tapestri

Tapestri® Single-Cell DNA + RNA Sequencing

User Guide

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Contact.

Mission Bio, Inc.
400 E Jamie Ct, Suite 100
South San Francisco, CA 94080
USA
www.missionbio.com

For technical support visit https://support.missionbio.com/hc/en-us
Email: support@missionbio.com

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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.

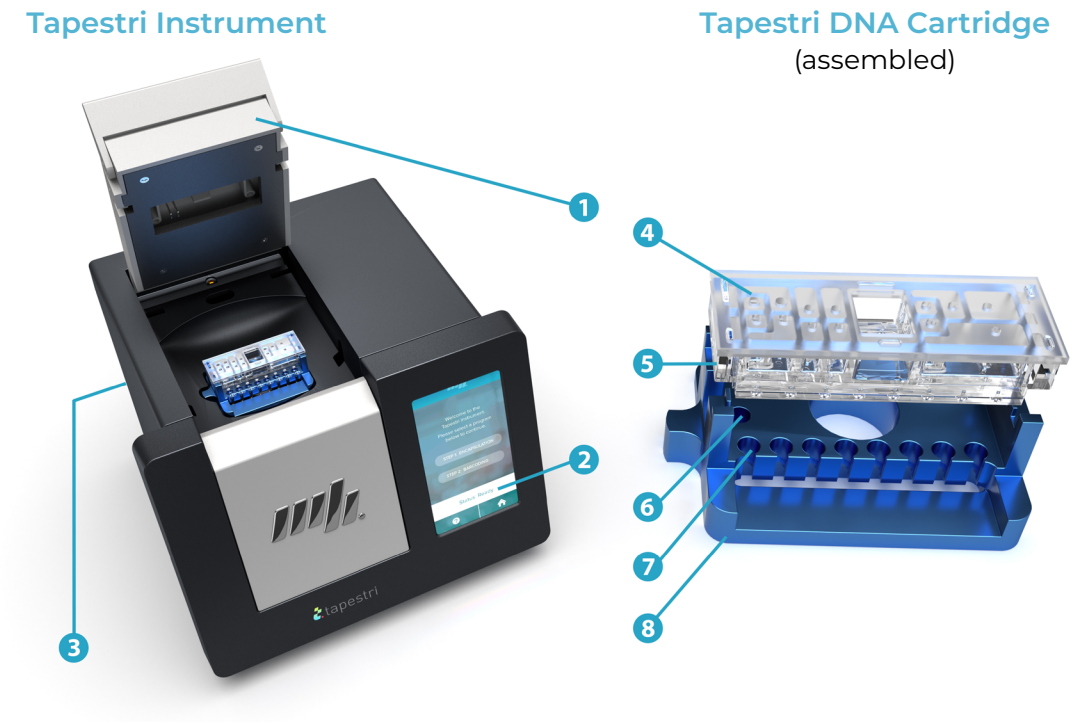


Figure 1. Tapestri Platform: Instrument and Assembled DNA Cartridge.

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 Targeted DNA + RNA Core Kit Configuration (MB03-0138)

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 Single-Cell Targeted RNA -20 Kit	MB03-0139	-20 °C
Tapestri Single-Cell Targeted RNA +4 Kit	MB03-0140	4 °C

Tapestri Single-Cell DNA Core Kit v3 Reagents

Component Name	Kit	Storage
Cell Buffer (*not used*)	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; *not used*)	Tapestri Single-Cell DNA Core -20 Kit v3	-20 °C
Barcoding Mix		-20 °C
Library Mix (green cap)		-20 °C
DNA Clean up Buffer (white cap)		-20 °C
Clean up Enzyme (pink cap)		-20 °C
Library Indices 1 – 8 (purple cap)		-20 °C
Barcoding Beads (blue cap)	Tapestri Single-Cell DNA Bead Kit v3	-20 °C

Tapestri Single-Cell Targeted RNA Kit Reagents

Component Name	Kit	Storage
RNA Enzyme (purple cap)	Tapestri Single-Cell Targeted RNA -20 Kit	-20 °C
RNA Enzyme Buffer (gray cap)		-20 °C
RNA Reagent 1 (yellow cap)		-20 °C
RNA Reagent 3 (red cap)		-20 °C
RNA Reagent 4 (white cap)		-20 °C
RNA Library Indices 1-8 (green cap)		-20 °C
RNA Reagent 3 (blue cap)	Tapestri Single-Cell Targeted RNA +4 Kit	4 °C
RNA Lysis Mix (brown 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
RNaseZap™	Invitrogen™ (AM9780)	RNase removal
TipOne RPT ultra low retention filter tips	USA Scientific (1180-8810) or Approved Supplier	Reagent handling
1.5 mL DNA LoBind Microcentrifuge Tubes	Eppendorf (0030108418) or Approved Supplier	Cell/Reagent handling
Sterile single-pack CellTrics™ filters, 30 µm (OPTIONAL)	Sysmex (04-004-2326)	Cell preparation
DPBS without Ca^{2+}/Mg^{2+} (1X)	Gibco (14190-144) or Approved Supplier	Cell preparation
15 mL DNA LoBind conical tubes	Eppendorf (30122208) or Approved Supplier	Cell preparation
Trypan Blue	Thermo Fisher (15250061) or Approved Supplier	Cell quantification
Propidium Iodide (OPTIONAL)	Thermo Fisher (P3566) or Approved Supplier	Cell quantification
* 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 µL 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
AMPure XP Reagent	Beckman Coulter (A63880)	AMPure purification
Serological pipettes (10 mL, 5 mL)	Any	AMPure purification
Nuclease-free water	Any	AMPure purification
Ethanol, Molecular Biology Grade	Sigma (E7023) or Approved Supplier	AMPure purification
E-Gel® EX 2% Precast Agarose Gel with SYBR® Gold II DNA Gel Stain	Thermo Fisher (G401002) or Approved Supplier	Gel purification
Invitrogen™E-Gel™ Sizing DNA Ladder	ThermoFisher (10-488-100)	Gel purification
Zymoclean Gel DNA Recovery Kit	Zymo Research (D4001)	Gel purification
Qubit® dsDNA HS Assay Kit	Qubit® (Q32851)	Library quantitation
Qubit Assay Tubes	Thermo Fisher (Q32856)	Library quantitation
Agilent DNA 1000 Kit or Agilent DNA High Sensitivity Kit	Agilent Technologies (5067-1504) Agilent Technologies (5067-4626)	Library quantitation
KAPA Library Quantification Kit Illumina Platforms (OPTIONAL)	KAPA (KK4873)	Sequencing
Paired-end Sequencing Reagent Kit, 300 cycles	Illumina or Approved Supplier	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)
Pipettes, 1 µL – 1000 µL	Mettler-Toledo, Rainin Pipettes, or Approved Supplier
Pipette Aid	Thermo Fisher (S1) or Approved Supplier
Tube Vortexer	Thermo Fisher (88880017TS) or Approved Supplier
Countess® II Automated Cell Counter or equivalent	Thermo Fisher (AMQAX1000)
Centrifuge with temperature control and swinging bucket (needs to support 15 mL conical tubes)	Eppendorf (5810 R) or Approved Supplier
Microcentrifuge (1.5 mL PCR tubes) with temperature control	Thermo Fisher (5406000240) 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
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
Horizontal Gel Electrophoresis Unit	Thermo Fisher (G9110) or Approved Supplier
E-Gel Camera	Thermo Fisher (G9200) or Approved Supplier
Safe Imager Viewing Glasses	Thermo Fisher (S37103) or Approved Supplier
Gel Knife (spatula)	Thermo Fisher (EI9010) or Approved Supplier
Surgical Design Disposable Scalpels (# 11)	Fisher Scientific (22-079-708) or Approved Supplier
Qubit Fluorometer	Thermo Fisher (Q33216)
Agilent 2100 Bioanalyzer or TapeStation	Agilent (G2939BA), (G2992AA), (G2991BA)
Paired-end Sequencing Platform	Illumina (MiSeq, HiSeq, NextSeq, NovaSeq) or Approved Supplier

