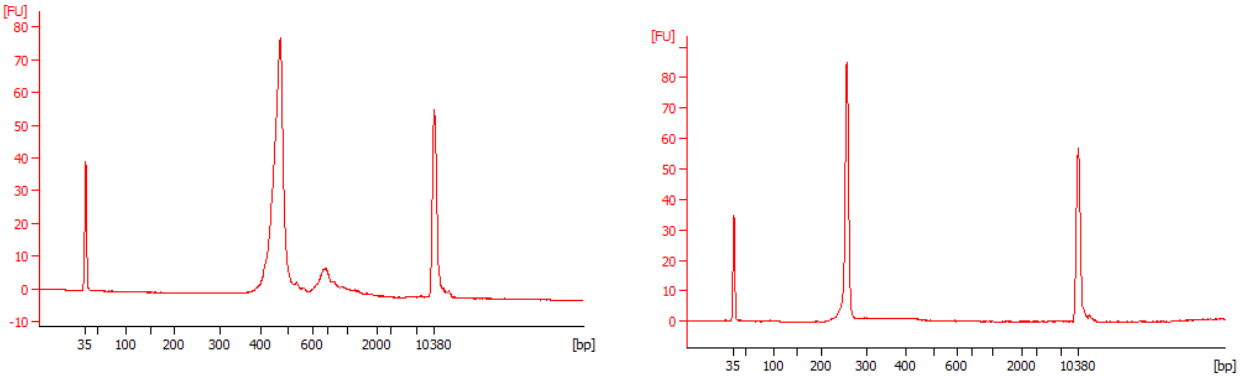
8.3 Verify the DNA and Protein Library product sizes and purity and quantify following manufacturer's instructions.

NOTE

- ***A final concentration of on-target product > 2 ng/µL can be expected for the DNA Library with a peak at ~460bp.***
- ***A final concentration of on-target product > 1 ng/µL can be expected for the Protein Library with a peak at ~250bp.***

Library Size Distributions

Libraries (DNA -left, Protein- right) generated with catalog panels will generally produce high-quality on-target amplicons 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 – 700 bp 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 8.4.*

NOTE ***If significant quantities (> 5%) of smaller products are seen at < 300bp (e.g., primer dimers), contact support@missionbio.com for additional support. An additional AMPure cleanup step may be required.***

Normalize and Pool Libraries

8.5 For DNA and Protein libraries use the ***Library Quantification and Pooling Tool*** to dilute each library.

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

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

DNA + Protein Protocol

9. Sequence Library

9. Sequence Library

Parameter	Specification
Final library size	**DNA Library: 350 bp – 550 bp** with peak at ~460 bp **Protein Library: 225 bp – 270 bp** with peak at ~250 bp
Supported sequencers	MiSeq, HiSeq 2500, NextSeq 1000/2000, NextSeq 550, HiSeq 3000/4000, NovaSeq 5000/6000, NovaSeq X
Index 1 (i7)	Yes (8nt). Index 1 – 8 sequences are different from Illumina's index sequences.
Index 2 (i5)	Yes (8nt). Index 1 – 8 sequences are different from Illumina's index sequences.
Number of unique i7/i5 index pair per sample	**1**
Custom sequencing primer?	**No**
Sequencing chemistry	**2 x 150 bp** recommended. **2 x 250** supported for DNA only.
PhiX %	**5 % – 20 %** see *Library Quantification and Pooling Tool*
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**.

Table 9. Sequencing Specifications

NOTE

- ***For expanded indexing options, refer to the Mission Bio Support Page DNA and Protein indexing guides.***
- ***For sequencing guidance, refer to the Mission Bio Support Page Library Quantification and Pooling Tool***
- ***For custom Protein Panels, refer to Mission Bio Support Page***
- ***DNA Libraries and Protein Libraries may be pooled and sequenced together. However, note that the average sizes differ between both libraries and particular care needs to be taken care when normalizing and pooling DNA and Protein libraries together for sequencing to ensure sufficient read coverage.***
- ***If using a patterned sequencing flowcell (eg. Nextseq2000), reduce Protein library input by 25% by volume to ensure sufficient DNA library reads.***
  Contact support@missionbio.com for additional support.

Sequence Information for Library Indices 1 – 8

Index	Sequence i7	Sequence i5	Reverse Sequence i5
1	CTGATCGT	ATATGCGC	GCGCATAT
2	ACTCTCGA	TGGTACAG	CTGTACCA
3	TGAGCTAG	AACCGTTC	GAACGGTT
4	GAGACGAT	TAACCGGT	ACCGGTTA
5	CTTGTCGA	GAACATCG	CGATGTTC
6	TTCCAAGG	CCTTGTAG	CTACAAGC
7	CGCATGAT	TCAGGCTT	AAGCCTGA
8	ACGGAACA	GTTCTCGT	ACGAGAAC

Table 10. Sequence nucleotide information for Library Indices 1 – 8.