Protocol Overview

Single cells are individually partitioned into nanoliter droplets. Barcoding Beads and PCR reagents are introduced using the Mission Bio Tapestri Instrument and DNA Cartridge. Cell lysis, reverse transcription, 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. DNA and RNA libraries are indexed and amplified separately. Final libraries are purified and can be sequenced on one of the supported sequencing instruments.

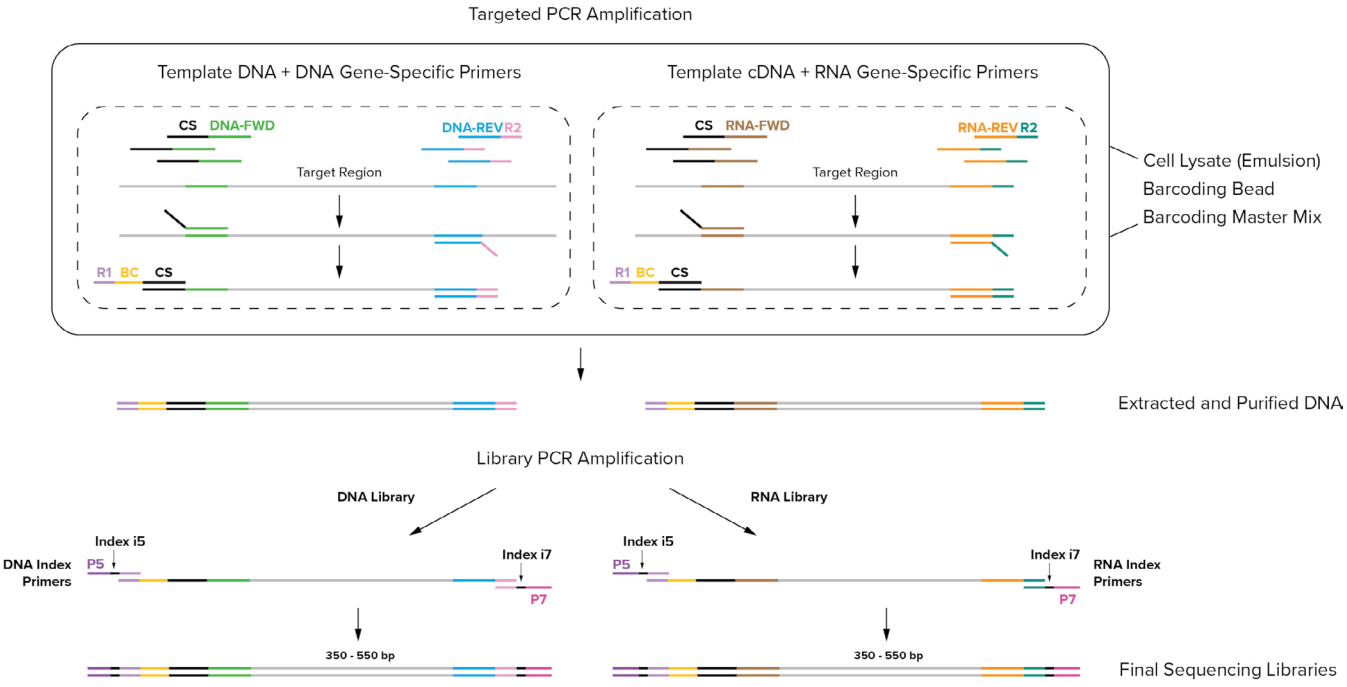


Figure 2. Overview of library construction. R1: Read 1, R2: Read 2, BC: barcode, CS: common sequence, FWD: forward primer, REV: reverse primer, P5: P5 Illumina adapter, P7: P7 Illumina adapter.

Best Practices

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.
- For RNA protocols, additionally clean bench areas and pipettes with RNaseZap™.

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 (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. Avoid direct contact with the side of the tube, where emulsions directly interface, and instead hold tubes by the lid.

Cell Recovery

- Always pipette slowly and carefully when removing the supernatant following centrifugation 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 a PCR skirt. For specific instrument operation, follow the instructions provided by the manufacturer.

1. Cell Lysis + Reverse Transcription		
Step	**Temperature**	**Time**
1	50 °C	120 min
2	80 °C	10 min
3	4 °C	HOLD

			2. Targeted PCR				
		DNA 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	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	

3. Library PCR			
Step	**Temperature**	**Time**	**Cycle**
1	95 °C	3 min	
2	98 °C	20 sec	**10** for DNA **15** for RNA
3	**62 °C** for DNA **65 °C** for RNA	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).
- The 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.

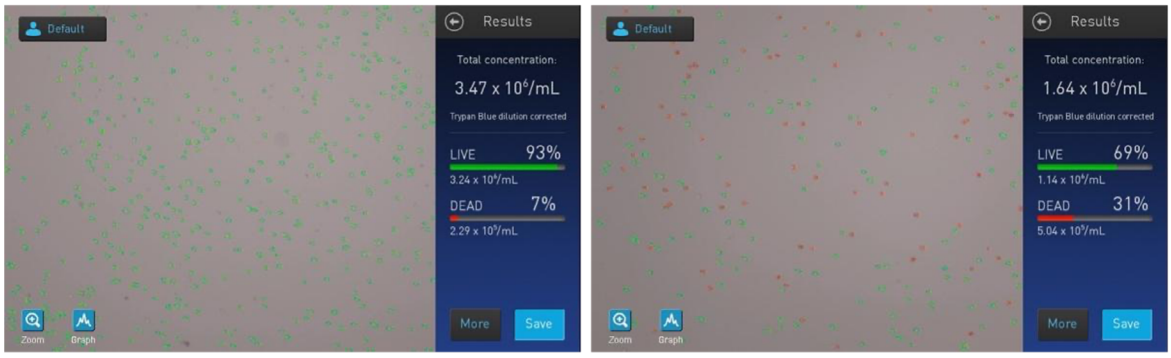


Figure 3. Representative images of high-quality cell suspension (left) and low-quality cell suspension (right).

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 + RNA Protocol

1. Prepare Cell Suspension

DNA + RNA 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 workflow is optimized for a single-cell suspension input of 4,000 – 4,500 cells/µL at > 90% viability in a total volume of 40 µL. Some cell loss is to be expected throughout the thawing and washing procedure for cryopreserved cell samples, and therefore a recommended 1 x 10^6 cells per cryovial is recommended.

NOTE

- ***Thaw reagents at room temperature unless directed to thaw them on ice.***
- ***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. If working with other sample types, please contact support@missionbio.com.***

Thaw Cells

1.1 Retrieve all reagents required for preparing the cell suspension:

- DPBS without Ca^{2+}/Mg^{2+} (1x) → keep at RT
- CellTrics™ 30 µm filter
- RNaseZap™

1.2 Clean all working areas and pipettes with RNaseZap™.

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

1.4 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.

1.5 **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.

1.6 Remove tube and clean with 70% ethanol.

1.7 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.

1.8 **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 the tube while adding.

1.9 **Add 0.5 mL of thawing media** to the 15 mL tube **every few seconds until 10 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 9.9 mL of supernatant, leaving 100 µL of thawing media behind. **Do not disturb the cell pellet.**

1.12 Add 900 µL of thawing media and gently resuspend the cell pellet by pipetting up and down five times.

1.13 Add 9 mL of DPBS to the tube.

1.14 If there are visible cell clumps, **filter the cells through a 30 µm CellTrics™ filter** into a new 15 mL conical tube:

- a. Place a 30 µm CellTrics™ filter over a 15 mL tube and **prime the filter by adding 1 mL of DPBS**. Allow all the liquid to flow through the filter.
- b. **Discard** the flow-through.
- c. **Transfer the cell suspension** from ***Step 1.13*** to the 15 mL tube through the primed 30 µm CellTrics™ filter.
- Discard the filter.

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

1.16 Immediately aspirate all supernatant. **Do not disturb the cell pellet**.

1.17 Resuspend the cells in **40 µL of DPBS.**

1.18 **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.**

1.19 Dilute cell suspension to **4,000 – 4,500 cells/µL** using DPBS. Confirm the final concentration using the automated cell counter.

IMPORTANT ***Use of cell concentrations outside the range of 4,000 – 4,500 cells/µL or viability below 90% may adversely affect results.***

1.20 **Place the 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 + RNA Protocol

2. Encapsulate Cells

2. Encapsulate Cells

In this step, cells are encapsulated with RNA Lysis Mix to create a cell emulsion. For input cell concentrations of 4,000 – 4,500 cells/µL, approximately 5% of all emulsion droplets will contain a cell, following a Poisson distribution.

IMPORTANT

- ***Do not use the Lysis Mix or Cell Buffer provided in the Tapestri Single-Cell DNA Core reagent kit.***
- ***Handle emulsions with caution, avoiding sources of static and pipetting slowly and carefully.***
- ***Use only the consumables (emulsion safe PCR tubes) validated by Mission Bio (see list of Required Third Party Consumables and 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
- RNA Enzyme (●) (-20 °C, RNA -20 Kit) → place on ice
- RNA Enzyme Buffer (●) (-20 °C, RNA -20 Kit) → place on ice
- RNA Reagent 1 (●) (-20 °C, RNA -20 Kit) → place on ice
- RNA Reagent 3 (●) (-20 °C, RNA -20 Kit) → place on ice
- RNA Reagent 4 (○) (-20 °C, RNA -20 Kit) → place on ice
- RNA Lysis Mix (●) (4 °C, RNA +4 Kit) → place on ice
- RNA Reagent 2 (●) (4 °C, RNA +4 Kit) → equilibrate to RT
- RNA Reverse Primer Pool (●) (-20 °C, RNA Oligo Pool Kit) → place on ice
- Encapsulation Oil (4 °C, +4 Kit) → equilibrate to RT
- Nuclease-free water → keep at RT
- Cell Suspension (prepared in ***Section 1 – Prepare Cell Suspension***) → keep on ice

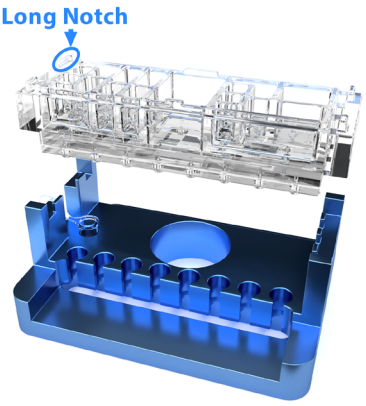
2.3 Add **7 µL of the RNA Reverse Primer Pool** a 0.2 mL PCR tube and **preheat to 70 °C for 5 minutes**, then store on ice for at least **10 minutes**.

2.4 **Preheat the thermal cycler** with the Cell Lysis + Reverse Transcription protocol and **hold at 50 °C**.

2.5 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.***



2.6 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.

2.7 **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.***

2.8 In a new tube on ice, prepare **RNA Mix** as follows:

Reagent	Handling	Volume (µL)
RNA Enzyme Buffer (⚪)	Vortex, spin down	12
RNA Reagent 1 (🟡)	Vortex, spin down	3
RNA Reagent 2 (🔵)	Spin down	5.4
RNA Reagent 3 (🔴)	Vortex, spin down	3
RNA Reagent 4 (⚪)	Spin down	3
Nuclease-free Water		23.3
Total		**49.7**

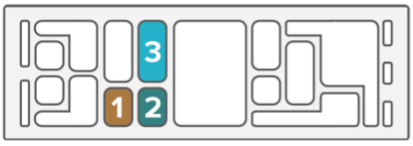
Table 2. Reagents for RNA Mix.

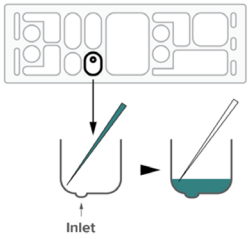
2.9 Add **6 µL of the preheated and chilled RNA Reverse Primer Pool** (from *Step 2.3*) to the RNA Mix (total volume is 55.7 µL).

2.10 In a new 0.2 mL PCR tube, combine **2 µL RNA Lysis Mix** (🟤) with **18 µL nuclease-free water**. Gently mix by pipetting up and down 5 times. *Do not vortex*. Store on ice.

IMPORTANT ***The next steps are time sensitive. Minimize the time between loading the DNA cartridge and running the Encapsulation program.***

2.11 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.

2.12 Pipette 200 µL of **Encapsulation Oil** into reservoir 3. Be careful not to spill oil into surrounding reservoirs while loading the cartridge.