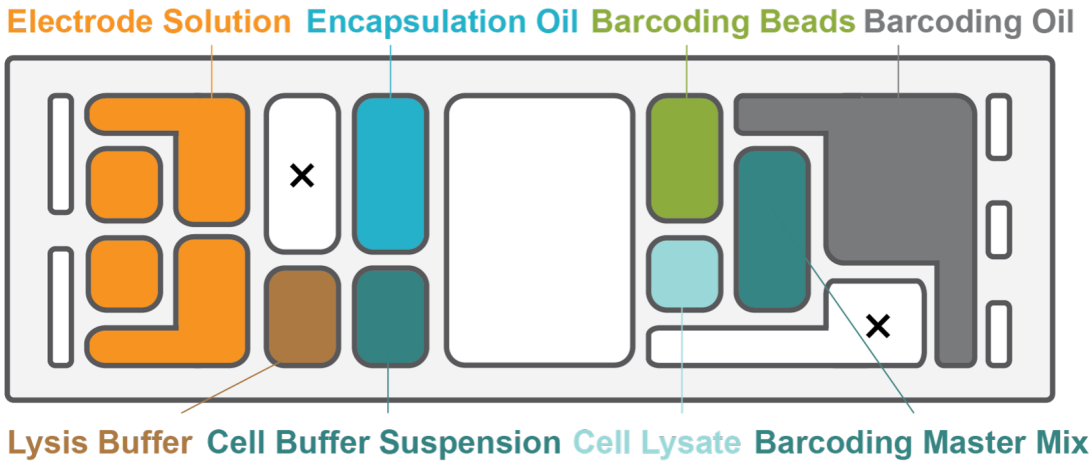
Sequence Information for Protein Library Indices 1 - 8

Index	Sequence i7	Sequence i5	Reverse Sequence i5
1	TAAGGCGA	ATATGCGC	GCGCATAT
2	CGTACTAG	TGGTACAG	CTGTACCA
3	AGGCAGAA	AACCGTTC	GAACGGTT
4	TCCTGAGC	TAACCGGT	ACCGGTTA
5	GGACTCCT	GAACATCG	CGATGTTC
6	TAGGCATG	CCTTGTAG	CTACAAGC
7	CTCTCTAC	TCAGGCTT	AAGCCTGA
8	CAGAGAGG	GTTCTCGT	ACGAGAAC

Table 11. Sequence nucleotide information for Protein Library Indices 1 – 8.

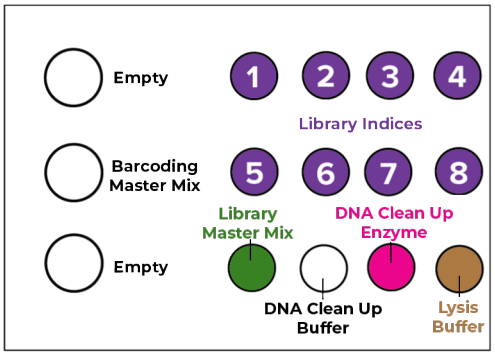
NOTE ***Protein i5 sequences are identical to the DNA i5 sequences.***

Cartridge Map

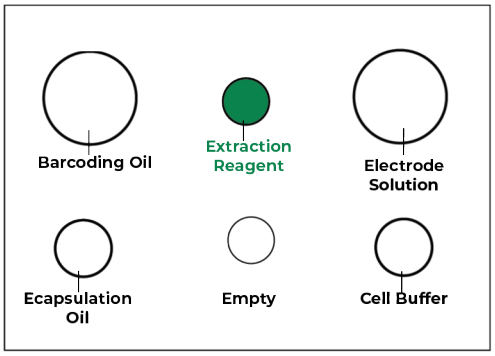


Kit Contents

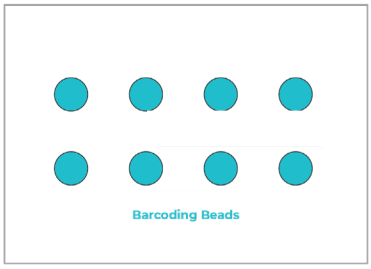
TAPESTRI SINGLE-CELL DNA CORE -20 KIT v3



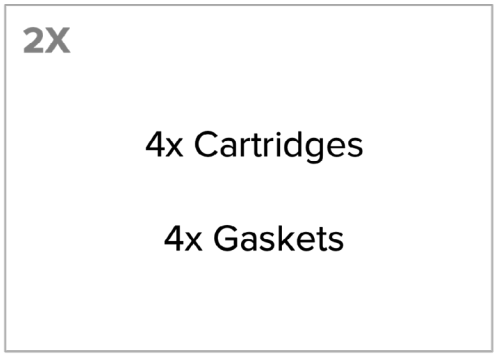
TAPESTRI SINGLE-CELL DNA CORE +4 KIT v3



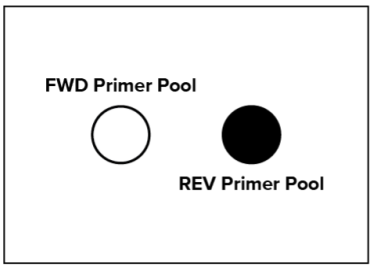
TAPESTRI SINGLE-CELL DNA BEAD KIT v3



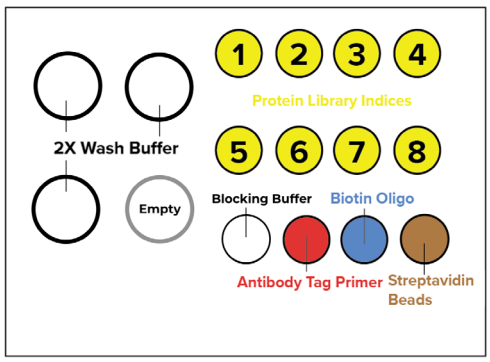
TAPESTRI SINGLE-CELL DNA CARTRIDGE KIT v3



PRIMER POOL KIT



TAPESTRI PROTEIN STAINING KIT v3



Tapestri Instrument Specifications

- Model: Tapestri Instrument
- Part Number: MB01-0020
- Mains Voltage: 115 VAC
- Frequency: 50/60 Hz
- Current: 1.0 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: 24” (1500 mm)
- Overall Dimensions. H/W/D: 10.6”/27 cm x 13.7”/35 cm x 13.2”/33.6 cm

References

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