2.13 Add **3 µL of RNA Enzyme** (●) **to the RNA Mix** (from *Step 2.9*; total volume is 58.7 µL). Gently mix by pipetting up and down 5 times. *Do not vortex*.

2.14 Preset a P200 pipette to 55 µL and a P10 pipette to 1.3 µL.

2.15 Place the ice bucket containing the RNA Mix (from *Step 2.13*) and the 10X diluted RNA Lysis Mix (from *Step 2.10*) near the Tapestri instrument.

2.16 Add **1.3 µL of diluted RNA Lysis Mix** (from *Step 2.10*) to the tube containing the **RNA Mix** (from *Step 2.13*). Mix by pipetting up and down 5 times with a P200 pipette tip set to 55 µL.

2.17 Pipette 55 µL of **RNA Mix + RNA Lysis Mix** into reservoir 1.

IMPORTANT ***Make sure to apply the DNA Gasket and start the Encapsulation program within 1 minute after loading the RNA Mix + RNA Lysis Mix.***

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

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

2.20 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.

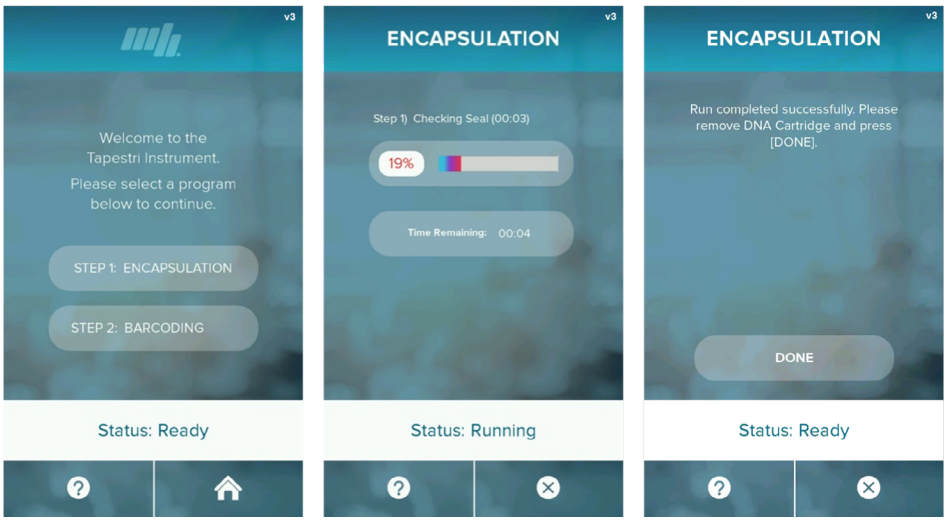


Figure 4. Touchscreen displays show main menu (left), screen after selecting 'Step 1: Encapsulation' program (middle), and final screen after Encapsulation is completed (right).

IMPORTANT ***The next steps are time sensitive. Encapsulation emulsions should be placed on the thermal cycler and the "Cell Lysis + Reverse Transcription" program should be resumed within one minute of completion of the Encapsulation program (Section 3 – Lyse Cells and Reverse Transcribe).***

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

2.22 **Carefully transfer the emulsion sample tube** to a 96-well plate holder.

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

2.24 Assess emulsion quality. Encapsulated cell emulsions are visible as a **white layer** on top of the oil layer. 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.

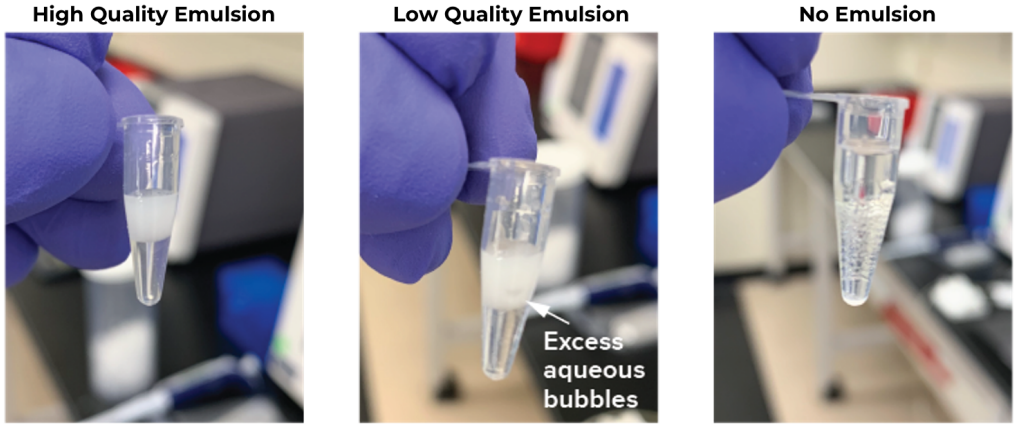


Figure 5. Emulsion Quality

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

2.25 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.***
- ***Proceed immediately to Section 3 – Lyse Cells and Reverse Transcribe.***

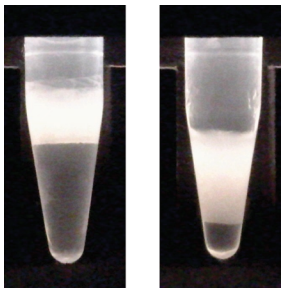


Figure 6. Encapsulation emulsions before (left) and after (right) excess oil removal.

DNA + RNA Protocol

3. Lyse Cells and Reverse Transcribe

3. Lyse Cells and Reverse Transcribe

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

3.1 Immediately place the sample tube on the **preheated thermal cycler.**

3.2 **Resume the "Cell Lysis + Reverse Transcription"** protocol on the thermal cycler according to the manufacturer's instructions, using the following parameters:

Step	Temperature	Time
1	50 °C	120 min
2	80 °C	10 min
3	4 °C	HOLD

Table 3. Thermal cycling protocol for 'Cell Lysis + Reverse Transcription'.

3.3 When the run completes, store the lysed and reverse transcribed 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 ***It is strongly recommended to proceed through Section 4 – Barcode Cells on day 1. If necessary, the lysed encapsulation emulsions may be stored at 4 °C overnight, upright in a sealed container to avoid condensation.***

DNA + RNA Protocol

4. Barcode Cells

4. Barcode Cells

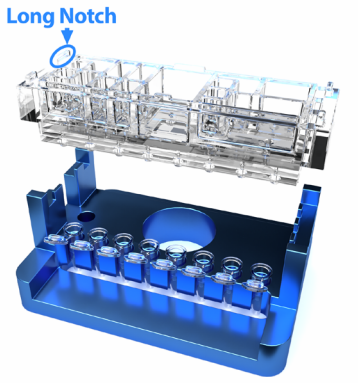
In this step, the droplets containing encapsulated cell lysate are combined with drops containing both Barcoding Mix and Barcoding Beads. These newly generated drops are then distributed into eight PCR collection tubes.

4.1 Retrieve all reagents required for Cell Barcoding:

- Barcoding Mix (-20 °C, -20 Kit) → place on ice
- Barcoding Beads (●) (-20 °C, Bead Kit) → ***thaw at RT and protect from light***
- DNA Forward Primer Pool (○) (-20 °C, DNA Oligo Pool Kit) → place on ice
- DNA Reverse Primer Pool (●) (-20 °C, DNA Oligo Pool Kit) → place on ice
- RNA Forward Primer Pool (○) (-20 °C, RNA Oligo Pool Kit) → place on ice
- Barcoding Oil (4 °C, +4 Kit) → equilibrate to RT
- Electrode Solution (4 °C, +4 Kit) → equilibrate to RT

IMPORTANT ***The Priming program should be started at most 50 minutes before the Cell Lysis + Reverse Transcription program completes on the thermal cycler.***

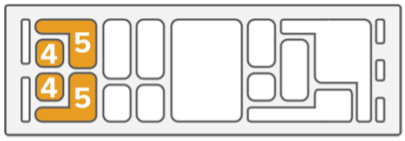
Prime the DNA Cartridge for Barcoding



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.

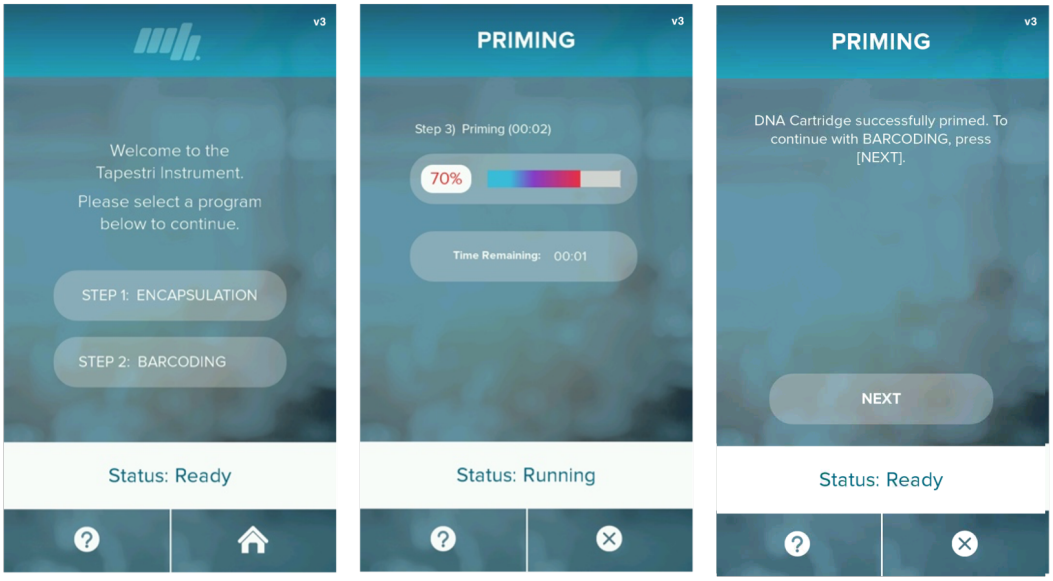


Figure 7. Touchscreen displays show main menu (left), screen after selecting 'Step 2: Barcoding' program (middle), and final screen after Priming is completed (right).

Prepare Barcoding Mix

4.8 In a new tube on ice, prepare **Barcode Mix** as follows, making sure to vortex and quick-spin each reagent before adding:

Reagent	Volume (µL)
Barcoding Mix	291.9
DNA Forward Primer Pool (○)	2.5
DNA Reverse Primer Pool (●)	3.1
RNA Forward Primer Pool (○)	2.5
Total	**300**

Table 4. Reagents for Barcode Mix.

4.9 **Briefly vortex the Barcode Mix** and quick spin to collect the contents. Store on ice.

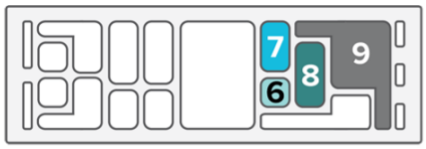
IMPORTANT ***After the Priming program has completed the Barcoding program must be started within 30 minutes.***

Load the DNA Cartridge

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

4.11 Add **67 µL** of the prepared **Barcode Mix (from *Step 4.8*) to the Barcoding 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 Cells and Reverse Transcribe***).

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; do not quick-spin*. Carefully pipette **250 µL of Barcoding Beads** (●) **into reservoir 7**.

4.15 Pipette **200 µL of the remaining Barcode Mix** (from ***Step 4.8***) 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. The program runs for 45 minutes.

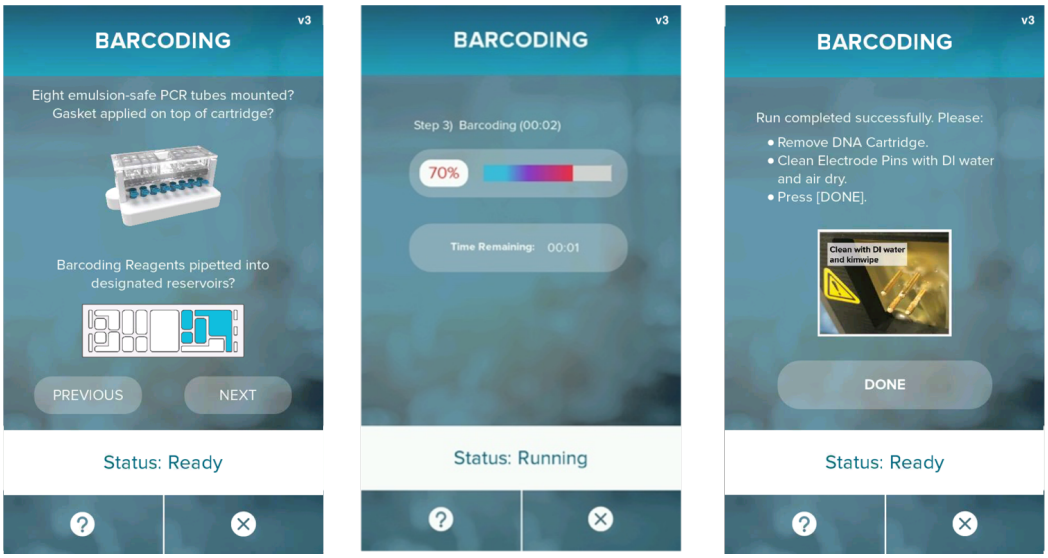


Figure 8. Touchscreen displays before the second part of Barcoding (left), the status during Barcoding (middle), and final screen after Barcoding is completed (right).

4.19 When the touchscreen displays **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 eight tubes. Occasionally, a tube may be empty; this is not cause for concern as all eight channels are connected. If more than 190 µL of Barcoding Beads or more than 15 µL of emulsions remain in the cartidge, proceed with the workflow and contact support@missionbio.com.***

4.20 Assess 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 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.***

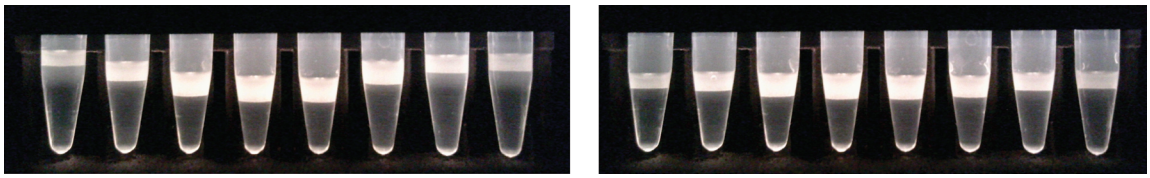


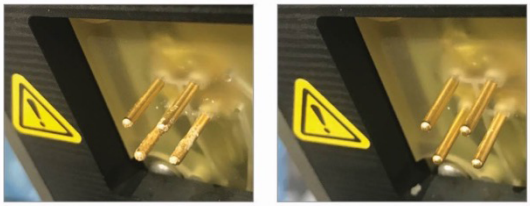
Figure 9. Barcoding emulsions before (left) and after (right) excess oil removal.

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 may 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 + RNA 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 5 below)** that are compatible with the targeted DNA and RNA panels 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.***

		DNA 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	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 5. Thermal cycling programs for Targeted PCR.

IMPORTANT ***Ensure ramp rate is set to 1 °C/s for emulsion stability.***

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

5.2 Following targeted PCR, remove the tubes from the thermal cycler and visually evaluate the emulsion quality. The barcoded emulsions are visible as a **white layer** on top of the oil layer.

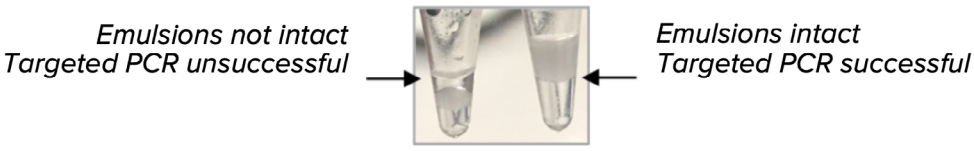


Figure 10. Post-PCR Emulsion Quality

NOTE ***If emulsions are not intact, please contact support@missionbio.com.***

Break Emulsions and Pool Tubes

5.3 Retrieve the following reagents needed for breaking the emulsions:

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

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

5.5 **Add 45 µL of nuclease-free water to each sample tube.** Vortex briefly and spin for at least 10 seconds.

5.6 Pipette the total volume of the **aqueous top layer from each tube** into one new 1.5 mL DNA LoBind Eppendorf tube.

5.7 Spin down at **3,000 x g for 5 minutes** at room temperature.

5.8 Pipette **336 µL of the aqueous top layer** into a new 1.5 mL DNA LoBind Eppendorf tube. *Do not transfer any oil or Barcoding Beads. If needed, add nuclease-free water to achieve a total volume of 336 µL.*

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

NOTE ***STOPPING POINT: This is a good place to stop in the protocol if there is not adequate time to cleanup the PCR products in one day (~ 3.5 hr). The amplified PCR products can be stored at 4 °C for < 24 hours or -20 °C for > 24 hours.***

DNA + RNA Protocol

6. Cleanup PCR Product

6. Cleanup PCR Product

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 and store at room temperature.

AMPure XP PCR Cleanup

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**. *Discard the remaining tube containing the pellet.*

6.8 Thoroughly **vortex AMPure XP reagent for 45 seconds** at high-speed.

6.9 Prepare **15 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 288 µL (0.72X) 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.

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

6.15 **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.

6.16 Keeping the tube on the magnet, using a P10 pipette, **remove all residual ethanol** from the tube without disturbing the AMPure XP beads.

6.17 **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.*

6.18 Remove the tube from the magnet.

6.19 **Add 70 µL** of nuclease-free water into the tube.

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

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

6.22 **Transfer 65 µL** of purified PCR product from the tube to a new 0.2 mL PCR tube. *Avoid transfer of AMPure XP beads.*

Gel Purification

In this step, the purified PCR products are run on a precast agarose gel, fragments between 250 and 450 bp are excised, and the on-target products (~300-350 bp) are purified accoring to the manufacturer's instructions. If using an alternative gel purification platform or DNA recovery kit, excise and purify the fragments between 250 and 450 bp according to the manufacturer's instructions, then proceed to ***Step 6.58***.

6.23 Retrieve all reagents required for running the agarose gel:

- E-Gel® EX 2% Precast Agarose Gel with SYBR® Gold II DNA Gel Stain → keep at RT
- E-Gel® 1 Kb Plus DNA Ladder → place on ice
- Zymoclean Gel DNA Recovery Kit → keep at RT
- Qubit™ dsDNA HS Kit → keep at RT
- Nuclease-free water
- Gel knife (Spatula)
- Surgical Design Disposable Scalpels (# 11)

6.24 Follow Qubit protocol using 1-2 µL of the purified PCR product to verify DNA yield. If the **concentration is > 7 ng/µL, add 20 µL of nuclease-free water to the sample tube**.

6.25 Remove the gel from its packaging and carefully detach the comb from the E-Gel® cassette.

IMPORTANT ***Gels must be loaded within 15 minutes of opening the package.***

6.26 **Insert the gel cassette** into the E-Gel® Power Snap Electrophoresis Device, **beginning with the right edge**.

6.27 **Press down on the left side** of the cassette to secure it into the device.

6.28 Label the appropriate number of wells for the sample, excluding the first and last wells. *If no dilution was needed in Step 6.24, three wells will be used. If dilution was needed, four wells will be used.*

6.29 **Load 20 µL of DNA sizing ladder** into the first and last wells of the gel.

6.30 **Load 20 µL each of the PCR product** into the number of wells indicated by *Step 6.28* above. *If running multiple samples, leave one empty well between wells of different samples. If space allows, leave one empty well between the ladder and sample wells.*

6.31 **Load 20 µL of nuclease-free water** into any empty wells.

IMPORTANT ***Gels must be run within 1 minute of being loaded.***

6.32 Run the gel as follows:

- a. Select '**Set up run**' to start the E-Gel™ protocol selection.
- b. Select the '**Gel Type**' dropdown and choose '**E-Gel® EX 1-2%**.'
- d. Select '**Start run**' to begin running the gel.
- e. Check the gel status any time by selecting the '**Back Light**' button to activate the blue light transilluminator.

6.33 Run the gel for **15 minutes**. The bands of the E-Gel™ 1 Kb Plus DNA Ladder should be **clearly separated between 200 bp and 500 bp**. *The on-target PCR product should be visible as a band between 300 and 350 bp (see **Figure 9** below).*

6.34 **Save an image** of the gel by connecting the E-Gel Power Snap Camera to the electrophoresis unit. From the Home Screen, press '**Capture**.'

IMPORTANT ***Ensure that Safe Imager viewing glasses are worn for Steps 6.35-6.46.***

6.35 **Open the filter lid** of the electrophoresis unit.

6.36 Using the spatula, **remove the gel cassette** from the unit. **Flip the gel cassette** and put it back in the unit.

6.37 Select the '**Back Light**' button followed by the '**Proceed**' button.

6.38 Identify the DNA ladder bands corresponding to **250 and 450 bp in the first and last wells**. Using a ruler, **trace two horizontal lines connecting the bands** on each side of the gel.

6.39 Mark the **edges of the sample wells with two vertical lines**, creating a box to be excised.

6.40 Using the spatula, **remove the gel cassette** from the unit. **Flip the gel cassette**. *The marked box should be visible.*

6.41 **Insert the spatula into the middle groove** on the edge of the cassette; carefully lever the spatula up and down to **crack the seal**. **Remove the top cover**, leaving the gel exposed over the bottom cover.

6.42 **Return the gel** into the electrophoresis unit.

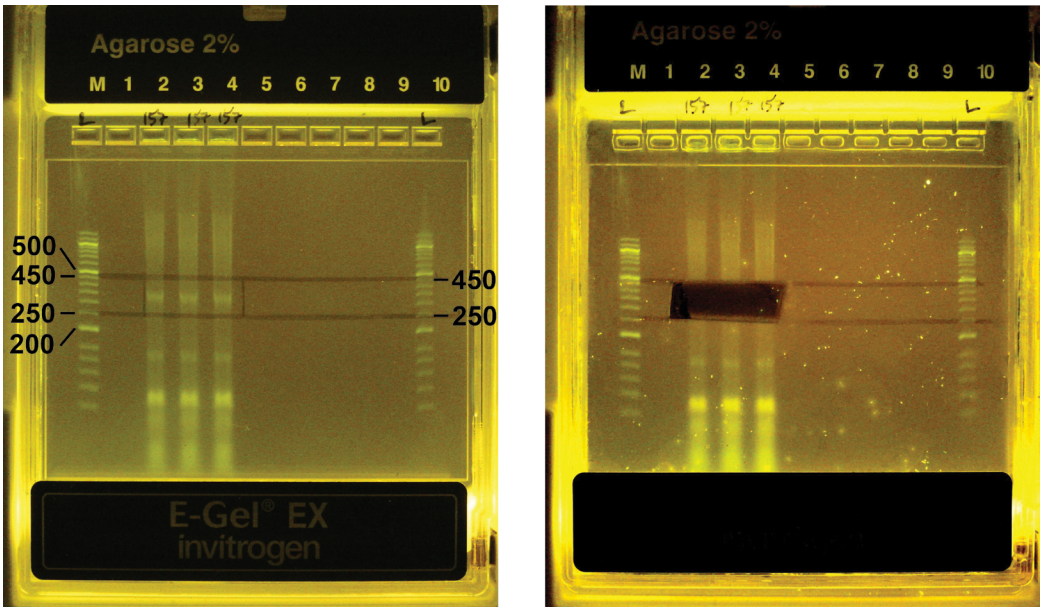


Figure 11. Gel images before (left) and after (right) excision of the fragments between 250 and 450 bp.

6.43 Weigh a 1.5 mL DNA LoBind Eppendorf tube and record the weight. *If the sample was run in four wells of the gel, two 1.5 mL tubes will be needed; record the weight of each tube.*

6.44 Ensuring that the Safe Imager viewing glasses are worn, open the filter lid and select the '**View Gel**' button. *Ensure the marked box still aligns with the 250 and 450 bp markers on the ladder, and adjust the position of the gel if necessary.*

6.45 Using a scalpel, **excise the gel fragments** corresponding to the marked box and transfer them to the 1.5 mL tube. *If the sample was run in four wells of the gel, divide the gel fragments between two 1.5 mL tubes.*

IMPORTANT ***When cutting the gel, maintain the scalpel in a vertical position to correctly excise the target products, avoiding cutting at an angle.***

6.46 **Save an image** of the gel by connecting the E-Gel Power Snap Camera to the electrophoresis unit. From the Home Screen, press '**Capture**.'

6.47 Weigh the 1.5 mL tube containing the excised gel and **calculate the weight of the gel** by subtracting the weight of the tube (from ***Step 6.43***). *If using two tubes, perform this step for each tube separately.*

6.48 **Add agarose dissolving buffer (ADB) in a 3:1 ratio relative to the mass of the gel fragment** (e.g. 300 µL of ADB for 100 mg of gel). Vortex briefly and quick-spin to collect contents.

6.49 Transfer the tube(s) to a thermo mixer and **incubate at 55 °C for 10 minutes** with **300 rpm shaking**. *Alternatively, incubate in a heat block and vortex briefly every 2 minutes.*

NOTE ***The gel should dissolve completely. If necessary, vortex vigorously and incubate for an additional 5 minutes.***

6.50 **Transfer the dissolved agarose solution to a Zymo-Spin™ I column** and centrifuge at **10,000 x g for 1 minute**. Discard the flow through. *If two tubes were used, use two separate spin columns.*

NOTE ***The maximum capacity of the column is 800 µL. If needed, apply 800 µL, spin, discard the flow through from the collection tube, and add an additional 800 µL until all of the volume has been bound to the column. If two tubes were used, process each tube separately according to the steps below.***

6.51 Wash the column by adding **200 µL of Zymo Wash Buffer.**

6.52 Centrifuge column for **1 minute at 10,000 x g**. Discard flow through.

6.53 Repeat *Steps 6.51-6.52* for a total of two washes.

6.54 Place the Zymo-Spin™ I column into a **new 1.5 mL DNA LoBind Eppendorf tube**.

6.55 Add **110 µL of Zymo DNA Elution buffer** directly to the column and incubate for **5 minutes at room temperature**. *If two tubes were used, divide the elution buffer between tubes (i.e., add 55 µL of elution buffer to each tube).*

6.56 Centrifuge for **1 minute at 10,000 x g** to collect the eluted PCR product.

6.57 Transfer **100 µL of the eluted product** to a new 1.5 mL DNA LoBind Eppendorf tube. *If two tubes were used, transfer and combine 50 µL from each tube into a single new 1.5 mL DNA LoBind Eppendorf tube.*

6.58 To the 100 µL of the eluted product add **400 µL of nuclease-free water.**

6.59 Thoroughly **vortex AMPure XP reagent.** Add **460 µL (0.92X)** of AMPure XP reagent to the tube with eluted PCR product (960 µL total). Vortex for 5 seconds and quick-spin to collect the contents.

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

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

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

6.63 **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.

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

6.65 **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.*

6.66 Remove the tube from the magnet. Add **55 µL** of nuclease-free water into the tube. Vortex and quick-spin to collect the contents.

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

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

6.69 Transfer **50 µL of purified PCR product** to a new 0.2 mL PCR tube.

6.70 Store the purified PCR product on ice and proceed to the next step, or store at -20 °C long term.

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.***

DNA + RNA Protocol

7. Library PCR

7. Library PCR

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

Use the following index combinations when indexing your DNA and RNA libraries:

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

Table 6. Index combinations for different sample multiplexing schemes.

7.1 Retrieve the following reagents required for Library PCR:

- Purified PCR product (from ***Section 6 – Cleanup PCR Product***) → keep on ice
- Library Indices 1 – 8 (🟣) (-20 °C, -20 Kit) → place on ice
- RNA Library Indices 1-8 (🟢) (-20 °C, RNA -20 Kit) → place on ice
- Library Mix (🟢) (-20 °C, -20 Kit) → place on ice
- Nuclease-free water → keep at RT

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 RNA Library as follows:

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

	DNA Library	RNA Library
Reagent	Volume (µL)	
Library Mix (🟢)	25	25
Library Indices (🟣)	10	-
RNA Library Indices (🟢)	-	10
Gel Extracted PCR product	15	15
Total Volume	**50**	**50**

Table 7. Reagents for Library PCR Reactions

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 **15** for RNA
3	**62 °C** for DNA **65 °C** for RNA	20 sec	
4	72 °C	45 sec	
5	72 °C	2 min	
6	4 °C	HOLD	

Table 8. Thermal cycling programs for Library PCR.

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

Library Cleanup

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

NOTE ***The DNA and RNA libraries can be processed in parallel according to Steps 7.7-7.35 below. Always use freshly prepared 80% ethanol.***

7.7 Transfer the library from the 0.2 mL PCR tube to a new 1.5 mL DNA LoBind Eppendorf tube.

7.8 Add **450 µL of nuclease-free water to the sample tube.**

7.9 **Add 345 µL (0.69X) of AMPure XP reagent** to the 500 µL sample.

7.10 **Vortex for 10 seconds** and quick-spin to collect the 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 **5 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 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.

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 **4 – 6 minutes.** *Over-dried beads may be more difficult to suspend.*

7.17 Remove the tube from the magnet.

7.18 **Add 510 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 500 µL of purified PCR product from the tube to a new 1.5 mL DNA LoBind Eppendorf tube. *Avoid transfer of AMPure XP beads.*

7.22 Thoroughly **vortex AMPure XP reagent. Add 345 µL (0.69X) of AMPure XP reagent** to the 500 µL sample.

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

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 **4 – 6 minutes**. *Over-dried beads may be more difficult to suspend.*

7.30 Remove the tube from the magnet.

7.31 **Add 12 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 10 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 and RNA libraries on ice until proceeding to the next step.

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 + RNA 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 RNA library) → equilibrate to RT
- Agilent DNA High Sensitivity Kit or Agilent DNA 1000 kit → equilibrate to RT
- Qubit™ dsDNA HS Kit → equilibrate to RT

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 RNA libraries.

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

NOTE

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

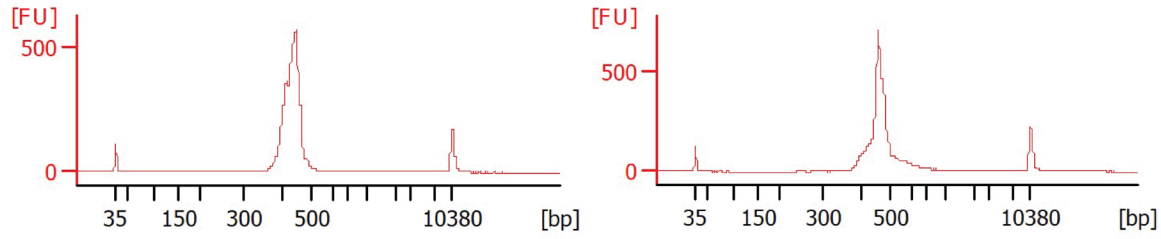


Figure 12. Library size distributions for DNA (left) and RNA (right) libraries.

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 8.5.*

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

Normalize and Pool Libraries

8.5 For DNA and RNA 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 + RNA Protocol

9. Sequence Library

9. Sequence Library

Parameter	Specification
Final library size	**DNA Library: 350 bp – 550 bp** with peak at ~460 bp **RNA Library: 450 bp – 550 bp** with peak at ~460 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, please contact support@missionbio.com.***

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

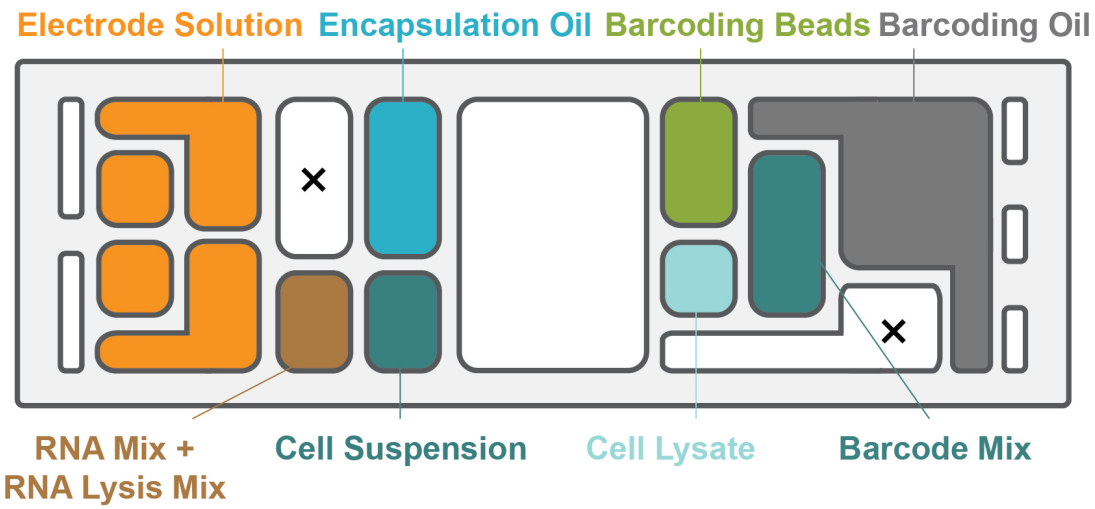
Table 10. Sequence nucleotide information for DNA Library Indices 1 – 8.

Sequence Information for RNA Library Indices 1 - 8

Index	Sequence i7	Sequence i5	Reverse Sequence i5
1	TGTGACTG	GATAGGCT	AGCCTATC
2	GTCATCGA	AGTGGATC	GATCCACT
3	AGCACTTC	TTGGACGT	ACGTCCAA
4	GAAGGAAG	ATGACGTC	GACGTCAT
5	GTTGTTCG	GAAGTTGG	CCAACTTC
6	CGGTTGTT	CATACCAC	GTGGTATG
7	ACTGAGGT	CTGTTGAC	GTCAACAG
8	TGAAGACG	TGGCATGT	ACATGCCA

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

Cartridge Map



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

Visit www.missionbio.com for additional support.